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CARLO BO

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CICLO XXX

SYNTHESIS OF VARIOUS AND INTERESTING HETEROCYCLES INCLUDED BERBERINO DERIVATIVES

Settore scientifico disciplinare: CHIM/06

RELATORE
Chiar.ma prof. De Crescentini Lucia

DOTTORANDO
Dott. Mari Giacomo

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SUMMARY

1. GENERAL INTRODUCTION

CHAPTER 1

1.1 The importance of heterocyclic compounds

Literature survey revealed that the history of heterocyclic chemistry began in the 1800's, consequentially to the first noteworthy developments like the works of Brugnatelli, Dobereiner, Runge… After World War II, there was an enormous increase of the research in the field of heterocycles. About one half of over six million compounds recorded in Chemical Abstracts are heterocyclic. Heterocyclic chemistry is one of the most complex and intriguing branch of organic chemistry and heterocyclic compounds constitute the largest and most varied family of organic compounds.[1]

Heterocyclic compounds are the cyclic organic compounds which contain at least one hetero atom; the most common heteroatoms the nitrogen, oxygen and sulphur but heterocyclic rings containing other hetero atoms are also widely known. Heterocyclic compounds are considered one of the vital classes of organic compounds, which are used in many biological fields, due their activity in multiple illnesses. Heterocyclic compounds played an important role in biological processes and are wide spread as natural products. They are widely found in nature particularly in nucleic acids, plant alkaloids, anthocyanins and flavones as well as in chlorophyll. Additionally some vitamins, proteins, hormones contain aromatic heterocyclic system (*Figure 1*).

Heterocyclic compounds have a wide range of application: they are predominant among the type of compounds used as pharmaceuticals, as agrochemicals and as veterinary products. Heterocycles are the key to biological activity in many small molecule drugs due to their ability to hydrogen bond, alter polarity and modulate lipophilicity at specific sites in the pathogen or host with the overall effect of inhibiting the biological processes that lead to programmed progression of diseases. These subunits thus have the ability to improve pharmacological, pharmacokinetic, toxicological and physicochemical properties of compounds, making them more effective in alleviating a variety of afflictions. In fact more than 90% of new drugs contain heterocycles and the interface between chemistry and biology, at which so much new scientific insights, discoveries and applications are taking place, is crossed by heterocyclic compound (*Figure 2*).[2]

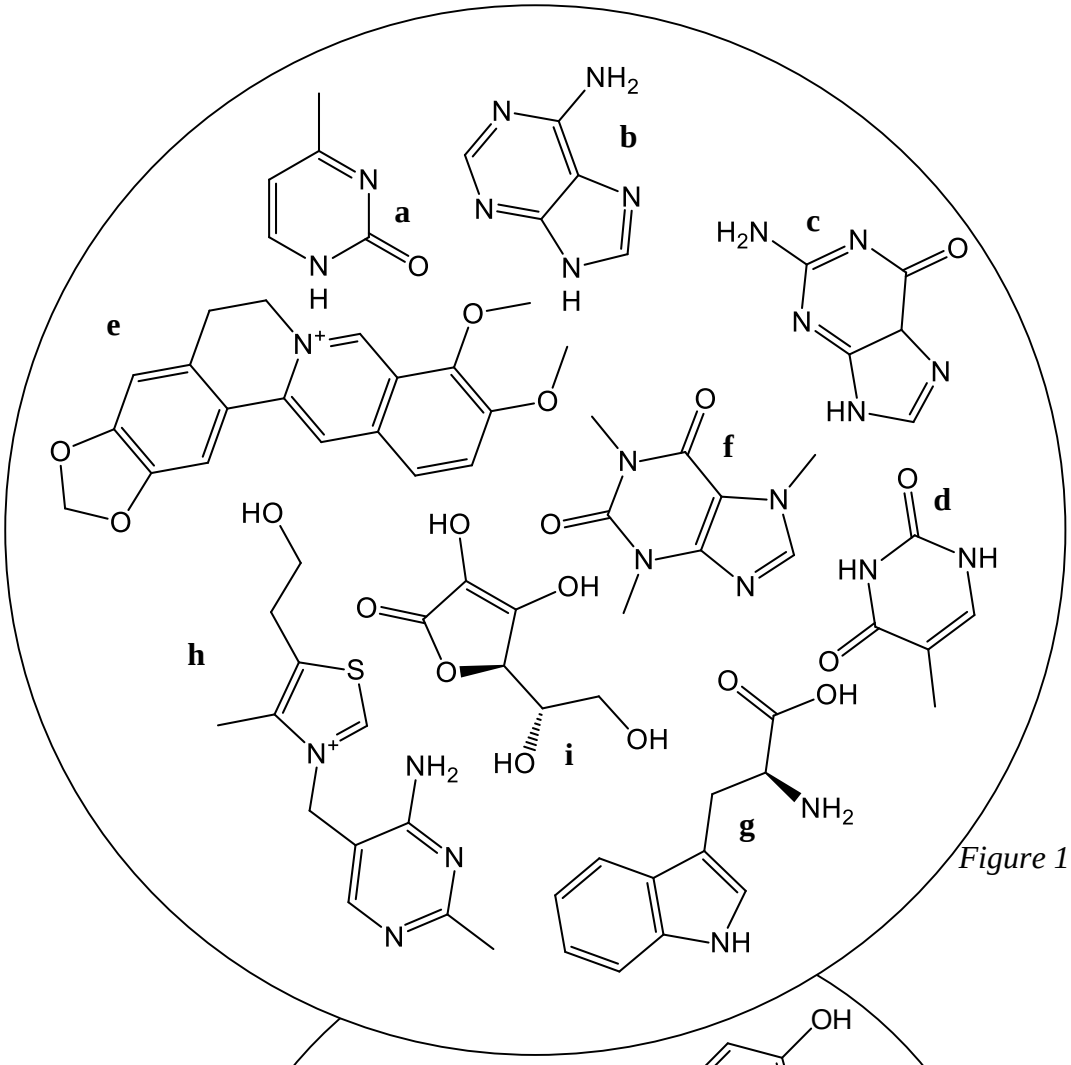


Figure 1

Figure 2

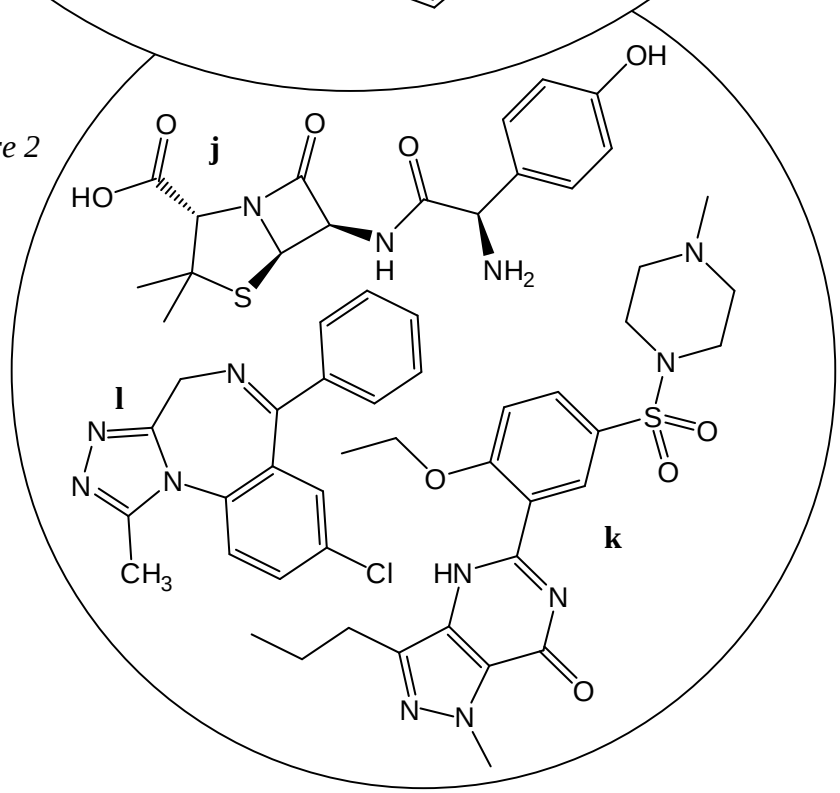
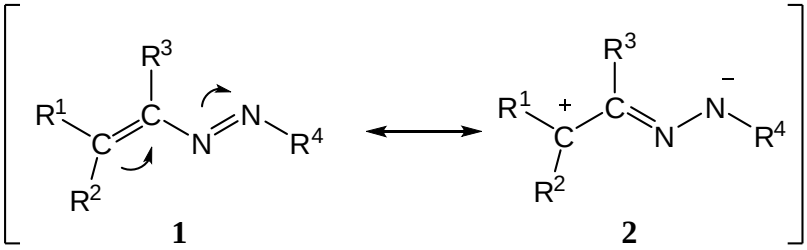


Figure 1: *Natural compounds contain heterocycles: a-Thymine b-Adenine c-Guanine d-Cytosine e-Berberine f-Caffeine g-Tryptophan h-Thiamine i-Vitamin C*

Figure 2: *Synthetic compounds contain heterocycles: j-Amoxicillin k-Sildenafil l-Alprazolam*

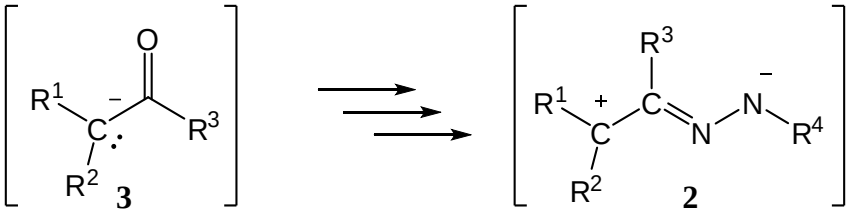
1.2 1,2-Diaza-1,3-dienes as useful building blocks

1,2-Diaza-1,3-dienes (DDs) are characterized by a carbon-carbon, nitrogen-nitrogen conjugate double bond system. Those compounds are extremely versatile: in fact, for over thirty years, they were used as building blocks for the synthesis of several five-, six- and seven-membered heteroring systems. The chemical properties of DD are strictly related to the electron-withdrawing effect of the azo group in the heterodiene system, as showed from the resonance structures in *Scheme 1* that make these compounds good Michael acceptors.



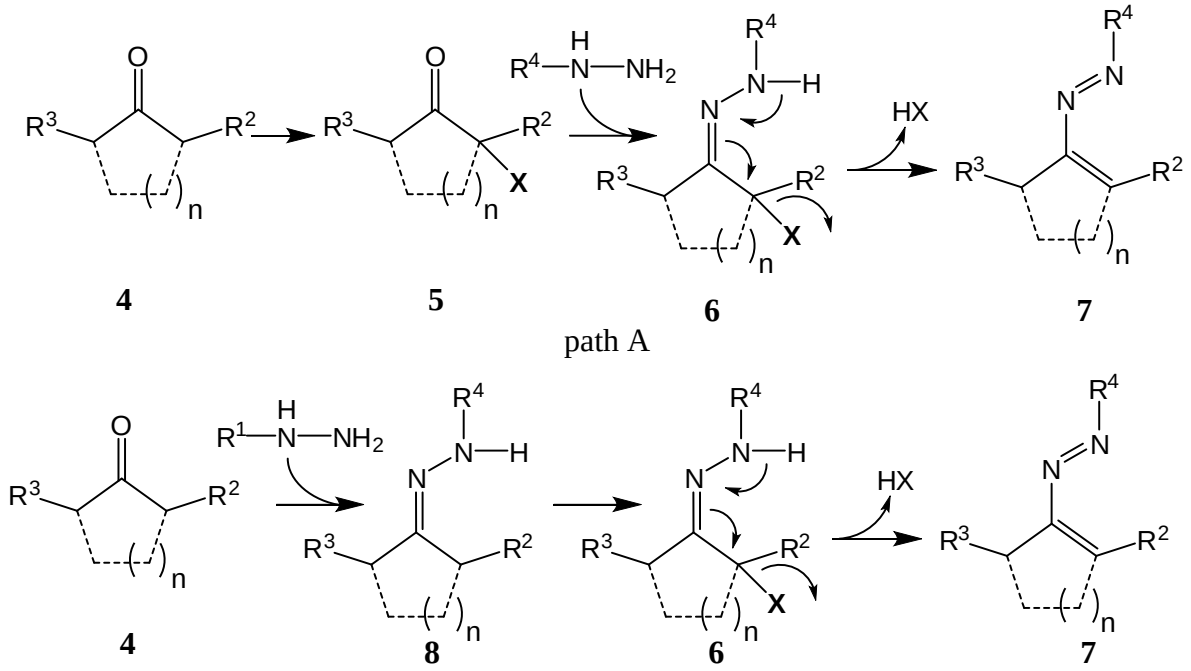
Scheme 1: *Resonance structures of DDs*

DDs feature an umpolung of the classical carbonyl reactivity, since these neutral compounds enable nucleophilic additions at the terminal carbon atom of the azoene system (*Scheme 2*). This atom is originally located in the α-position to the ketone function from which DDs are prepared. It is well known that such a carbon atom is a nucleophilic rather than an electrophilic site. Therefore, the reactivity of DD proceeds contrary to the natural polarity of the parent carbonyl derivatives and their employment represents a valid approach to reverse the normal polarity of carbon atom in the α-position to the carbonyl group.



Scheme 2: *DDs as umpolung of the classic carbonyl reactivity*

DDs are usually prepared by means of 1,4-elimination of a good leaving group X (frequently chloride or bromide) in the α-position with respect to an hydrazone function. The leaving group can be present in the starting carbonyl derivatives (*Scheme 3*, Path A) or introduced later in the hydrazone compounds (*Scheme 3'*, Path B). Both cyclic and acyclic ketones can be used in the preparation of DDs.



Scheme 3: *Synthetic pathways to prepare DDs*

Substituents influence the physical and chemical properties of DD. Electron-withdrawing groups (e.g. esters or amides) on the terminal carbon and/or nitrogen atom favor their stability and enhance the electrophilic character of the heterodiene system (*Figure 3*).[3] In addition the yellow, orange, red or amaranth color of DDs, due to their conjugation, is a convenient and useful "internal litmus" to check the progress of the reaction. In fact, the transformation of these compounds is accompanied with the change of the initial color of the reaction mixture to the final colorless or pale-yellow state.

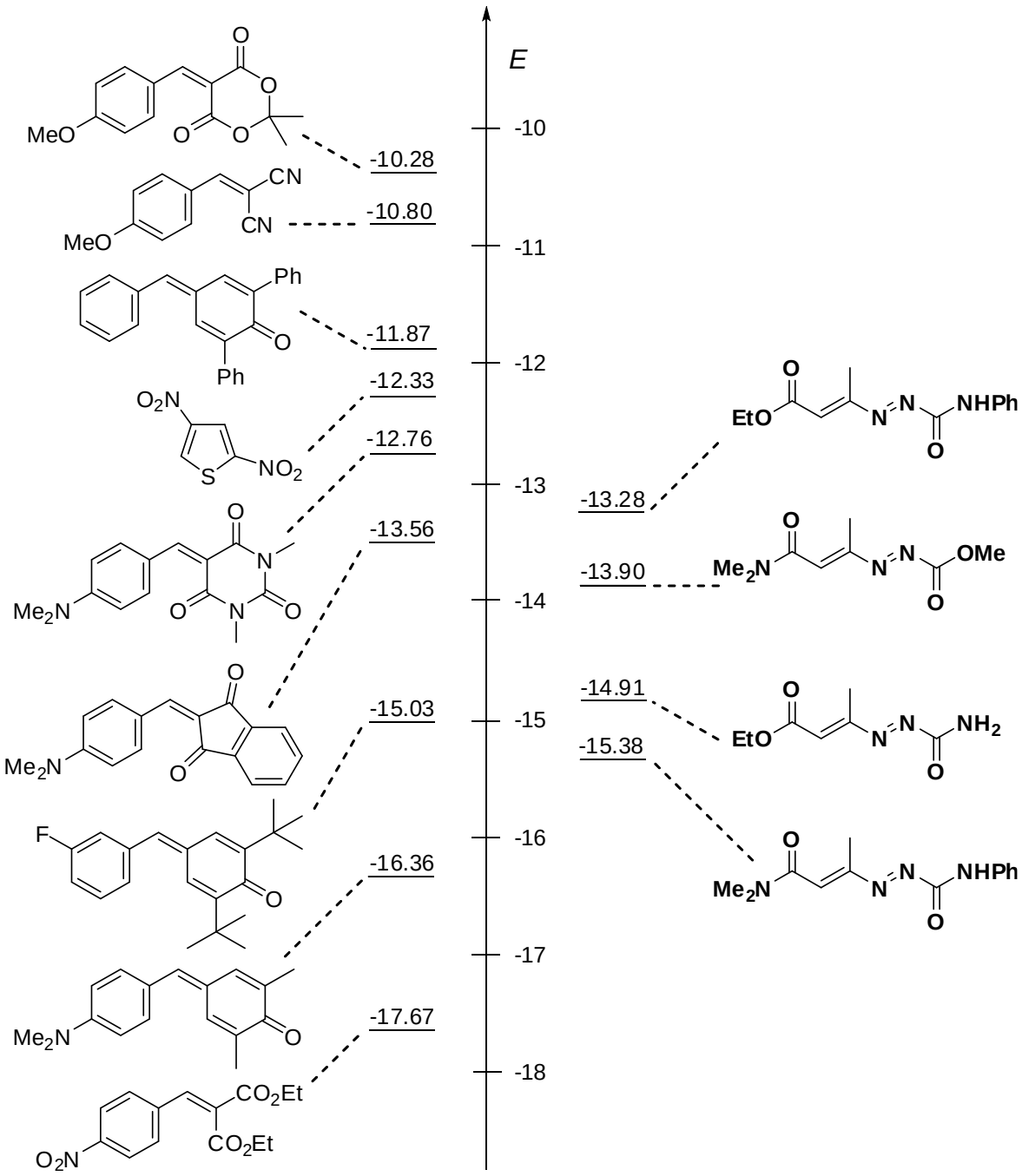
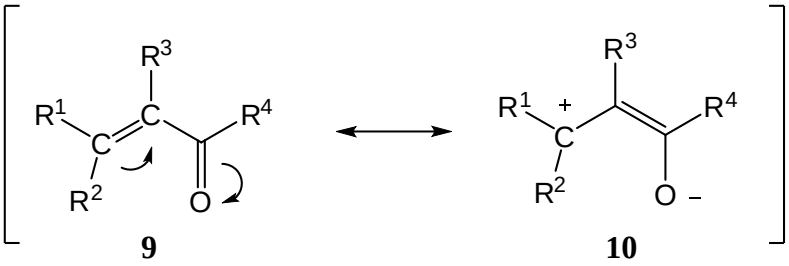


***Figure 3**: Reactivity scale: influence of substituents on the electrophilicity of DDs*

1.2.1 1,2-Diaza-1,3-dienes as acceptors in 1,4-conjugate additions

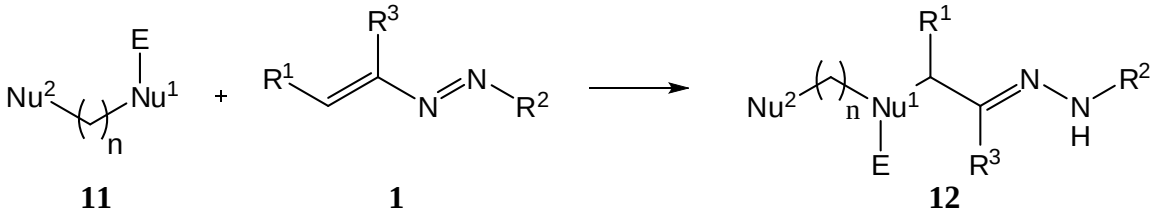
Nucleophilic conjugate addition is one of the most famous organic reactions to form C-C bond. Ordinary nucleophilic additions or 1,2-nucleophilic additions deal mostly with additions to carbonyl compounds. With the introduction of an α,β-unsaturation to a C=O, we can have a different regioselectivity. The β position is an electrophilic site as shown on *Scheme 4* which can react with a nucleophile generating a new bond.

These reactions are usually called nucleophilic conjugate additions or simply 1,4 nucleophilic additions.



Scheme 4: *Resonance structure of conjugate olefin*

The most famous nucleophilic conjugate addition is the Michael reaction,[4] where the nucleophile is an activated methylene able to react with a conjugate olefin. Also the terminal carbon atom of the azo-ene system of DDs, can undergo a nucleophilic attack by a variety of carbon and hetero nucleophiles such as oxygen, nitrogen, sulfur, selenium and phosphorus (Nu^1, *Scheme 5*), making this substrate a good Michael acceptor. These 1,4-additions produce highly functionalized hydrazones (*Scheme 5*). In this key step, together with the attacking nucleophiles, we can introduce various other nucleophiles (Nu^2, *Scheme 5*) or electrophile (E, *Scheme 5*) functions.



Scheme 5: *DDs as Michael acceptors*

We studied this versatile compound **12**, because is a potential starting material for further interesting structural modifications through controlled regioselective reactions leading to complex heterocyclic systems. In fact, different intramolecular ring closures are possible (*Scheme 6*).

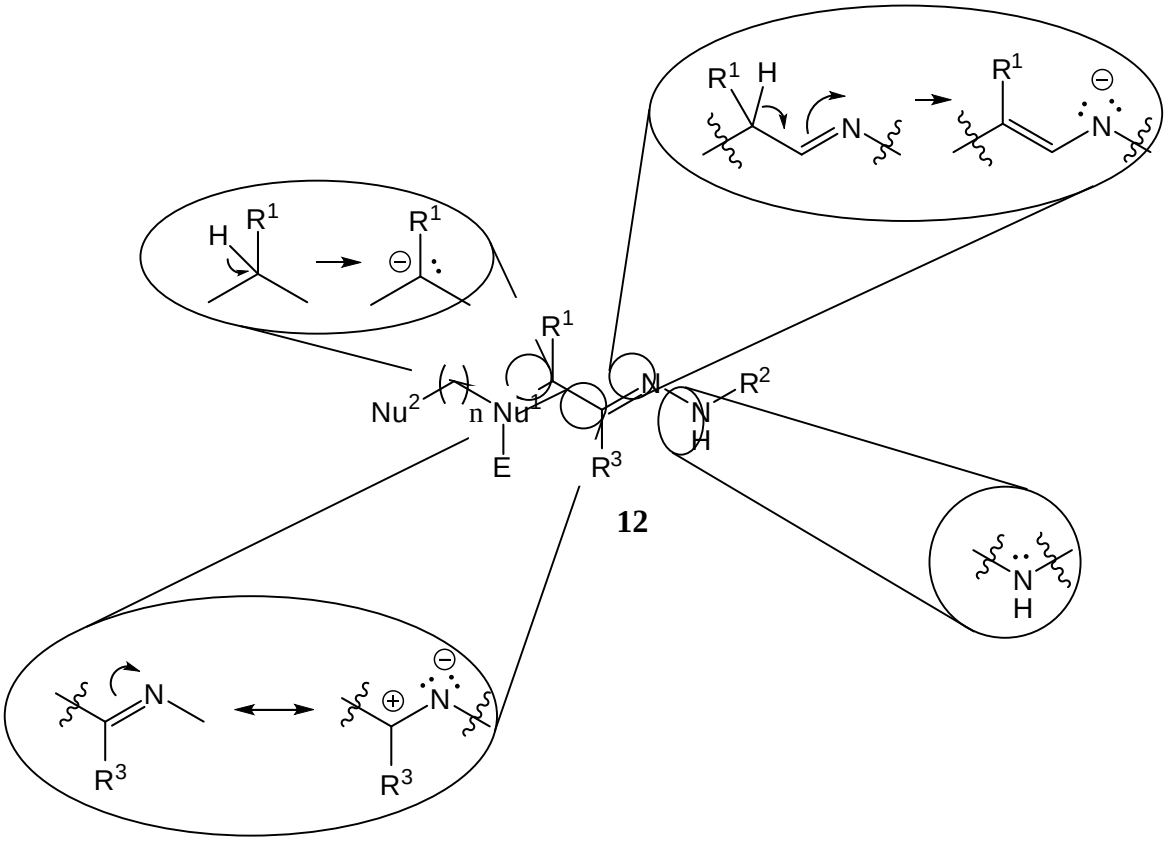


Figure 4: *Exploitable reaction sites in the highly functionalized hydrazones*

As we can observe in *Figure 4,* the most important reactive sites of these compounds are focused in:

- The hydrazone: the sp^2 hydrazonic nitrogen could act like nucleophile: the loss of the hydrogen in α-position to the hydrazone moiety promotes its nucleophilic behavior.
- The C atom in position 4: the loss of acidic hydrogen in α-position, not always promotes the activation of hydrazonic N atom, but sometimes can react like a nucleophile.
- The sp^3 hydrazine nitrogen: also this N could act like nucleophile thanks to its electronegativity.
- The hydrazone C=N bond: this function can be involved as electrophile for a further addition.

If during the first Michael addition, we have introduced other functionalities, we can exploit different intramolecular reactions and combinations to form several cyclization products.

1.2.2 1,2-Diaza-1,3-dienes as dienes in IEDDA

On the other hand, cycloaddition reactions, probably, is the most widely used methodology in organic synthesis; these synthetic methodologies make possible to widely plan ab initio the substituents of the rings by means of the preliminary preparation of simple and more accessible starting materials. In general, these reactions do not require anhydrous solvents or inert atmospheres, occur under mild conditions and need simple work-up procedures.

Electron-donating substituents in the diene influence the rate of cycloaddition. Electron-donating substituted dienes accelerate the reaction with electron-withdrawing substituted dienes: this is the normal electron-demand Diels-Alder reaction. On the other hand, electron withdrawing groups in the diene accelerate the cycloaddition with dienophiles having electron-donating groups (*Figure 5*).[5]

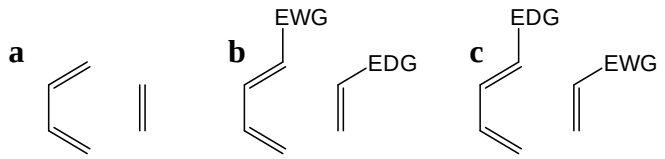


Figure 5 *(a)- neutral; (b)- inverse electron demand; (c)- normal electron demand*

The cycloadducts are usually obtained with a high degree of regio- and stereo-selectivity, as expected by the molecular frontier orbital interactions in which the dienophile is the part of donors (HOMO) and the diene is the acceptor (LUMO).

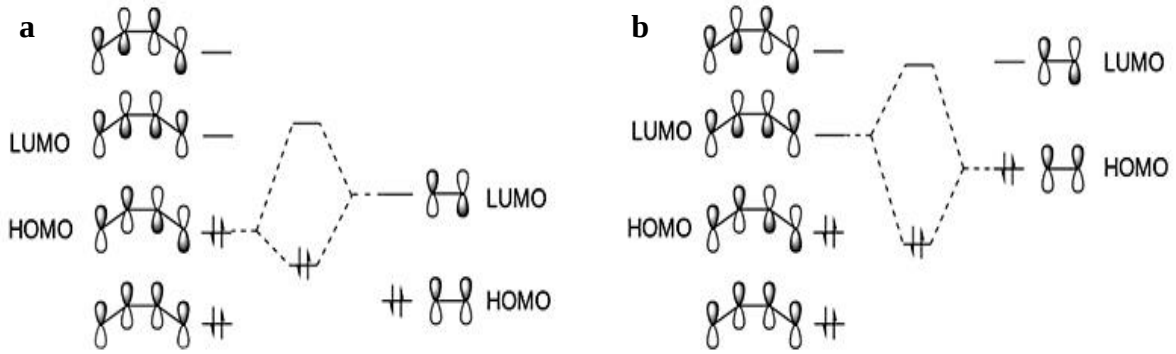


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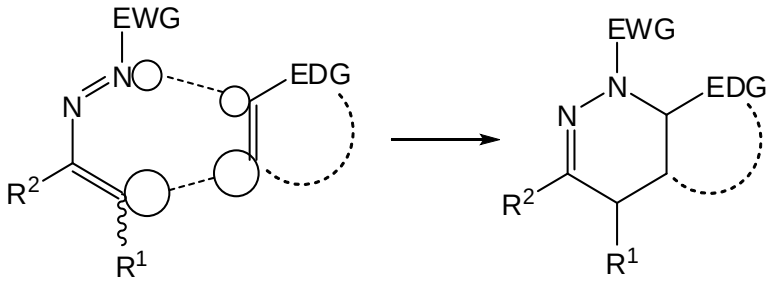
Figure 6 *According to frontier orbital theory, normal Diels–Alder additions (a) show a reduced HOMOdiene–LUMOdienophile separation (compared to the HOMOdienophile–LUMOdiene separation) which dominates the reactivity and finally, inverse electron demand (b) Diels–Alder reactions are governed by the HOMOdienophile–LUMOdiene gap.*[6]

The presence of two nitrogen atoms, combined with one or, more often, with two electron-withdrawing substituents, makes DDs very good starting materials in the "inverse-electrondemand" Diels–Alder reactions. Consequently, the main regioisomer obtained results from the terminal atom interaction of the electron rich dienophile (greater coefficient HOMO) with the terminal carbon atom of DDs (greater coefficient LUMO).

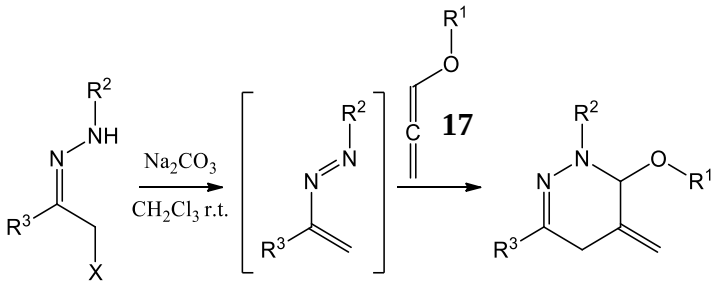
The reaction with carbon dienophiles[7] produces 2,3,4,5-tetrahydropyridazines **14** (*Scheme 6*) and follows the scheme of the donor-acceptor, in which the electron-rich alkenes **13** is the partner of the electron-deficient DDs **1**.



1 **13** **14**

Scheme 6: *Inverse electron demand Dies Alder (IEDDA) of DDs and electron rich olefin*

An example of this mechanism is reported in the work of Favi et al.[8] The synthesis of substituted tetrahydropyridazines shows the regioselectivity of IEDDA reaction (*Scheme 7*).



15 **16** **18**

Scheme 7: *Significative example of IEDDA reaction*

In this work DDs are not directly used, but they were generated in situ from α-hydrazones in basic conditions.

1.3 Results

Using DDs as building blocks, we have obtained a great number of useful heterocycles. Thanks to their reactivity, previously showed in paragraphs 1.2.1 and 1.2.2, those versatile compounds are involved in several annulations (*Figure 7*).

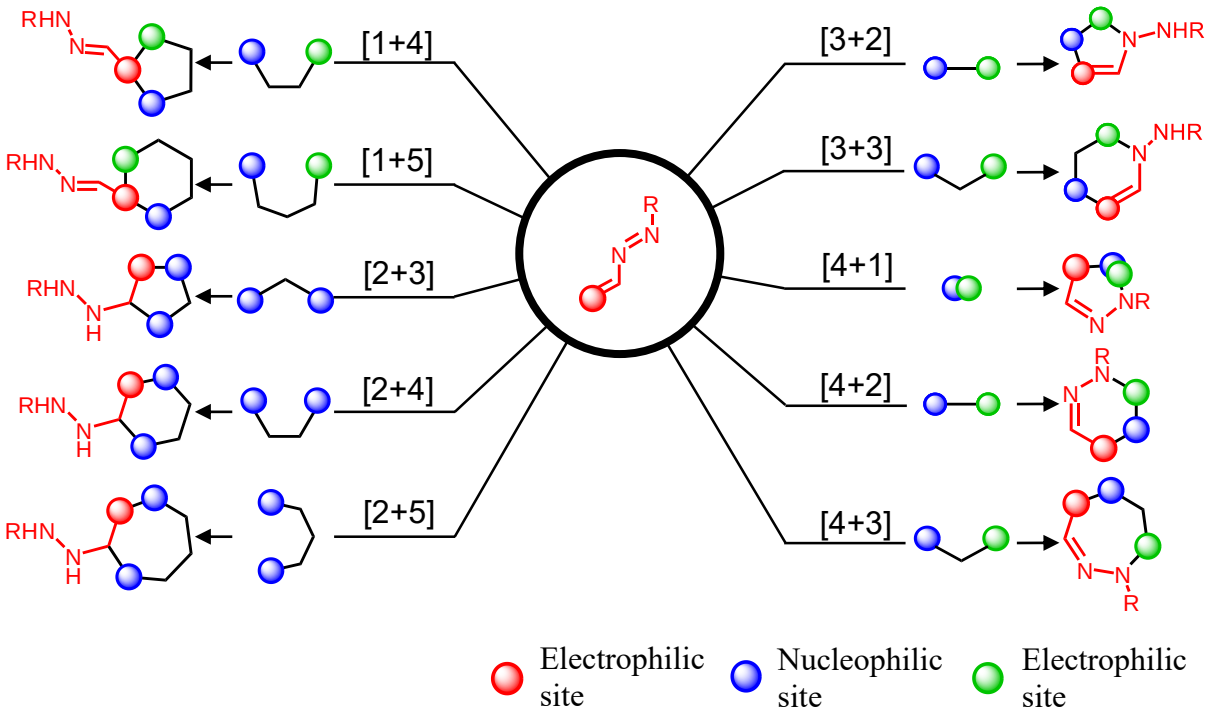
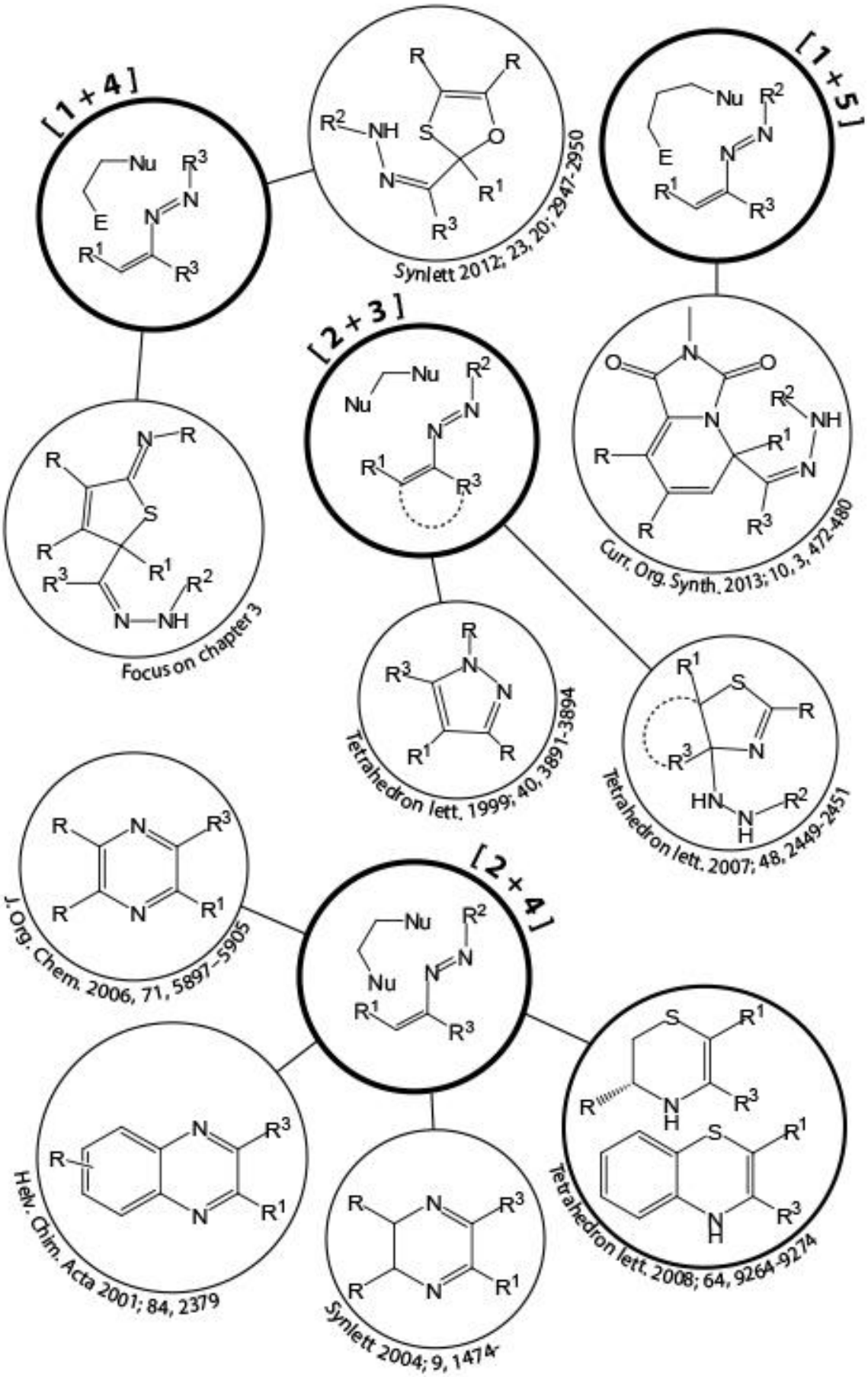
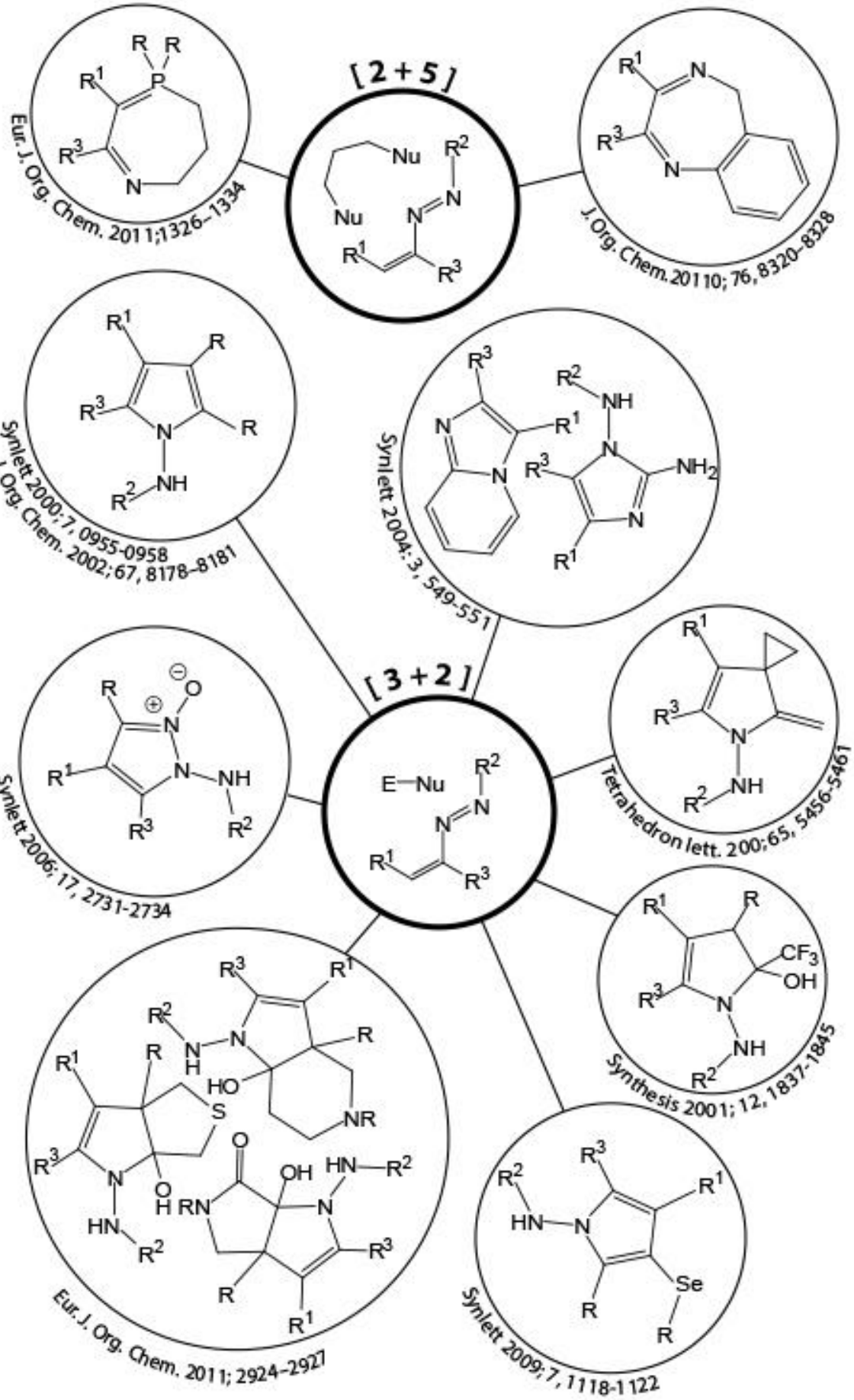


Figure 7 *DDs as building blocks*

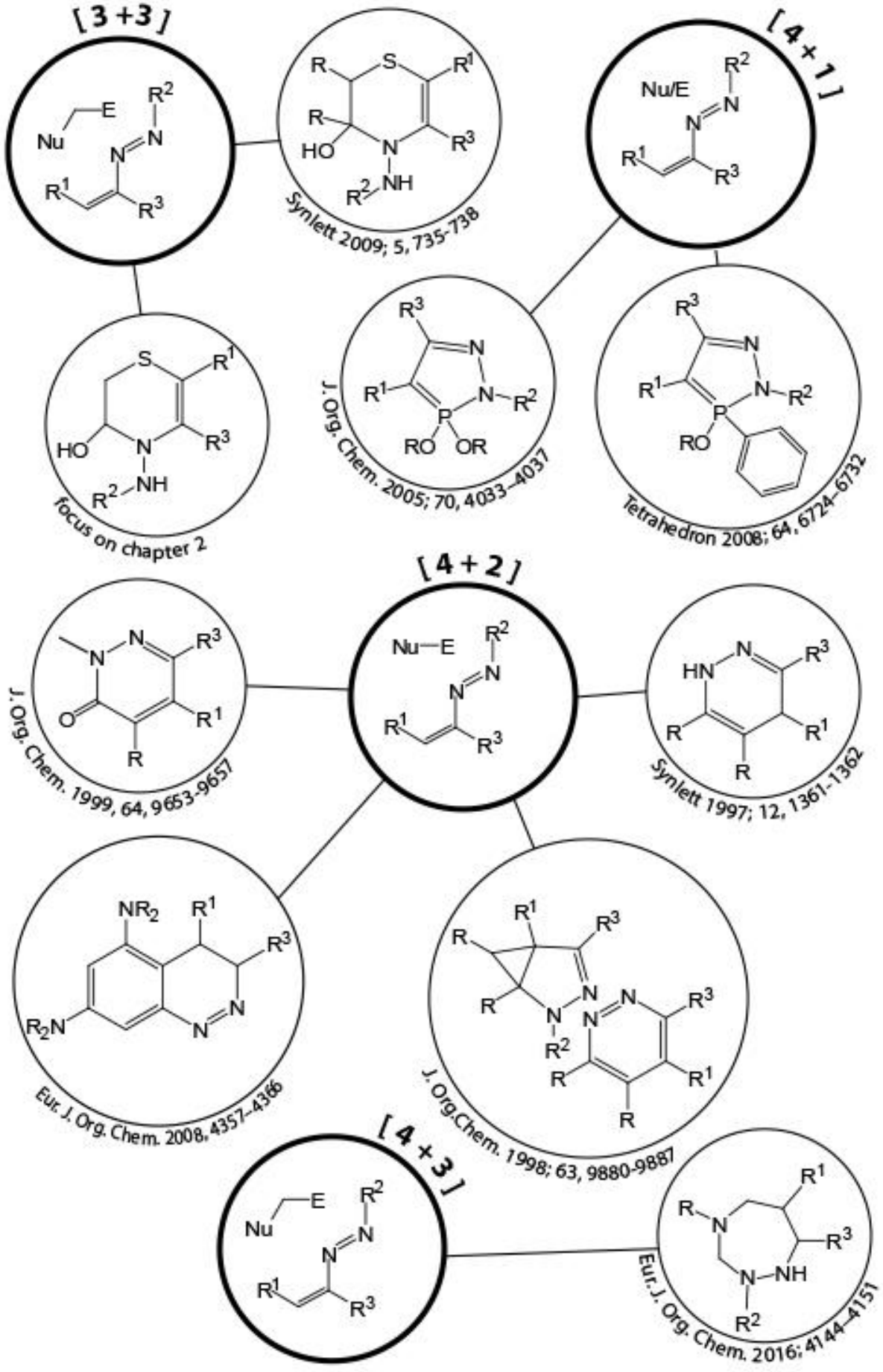
In this paragraph, we are going to have a focus to the most important reactions involved DDs with 1, 2, 3 or 4 atoms included in the final heterocyclic products. The next info graphic schedule reports some papers published in the past years.



Info graphic 1: *Representative examples of our group works*



Info graphic 2: *Representative examples of our group works*



Info graphic 3: *Representative examples of our group works*

2. FACILE ODOURLESS QUANTITATIVE SYNTHESIS OF 3-HYDROXY-3,4-DIHYDRO-2H-1,4-THIAZINES

CHAPTER 2

G. Mari, G. Favi, L. De Crescentini, S. Santeusanio, O. A. Attanasi, F. Mantellini
Asian J. Org. Chem., **2016**, 5, 705–709

2.1 Introduction

One of the main objectives of the modern organic chemistry is the easy transformation of readily available precursors into target-relevant products in a rapid, economical, efficient and eco-friendly manner. In particular, in the last time, the environmental protection and waste prevention have increased interest and relevance in an overcrowded world where demand becomes more pressing. The efficiency in atom economy combined with quantitative reaction can play a key role in the environmental field. In fact, waste-free synthesis (total atom economy) yielding single product in pure form that does not require solvent-consuming purification steps, avoiding further procedures such as chromatography, extraction or recrystallization corresponds to a fundamental benefit both from the environmental and the economical point of view.[9]

The 1,4-thiazine core (thiomorpholine) represents a recurrent structure in organic chemistry because it is present in some numerous compounds that found application both in medicinal/pharmaceutical chemistry, as well as in chemical manufacturing.

In fact, the biological properties shown by these aza-sulphur heterocycles range from antibacterial[10] or antimicrobial[11] to anti-inflammatories characteristic,[12] from hepatoprotective features[13] to calcium antagonistic,[14] from vasopressin receptor antagonistic[15] to anticancer activities,[16] from antioxidant to hypolipidemic agents.[17]

Besides, the 1,4-thiazine derivatives are commonly used as stabilizer or inhibitor, [18] and it is well known how this heterocyclic nucleus plays an important role in numerous pigments[19] and dyestuffs.[20]

The thiomorpholine core contributes to constitute also the skeleton of different natural products (*Figure 8*): for example, it is present in the structure of Adaciaquinones B,[21] a compound extracted from marine sponge *Petrosia alfiani* that showed antitumor activities, or in a metabolite produced by endophytic fungus *Paraphaeosphaeria neglecta FT462* isolated from the Hawaiian plant *Lycopodiella cernua*,[22] or in cytotoxic terpene derived Conicaquinones A or B isolated from Mediterranean ascidian *Aplidium conicum*.[23]

By virtue of these numerous applications, different methods have been developed for the preparation of thiomorpholine cores.[11,24] Usually, these approaches require harsh conditions, elaborated work up procedures and the thiols employed as starting materials are flammable, harmful, and strong smelly reagents, which can lead to serious environmental and safety problems. The most common synthetic route that involves 1,2-dihaloderivatives as starting materials furnishes low yields due to side-reactions such as elimination, requiring different purification procedure.[25]

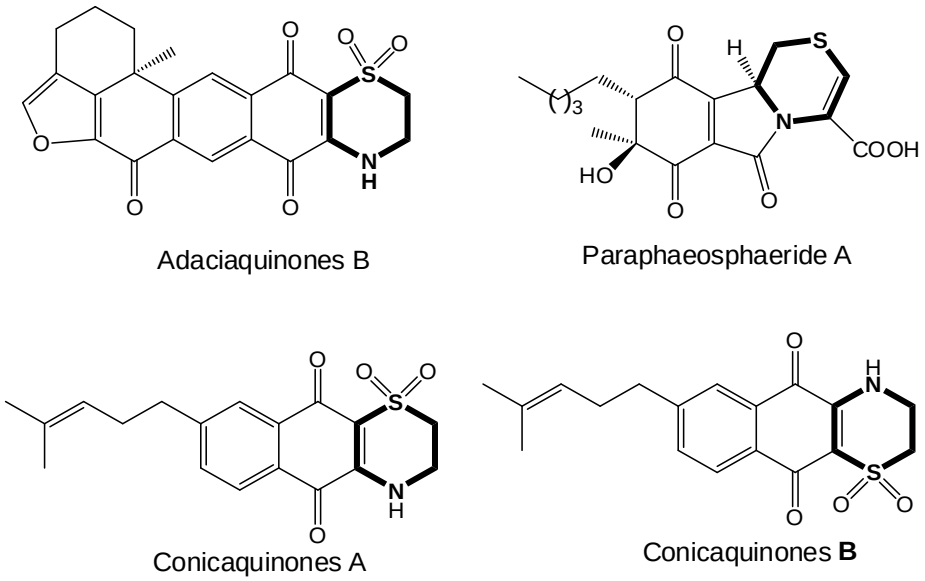


Figure 8: *Representative examples of natural products containing a 1,4-thiazine core.*

Aware of this interest, in the last years, we have proposed a synthetic route to obtain dihydro- or benzo-1,4-thiazine derivatives employing 1,2-diaza-1,3-dienes and 1,2-mercaptoamines as starting materials. In this case, the desired heterocycles were obtained in good yields through a formal [4+2] cyclization, in which the DDs contribute with 2 carbon atoms.[26]

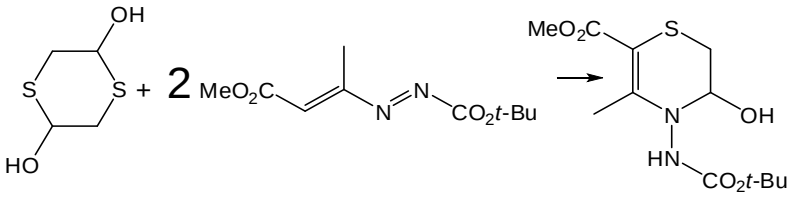
Also, the synthesis of containing 1,4-thiazine core bis-heterocycles such as 2-oxo[1,2,4]triazolo[5,1-*c*][1,4]thiazines was developed by our group using DDs and mercaptoketones in acidic medium.[27] Some limits affect this methodology: the overall yields were moderate, the 1,4-thiazine intermediates cannot be isolated, and, employing phosphonyl-substituted-DDs, the target heterocycles were not obtained.

Furthermore, despite of the variety of synthetic methodologies which are available for the construction of thiazine ring, dihydrothiazines bearing a quaternary carbon center such as emiheminal have been difficult to obtain. Until now, only one example of 3-hydroxy-dihydro-1,4-benzothiazine has been reported.[28]

For these reasons, we decided to find out new reagents able to react with DDs[29] to overcome these issues. The air stable, and odorless 1,4-dithiane-2,5-diol that has shown to be an useful synthon for the preparation of various sulphur containing heterocycles such as tetrahydrothiophenes,[30] 1,3-oxathiolanes,[31] thiazoles,[32] tetrahydrothiopyranols[33] should be a valid candidate.

2.2 Results and Discussion

Initially, we set out to identify the possible mild conditions under which the reaction of DDs with dithiane-2,5-diol **19** would proceed choosing DD **1a** as representative compound.



19 **1a** **21a**

Scheme 8: *Used reagent for the optimization of the synthesis of 3-hydroxy-3,4-dihydro-2H-1,4-thiazine*

Entry	Base	Equivalent of base	Solvent	Vol. solvent (mL)	Time (h)	Yield (%) of **21a**[b]
1	DIPEA	0.1	CH_2Cl_2	10.0	0.15	37
2	TEA	0.1	CH_2Cl_2	10.0	0.15	68
3	DABCO	0.1	CH_2Cl_2	10.0	0.15	66
4	K_2CO_3	0.1	CH_2Cl_2	10.0	0.15	21
5	TEA	0.2	CH_2Cl_2	10.0	0.15	75
6	TEA	0.3	CH_2Cl_2	10.0	0.15	73
7	TEA	0.2	MeCN	10.0	0.15	51
8	TEA	0.2	EtOAc	10.0	0.15	49
9	TEA	0.2	EtOH	10.0	0.15	36
10	TEA	0.2	THF	10.0	0.15	81
11	TEA	0.2	Et_2O	10.0	2.15	93
12	TEA	0.2	Et_2O	25.0	3.50	86
13	TEA	0.2	Et_2O	5.0	1.50	95
14	TEA	0.2	Et_2O	2.0	1.15	Quant.
15	TEA	0.2	SFC	0	5.00	31[c]
16	TEA	0.2	SFC	0	5.00	34[d]
17	Resin[e]	0.2	Et_2O	2.0	1.00	38
18	Resin[e]	1.0	Et_2O	2.0	1,00	42
19	**DMEA**	**0.2**	**Et_2O**	**2.0**	**1.50**	**Quant.**

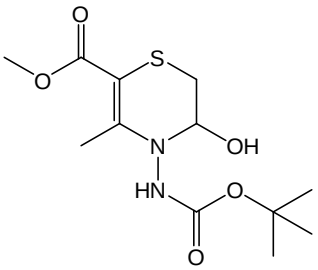
Table 1: *Optimization of reaction conditions for the synthesis of 3-hydroxy-3,4-dihydro-2H-1,4-thiazine. All the reactions were carried out under the following conditions: 1,4-dithiane-2,5-diol **19** (0.5 mmol), DD **1a** (1.0 mmol), room temperature. [b] The yields were determined by ^{1}H NMR spectroscopy of the crude (4-nitrotoluene as internal standard).[34] [c] DD **1a** was employed as solvent-reagent in the molar ratio of 1:0.5 referred to **1**. [d] DD **1a** was employed as solvent-reagent in the molar ratio of 3:0.5 referred to **19**. [e] Merck Millipore 104767 Ion exchanger III.*

Our investigation began evaluating different bases, necessary to generate in situ the 2-mercaptoethanal **19'** (*Scheme 8, Table 1, entries 1-5*). The desired 3-hydroxy-3,4-dihydro-2*H*-1,4-thiazine **21a** was obtained with higher yields using 1,4-diazabicyclo[2.2.2]octane (DABCO) and triethylamine (TEA) in dichloromethane (*entries 2,3, Table 1*). Between these two bases, the cheapest TEA has been chosen. We have observed that product **21a** during the chromatographic procedure spontaneously gave decomposition reactions. For this reason, the yield of the thiazine **21a** was determined directly on the crude mixture by ^{1}H-NMR analisys.[34] By increasing the amount of TEA to 0.2 eq, the yield of the product **21a** was enhanced to 75% (*entry 5, Table 1*). No further improvement in the yield was observed when TEA was

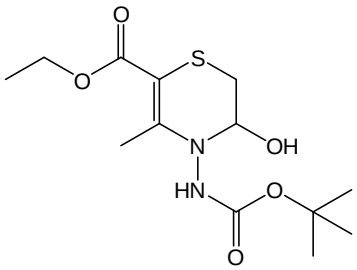
incremented to 0.3 eq. (*entry 6, Table 1*). Then we proceeded testing different solvents such as acetonitrile, ethyl acetate, ethanol, tetrahydrofuran and diethyl ether (*entries 7-11, Table 1*). The best outcome was in diethyl ether (*entry 11, Table 1*). An increment of the solvent amount generates lower yields with longer reaction time (*entry 12, Table 1*), while more concentrated solutions provide better yields and faster reactions (*entries 13, 14, Table 1*). Encouraged by these findings, we have carried out the reaction in solvent free conditions (SFC), in which DD **1a** acts as solvent-reagent (mp of **1a** < 15 °C). The desired thiomorpholine derivative **19a** was formed only in poor yields, employing either 1 or 3 equiv. of **1a** (*entries 15, 16, Table 1*).

These latter results probably are due to the low solubility of 1,4-dithiane-2,5-diol **19** in these conditions. With our delight, we have found that, at room temperature, in 2 mL of diethyl ether and in the presence of 0.2 equiv. of TEA, the reaction between 1,4-dithiane-2,5-diol **19** (0.5 mmol) and DD **1a** (1.0 mmol) furnished quantitatively the desired 3-hydroxy-3,4-dihydro-2*H*-1,4-thiazine **21a** (entry 14, Table 1). Taking advance of this fact, to facilitate the separation of the desired thiomorpholine **21a** from the promoter, we have tested other bases easily removable from the crude such as a basic resin, readily turned away by filtration, or *N*,*N*-dimethylethylamine (DMEA) (bp 36-38 °C) easily and fully evaporable under vacuum. While the use of the resin both in a catalytic (0.2 equiv.) or in stoichiometric amount provided the desired **21a** only in moderate yields (*entries 17, 18, Table 1*), the employ of the DMEA produced **21a** in quantitative manner without formation of any by-product (*entry 19, Table 1*). It is remarkable that in this latter case, to obtain the crude and pure desired product **21a**, solely the evaporation of the solvent and DMEA was necessary. Avoiding the chromatographic separation, the degradation processes previously observed during the development of the procedure did not take place. In fact, the direct ^{1}H- and ^{13}C-NMR analyses of the crude showed exclusively the signals related to the proposed structure of compound **21a** confirming that the yields are quantitative.

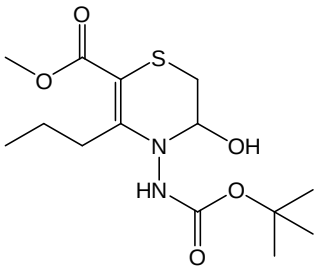
The gradual disappearance of the red colour imparted to DDs by their internal conjugation permits the visual check of the reaction progress. Therefore, this occurrence contributes to make this methodology very facile, eventually requiring only the evaporation step at the end of the reaction shown by a colour change of the solution. Identified the optimal conditions, reactions of 1,4-dithiane-2,5-diol **19** with a range of different DDs **1a–k** were evaluated (*Table 2*). In all cases, products **21a–k** were quantitatively obtained simply by under vacuum evaporation at room temperature of the solvent and DMEA without any other purification step. Notably, the synthesis of **3b** was scaled up to the gram-scale without any noticeable drawback (*Table 2*).



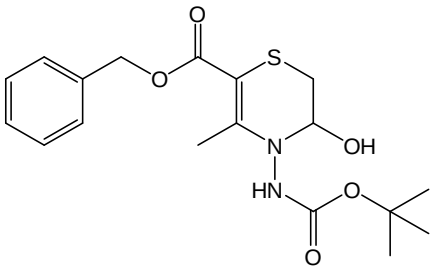
21a quantitative yeld



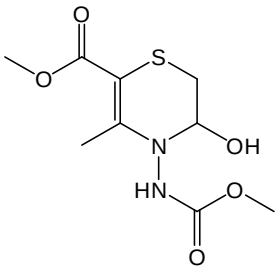
21b quantitative yeld



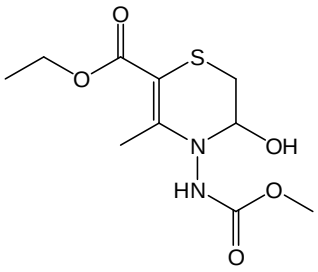
21c quantitative yeld



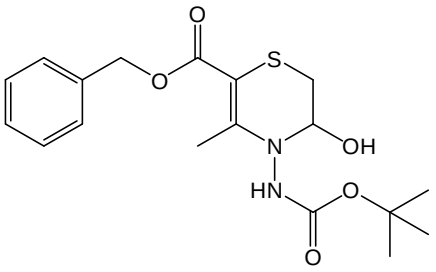
21d quantitative yeld



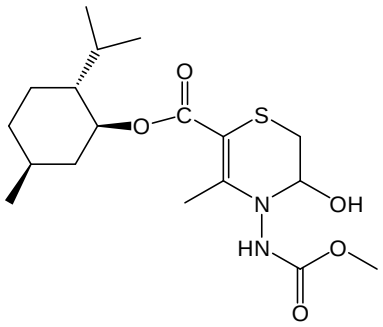
21e quantitative yeld



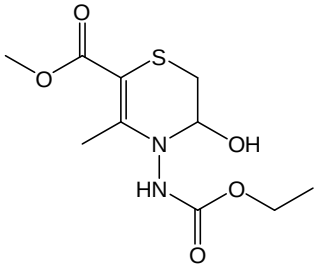
21f quantitative yeld



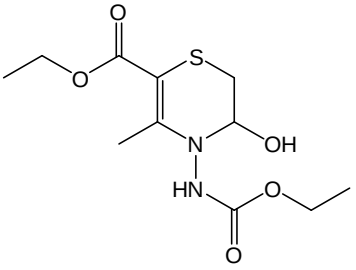
21g quantitative yeld



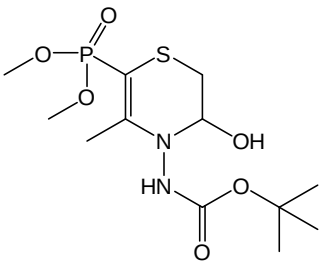
21h quantitative yeld



21i quantitative yeld



21j quantitative yeld

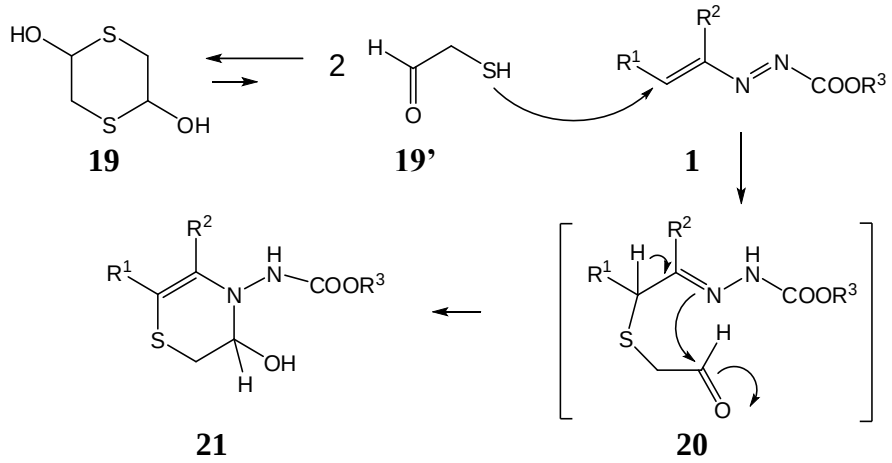


21k quantitative yeld

Table 2: *The yields were determined by ^{1}H NMR spectroscopy of the crude pure products.[34] Reaction was carried out on gram scale with 1.0 g of **1b***

Employing 4-phosphonyl-DD **1k**, the corresponding phosphorylated 1,4 thiazine **21k** was achieved. It is noteworthy that the introduction of organophosphorus moieties in simple synthons may provide valid substrates for the preparation of biologically active compounds.

The plausible mechanism contemplates the in situ generation of 2-mercaptoethanal **19'** that is readily captured from the terminal carbon atom of the azo-ene system of DDs **1**. This initial sulpha-Michael addition leads to the formation of the odourless α-sulpha-functionalized hydrazone intermediates **20**. Intramolecular nitrogen nucleophilic attack on carbonyl group furnishes the final **21a–k** through a formal [3+3] cycloaddition. In this way, the reaction comes to completion without any formation of unpleasant stench.



Scheme 9: *Plausible mechanism for the synthesis of 3-hydroxy-3,4-dihydro-2H-1,4-thiazines **21a–k**.*

2.3 Conclusions

In conclusion, here we report a new simple and convenient methodology for easy access to a series of 3-hydroxy-3,4-dihydro-2*H*-1,4-thiazines. This procedure proceeds rapidly to completion, produces only the desired compounds without formation of by-products, and offers the final pure products without any chromatography process. The in situ generation of 2-mercaptoethanal using odourless, and inoffensive dithiane-2,5-diol **19** is an appropriate solution to serious environmental and safety problems due to the utilization of volatile and unpleasant smelling thiols.Then, quantitative yields, absence of tedious separation procedures, total atom efficiency, mild reaction conditions, readily available and inexpensive starting materials, operational simplicity, clean reaction profiles as well as energy efficiency, are the key advantages of the present method.

3. UNEXPECTED SYNTHESIS OF 2,3,5,6-TETRAHYDRO-1*H*-PYRROLO[3,4-*C*]PYRIDINE-1,3,6-TRIONES

CHAPTER 3

G. Mari, L. De Crescentini, G. Favi, S. Santeusanio, S. Lillini, F. Mantellini
Eur. J. Org. Chem., **2017**, 42, 6291–6298

3.1 Introduction

One of the most intriguing targets of organic chemists is the possibility to obtain complex transformations in a single-step reaction, starting from a set of various reagents to give directly the final product.

This kind of transformations in which several bonds are formed in one sequence in the same reaction conditions without isolating the intermediates and without adding additional reagents or catalysts are commonly called domino or cascade reactions.[35,36]

It appears clear that the advantages of this synthetic methodology can be of economical, ecological and operative interest, as it increases the synthetic efficiency and in parallel decreases the amount of solvents, reagents, catalysts used and the number of laboratory operations required.[35,36]

The utility and versatility of 1,2-diaza-1,3-dienes in the construction of a variety of heterocyclic rings are well known and are demonstrated by significant activity in this field over recent years.[3,29,37] In particular, they can react not only as Michael acceptors in conjugated additions but they can also be employed in cycloaddition reactions with a wide range of partners.[3,29,37,39]

In most cases reported in the literature for the Michael addition, DD participates with one unit in the construction of the final heterocycle, while there are only few examples in which it takes part with two units. In particular, in the spiroheterocycle-pyridines previously synthesized by our group starting from DDs and some oxindole or barbiturate derivatives, the two molecules of DDs contribute to the final pyridine core with 3 and 2 atoms (Structure A, *Figure 3*).[29b,29d]

Another example is represented by pyridazines derived from the cyclodimerization of two molecules of DDs that results in formal [4+2] cycloaddition (Structure B, *Figure 3*).[39]

Recently, Shelke and Suryavanshi have reported the synthesis of tetrazocine derived from [4+4] cycloaddition (Structure C, *Figure 3*).[39a]

In the present work, for the first time, two units of DDs participate in the construction of a system containing two fused heterocycles of the formed pyrrolo-pyridines with 4 and 2 atoms (Structure D, *Figure 3*). In particular, one unit of the DDs contributes in both the pyridine and pyrrole nuclei of the final molecule. Our initial intent was to obtain 4-oxo-2-thioxo-1-thia-3,8-diazaspiro[4.5]deca-6,9-diene-6,10-dicarboxylates **A** (*Scheme 10*, Route B), since rhodanines represent a privileged scaffold in drug discovery, and they are known to exhibit a wide spectrum of pharmacological properties.[40]

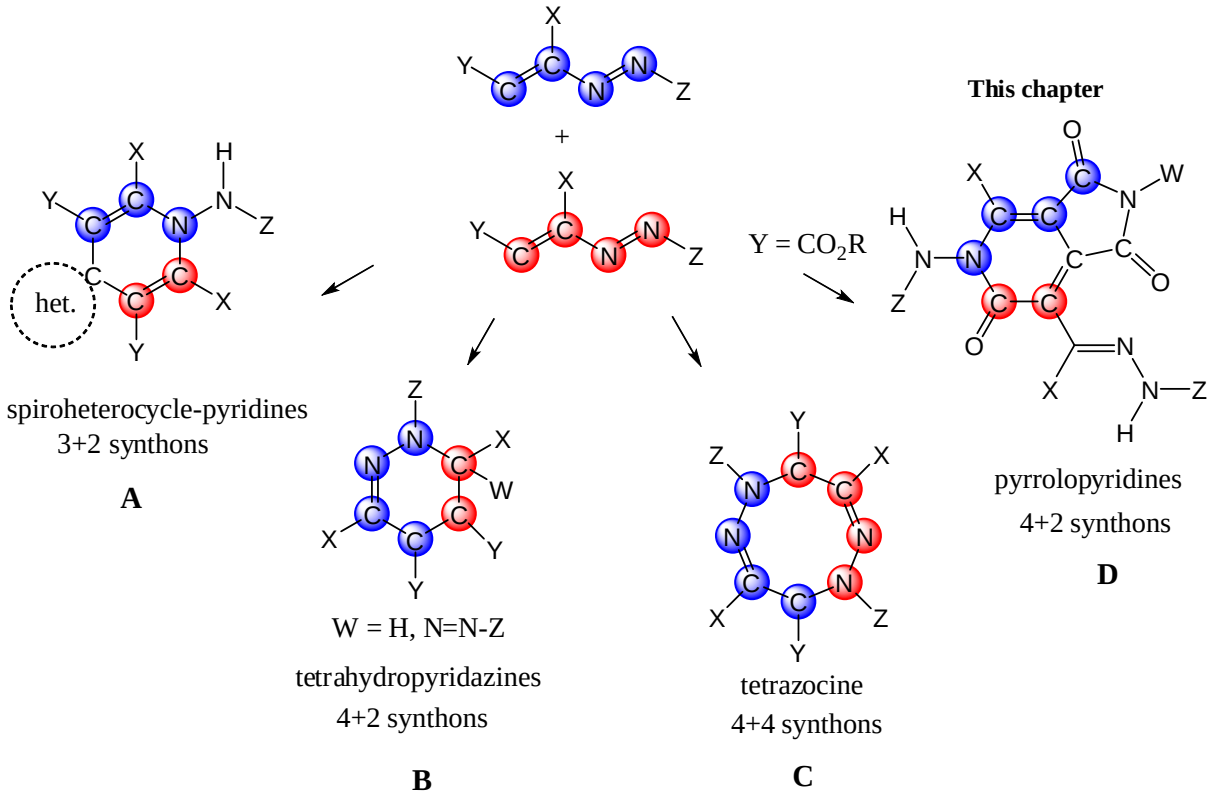
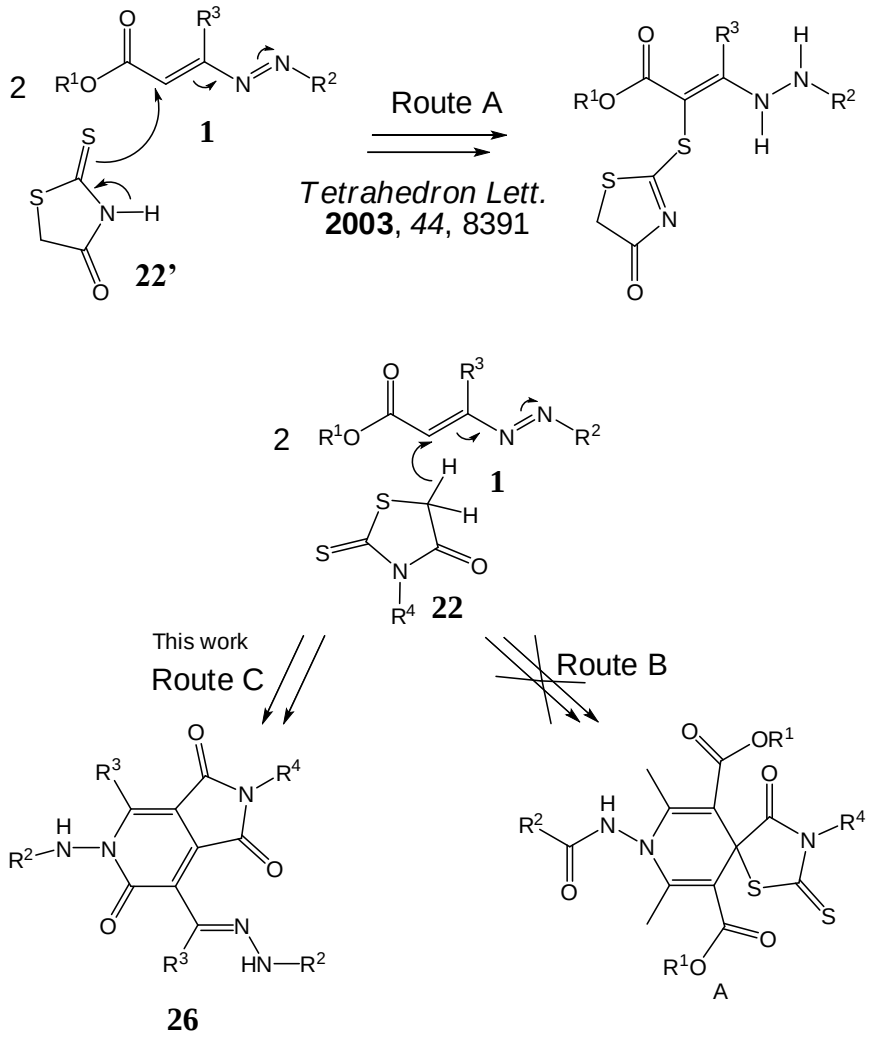


Figure 3: *Participation of two DDs units in heterocyclic assembly.*

Previously, some of us investigated the reaction between DDs **1** and *N*-unsubstituted rhodanines **22'** (*Figure 1*, Route A).[41] In this case, the sulphur of the rhodanines acted as nucleophile. To avoid this eventuality, we have planned to explore the reaction between two equivalents of DDs **1** and some *N*-substituted rhodanine derivatives **22** in which carbon at position 5 should act as nucleophile.

After the formation of bis-adducts by means of a double Michael addition of the rhodanine **22** to two molecules of the DD **1**, the intramolecular ring closure could have furnished the desired spiro derivatives **A** in analogy to what previously observed [29b,29d] (*Scheme 10*, Route B).

But things went different and the reactions have surprisingly furnished interesting 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridine-1,3,6-triones **26** (*Scheme 10*, Route C). These bicyclic compounds represent a new family of HIV-1 integrase inhibitors that showed low micromolar inhibitory potency in vitro HIV-1 integrase assays, with good selectivity for strand transfer reactions as compared with 3'-processing inhibition.[42] They have reported in literature to be prepared by 'Plummer cyclization-deprotonation-cycloaddition' cascade reactions of imidosulfoxides.[42]

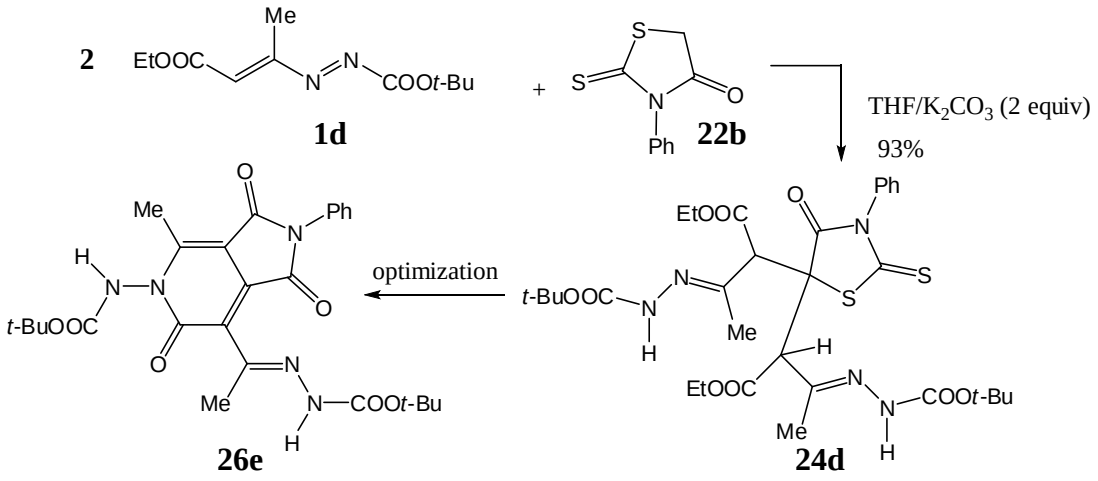


Scheme 10: *Our intent to synthesize 4-oxo-2-thioxo-1-thia-3,8-diazaspiro[4.5]deca-6,9-diene-6,10-dicarboxylates* ***A****: the supposed (Route B), the obtained (Route C).*

3.2 Results and Discussion

Several methods are reported in the literature for the synthesis of rhodanines.[43] To start our investigations, we have prepared 3-ethyl, 3-phenyl-, 3-(4-chlorophenyl)- and 3-(4-methoxy)-2-thioxothiazolidin-4-ones **22a–d** according to the procedure reported by Ravi et al.[43a]

So, we began our studies by carrying out a preliminary reaction between two equivalents of DD **1d** and 3-phenyl rhodanine **22b**, chosen as examples, in basic conditions, using two equivalents of K_2CO_3. As expected, bis-hydrazone-functionalized rhodanine **24d** was formed in 93% yield, resulting from a double Michael-type addition of the rhodanine **22b** to two equivalents of DD **1d** (*Table 3*).



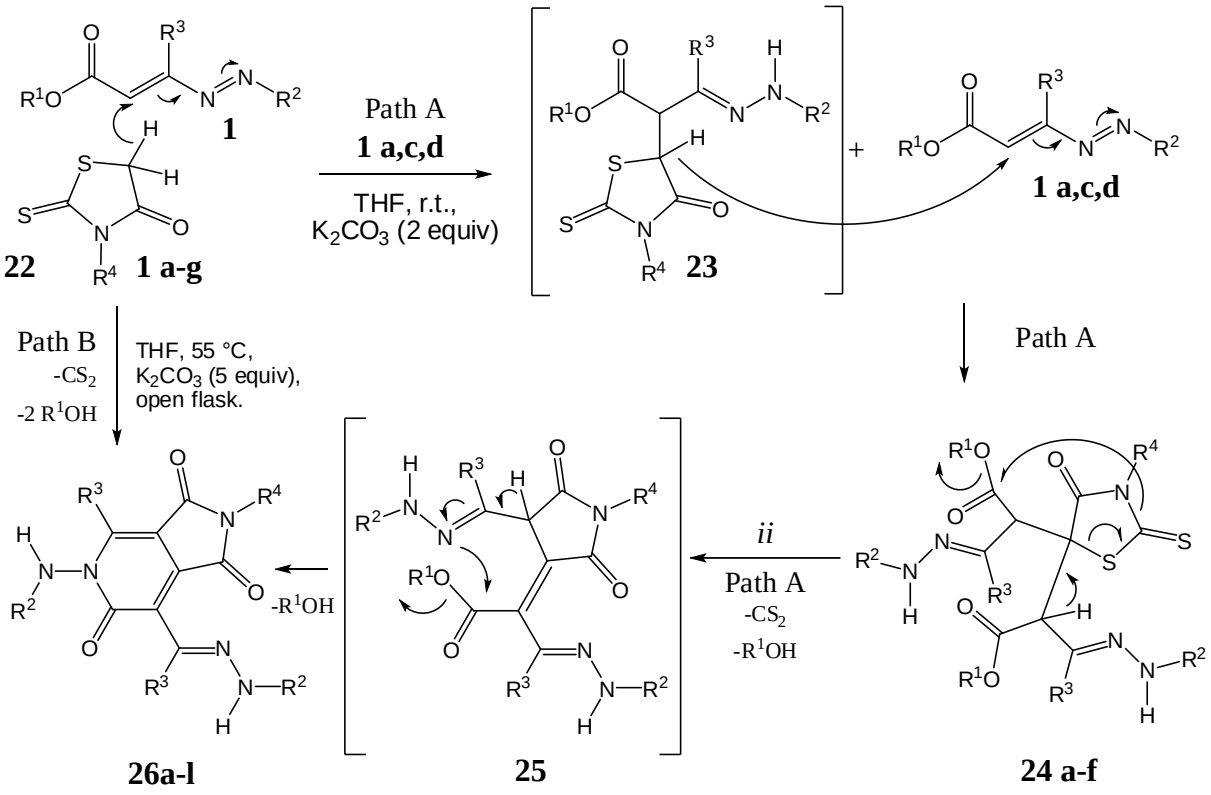
Scheme 11: *Used reagents for the optimization of the synthesis of 2,3,5,6-tetrahydro-1H-pyrrolo[3,4-c]pyridine-1,3,6-trione*

entry	Solvent	Temp. (°C)	Catalyst (C)	Molar ratio **24d**/C	**26e** yield (%)[b]
1	DCM	r.t.	TFA	1/0.1	Complicated mixture
2	THF	r.t.	NaH	1/0.1	14
3	THF[c]	r.t.	NaH	1/1.5	14
4	THF	r.t.	NaH	1/2	16
5	THF	r.t.	NaH	1/3	13
6	THF	r.t.	NaH, then Amberlyst 15H	1/2/2	25
7	THF	Reflux	NaH, then Amberlyst 15H	1/0.75/0.75	15
8	EtOH	r.t.	NaH	1/1	Complicated mixture
9	CH_3CN	r.t.	NaH	1/1	Complicated mixture
10	THF	r.t.	NaOMe	1/0.1	8
11	THF	r.t.	NaOMe	1/1.5	32
12[c]	THF	r.t.	NaOMe	1/5	21
13	DCM	r.t.	$CuCl_2$	1/0.1	No reaction
14	DCM	r.t.	$ZnCl_2$	1/0.1	No reaction
15	THF	55 °C	NaH	1/1	21
16	THF	55 °C	NaOMe	1/1	52
17	**THF**	**55 °C**	**K_2CO_3**	**1/5**	**61**
18[d]	**THF**	**55 °C**	**K_2CO_3**	**1/5**[e]	**66**[f]

Table 3 *Screening of different conditions in the cyclization reaction of bis-hydrazone functionalized rhodanine **24d** for the synthesis of 2,3,5,6-tetrahydro-1H-pyrrolo[3,4-c]pyridine-1,3,6-trione **26.** The reactions were performed at 0.5 mmol scale of **24d** in 3 mL of solvent. [b] Yields of isolated **26e**, based on **24d**. [c] The reaction was performed at 0.5 mmol scale of **24d** in 100 mL of solvent. [d] The reaction was performed in a one pot procedure starting from **1d** (1.0 mmol) and **22b** (0.5mmol). [e] Molar ratio between rhodanine **22b** and K_2CO_3. [f] Yield of isolated **26e**, based on **22b**.*

At this point, we have dealt with the development of a procedure for cyclization of **24d** testing several solvents, such as DCM, THF, EtOH, and acetonitrile, at different temperatures (r.t., reflux). Furthermore, a series of catalysts were used, such as K_2CO_3, NaH, NaH/Amberlyst 15H, MeONa, TFA, $CuCl_2$ and $ZnCl_2$, as well as different molar ratios between **24d**/catalyst were employed (*Table 3*). We have observed that, using EtOH or CH_3CN and NaH in equimolar ratio (*Table 3, entries 8 and 9*), the reactions gave complicated mixtures. The same behaviour

was pointed out in the reaction in DCM, using TFA as catalyst (*Table 3, entry 1*), while, employing $CuCl_2$ or $ZnCl_2$ in catalytic amount, no reactions were detected (*Table 3, entries 13 and 14*). In all the other cases (*Table 3, entries 2-7 and 10-12*), instead of the expected 4-oxo-2-thioxo-1-thia-3,8-diazaspiro[4.5]deca-6,9-diene-6,10-dicarboxylate, a different product was achieved, that was isolated and characterized as 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-*c*]pyridine-1,3,6-trione **26e**, whose formation took place with the loss of CS_2. Considering that its boiling point is 46.3 °C, we have decided to perform other tests at 55 °C, keeping open the reaction flask, in order to facilitate the removal of CS_2 and using the best conditions found at room temperature, such as THF and base (*Table 3, entries 15-17*). The best results in terms of highest yield of **26e** (61%) involve the use of 5 equivalent of K_2CO_3 (*Table 3, entry 17*). At this point, we have tested the same reaction between **1d** and **22b** using from the beginning 5 equivalent of K_2CO_3 at 55°C, in order to obtain 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-*c*]pyridine-1,3,6-trione **26e** directly in one pot procedure. Indeed, product **26e** was formed in 66% yield (*Table 3, entry 18*). So, with these optimal conditions in hand, we explored the reactions of various DDs **1a–h** with rhodanines **22a–d**, both in step by step *(Scheme 12*, Path A) and in one pot way (*Scheme 12*, Path B).



Scheme 12: *Synthesis of dialkyl 2,2'-((3-substituted-4-oxo-2-thioxothiazolidine-5,5-diyl)bis(4-alkoxy-4-oxobutan-3-yl-2-ylidene)) bis(hydrazine-carboxylates) **24a–f** and of 2,3,5,6-tetrahydro-1H-pyrrolo[3,4-c]pyridine-1,3,6-triones **26a–l**: the proposed mechanism.*

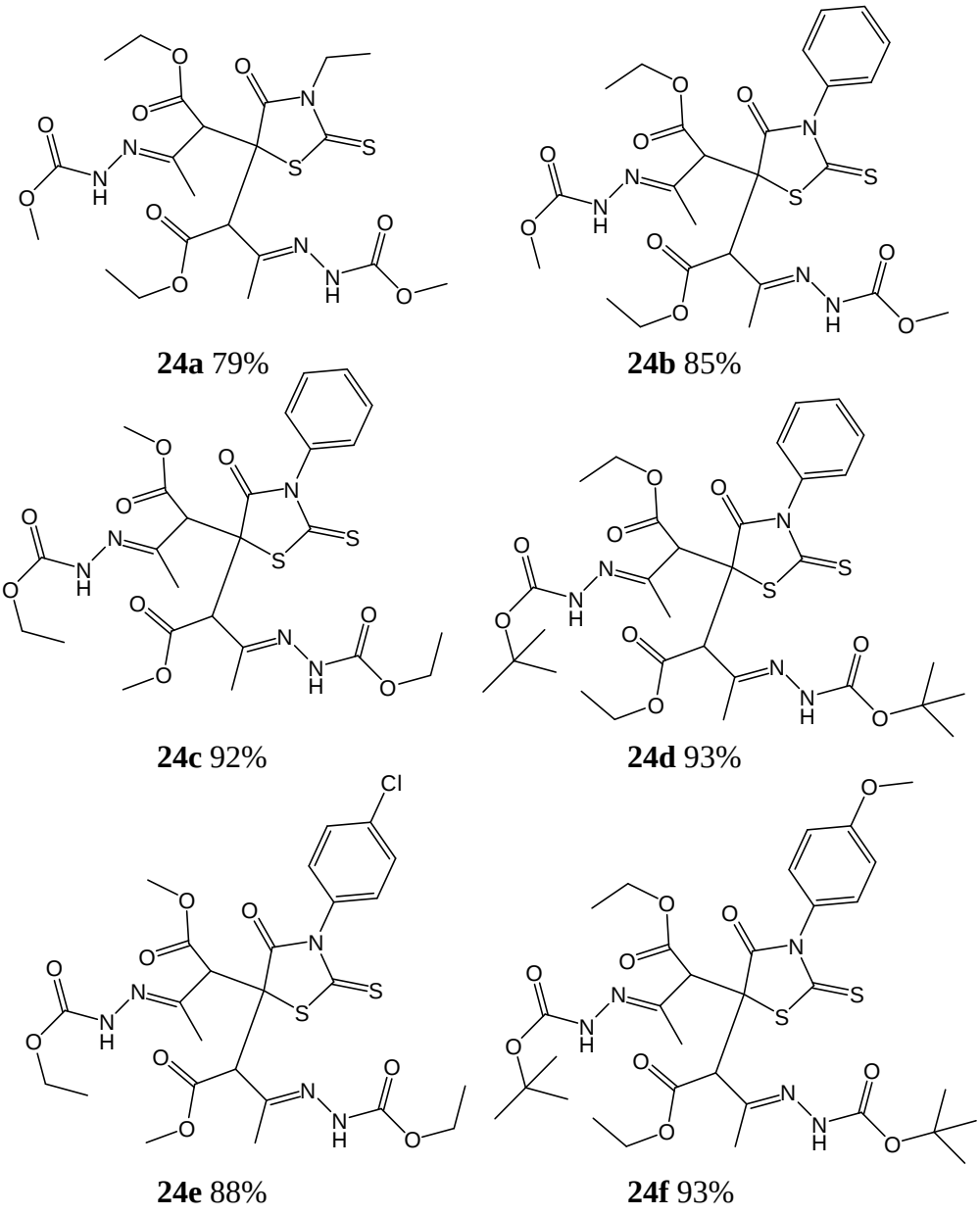
Entry	**1**	R^1	R^2	R^3	**22**	R^4
1	**1a**	Et	CO_2Me	Me	**22a**	Et
3	**1b**	Me	CO_2tBu	Me	**22a**	Et
4	**1a**	Et	CO_2Me	Me	**22b**	Ph
5	**1c**	Me	CO_2Et	Me	**22b**	Ph
6	**1d**	Et	CO_2t-Bu	Me	**22b**	Ph
7	**1e**	Me	CO_2Et	Bu	**22b**	Ph
8	**1f**	Et	CO_2Bn	Me	**2b**	Ph
9	**1g**	Me	CO_2Me	Me	**22c**	4-Cl-Ph
10	**1b**	Me	CO_2t-Bu	Me	**22c**	4-Cl-Ph
11	**1c**	Me	CO_2Et	Me	**22c**	4-Cl-Ph
12	**1c**	Me	CO_2Et	Me	**22d**	4-OMe-Ph
13	**1d**	Et	CO_2t-Bu	Me	**22d**	4-OMe-Ph

Table 4 *Substrates used for the synthesis of bis-hydrazone functionalized rhodanine **24d** and for the synthesis of 2,3,5,6-tetrahydro-1H-pyrrolo[3,4-c]pyridine-1,3,6-trione **26e***

In particular, following the Path A, by reacting DDs **1a,c–e** with rhodanines **22a–d**, in THF, with 2 equivalents of K_2CO_3, at room temperature, bis-hydrazone-functionalized rhodanines **24a–f** were obtained in good to excellent yields (79-93%) and the reactions were completed in 0.1–0.5 h (*Scheme 12, Path A, Table 5*).

Their basic treatment with 5 equivalents of K_2CO_3 in THF at 55 °C in open flask furnished the corresponding 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-*c*]pyridine-1,3,6-triones **26a,c–e,j,l**, in 2.0–5.0 h in acceptable yields (39–61%) (*Scheme 12, Path A, Table 5*).

More conveniently, the 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-*c*]pyridine-1,3,6-triones **26a–l** were obtained in good yields (43–66%) by means of one pot procedure, carrying out the reaction between DDs **1a–g** and rhodanines **22a–d**, in THF, with 5 equivalents of K_2CO_3, at 55 °C in open flask. Under these conditions the reactions were completed in 1.0–5.0 h (*Scheme 12, Path B, Table 5*).



24a 79% **24b** 85%

24c 92% **24d** 93%

24e 88% **24f** 93%

Table 5: *Yields of pure isolated products referred to rhodaines* ***22a–d****.*

Any attempt to isolate the mono-adduct **23**, deriving from the Michael-type addition of the rhodanine **22** to one equivalent of DD **1** failed (*Scheme 12*); in fact, in all the tests carried out in different conditions and in the DD/rhodanine equimolar ratio, only bis adducts **24** were formed. Having in hand the products **23** would have given us the opportunity to add them to DDs molecules different from the starting ones.

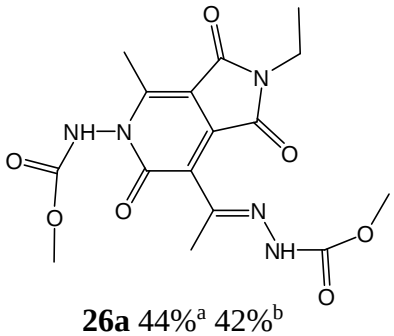
The plausible mechanism of this cascade reaction involves the preliminary double attack (Michael-type) of the carbon atom in 5 position of the rhodanine **22** to the terminal carbon atom of the azo-ene system of two molecules of the DD **1**, with the formation of the bis-hydrazonic intermediate **24**. The key step in the construction of the tetrahydro-1*H*-pyrrolo[3,4-c]pyridine-1,3,6 trione system is the opening of the 2-thioxothiazolidin-4-one ring which results in the loss

of CS_2. This process is triggered by the removal from the base of the acidic proton originally located in position 4 of the first azo-ene molecule, with the consequent activation of the nitrogen deriving from rhodanine core. Its nucleophilic attack on the ester function derived from the second DD molecule promotes the formation of the pyrrolidine-2,5-dione core of the non-isolable intermediate **25**. The further cyclization to obtain pyridin-2(1*H*)-one nucleus occurs as a result of the basic removal of the analogous proton deriving from the second molecule of DD that promotes the nucleophilic attack of the sp^2 nitrogen at the ester carbonyl function originally located in the first DD (*Scheme 12*).

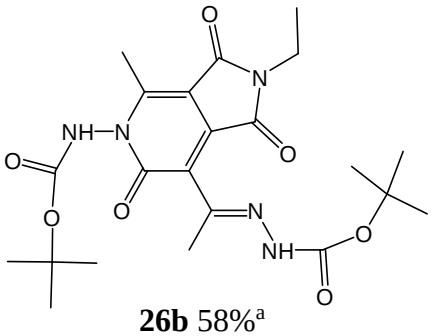
It is noteworthy that a double interaction between the two fragments derived from the two DDs occurs and in the one pot procedure, this cascade reaction furnishes two new single carbon-nitrogen bonds as well as a single and a double carbon-carbon bonds.

The two activated protons play a crucial role by promoting the double nitrogen nucleophilic attack onto the ester function of the other fragments.

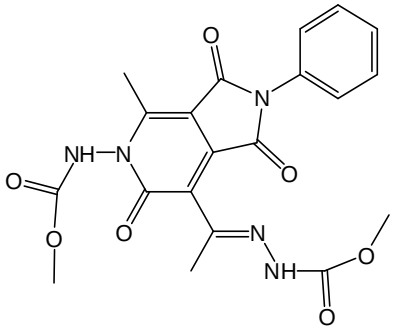
To the best of our knowledge, just one example of ring-opening/ring-closing process of the rhodanine core is reported in literature.[44] Differently from this, our sequence occurs with the loss of a molecule of carbon disulphide so activating the substituted nitrogen of the rhodanine as nucleophile in the first cyclization event.



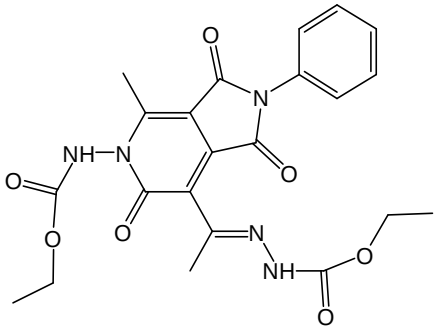
26a 44%[a] 42%[b]



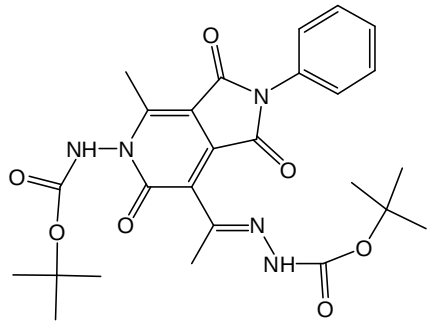
26b 58%[a]



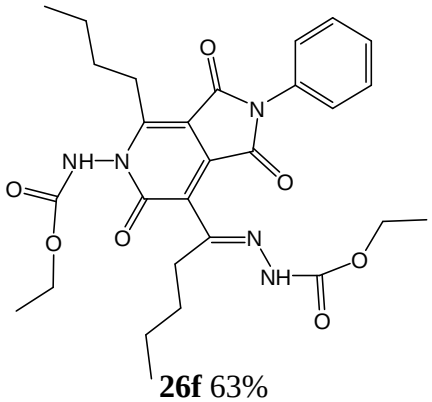
26c 60%[a]



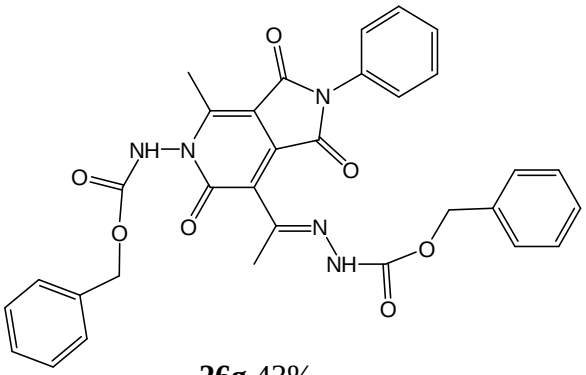
26d 55%[a] 53%[b]



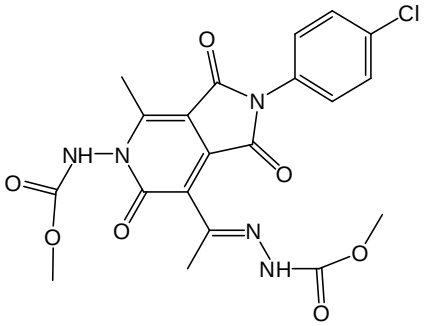
26e 66%[a] 61%[b]



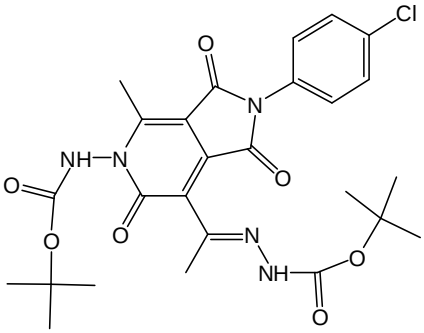
26f 63%



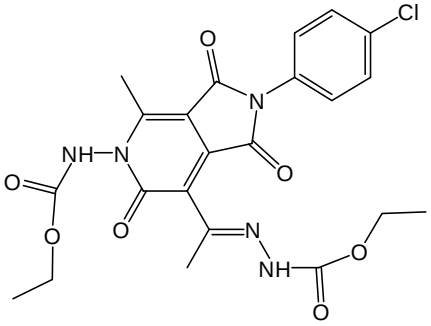
26g 43%



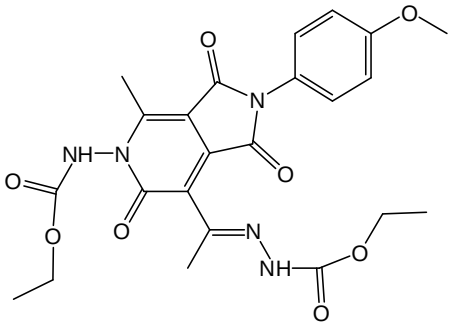
26h 46%



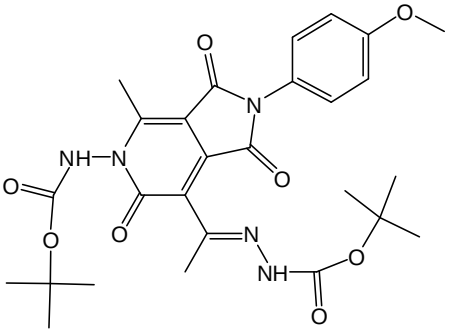
26i 51%



26j 58%[a] 39%[b]



26k 59%

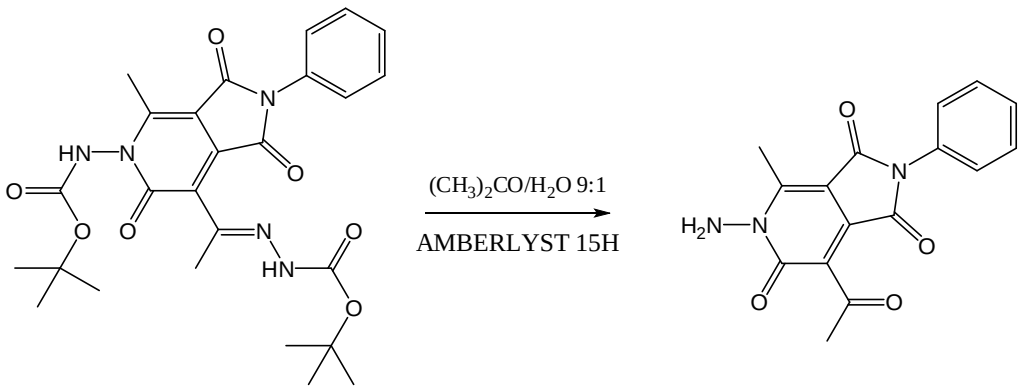


26l 64%[a] 64%[b]

***Table 5*:.** *[a] Yields of pure isolated products referred to rhodanines **22a–d**, in the one-pot procedure (Path B of Scheme 2) [b] Yields of pure isolated products referred to bis-hydrazones **24a–f** (Path A of Scheme 2).*

The structure of compounds **24** and **26** was confirmed by mono and bidimensional NMR measurements and the data are perfectly comparable to those reported in the literature for similar compounds.[42,45]

Further evidence of the proposed structure for compounds **26** was furnished by the hydrolytic cleavage of the hydrazone moiety in position **27** of the pyridine ring of compound **26e**, chosen as an example, that was obtained under heterogeneous conditions, using acetone/water (9:1) and Amberlyst 15H.[46] In these acidic conditions also the loss of the *tert*-butoxycarbonyl moiety occurred to give the corresponding 7-acetyl-5-amino-4-methyl-2-phenyl-1H-pyrrolo[3,4-c]pyridine-1,3,6(2*H*,5*H*)-trione **27a** in very good yield (83%) (*Scheme 13*).



26e **27a**

Scheme 13: *Hydrolytic cleavage of 2,3,5,6-tetrahydro-1H-pyrrolo[3,4-c]pyridine-1,3,6-trione **26e** to 7-acetyl-5-amino-4-methyl-2-phenyl-1H-pyrrolo[3,4-c]pyridine-1,3,6(2H,5H)-trione **27a***

3.2 Conclusions

In conclusion, starting from 1,2-diaza-1,3-dienes and rhodanine derivatives, we have developed a synthetic strategy to have access to 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridine-1,3,6-triones via double Michael addition/CS_2 extrusion/double cyclization sequence. To the best of our knowledge, this work represents the first example of ring-opening/ring-closing process with concomitant extrusion of carbon disulfide from the rhodanine core. The easy availability of the starting materials, the simplicity of the experimental method, as well as the potential biological activities and utilities of products can increase the synthetic usefulness of this novel procedure. Furthermore, employing the step by step approach, also highly functionalized rhodanines could be easily obtained in satisfactory yields

4. SEQUENTIAL ONE POT SYNTHESIS OF 2,5-DIHYDROTHIOPHENES AND THIOPHENES

CHAPTER 4

4.1 Introduction

One of the main aim for a synthetic chemists is the development of highly efficient procedures to assemble valuable compounds with high structural complexity resorting to simple available materials.[47] This request can be meet by exploiting the multicomponent reactions (MCRs) that allows incorporation into the final products of three or more reactants. MCRs lead to wide molecular diversity, minimizing the work-up procedures and consequently saving both time and solvents.[48] A class of MCR is the sequential reactions, where the reagents are added consecutively in a defined order in the same reaction environment under constant conditions In this way, it is possible to stop the synthetic sequence at every step, and so the intermediates can be isolable. Thereby, the intermediates can be isolable by stopping the sequence at the right moment.[49] Thiophene is the key structural unit of numerous pharmacophores such as Plavix, (adenosine diphosphate $P2Y_{12}$ receptor antagonist),[50] Raloxifene (selective estrogen receptor modulator),[51] PaTrin 2 (MGMT inactivating drug),[52] articaine (local anaesthetic),[53] Cymbalta (selective serotonin norepinephrine reuptake inhibitor).[54]

Furthermore, its peculiar electronic properties and the relevant structural rigidity make the thiophenes derivatives interesting cores in the production of innovative organic materials like organic semiconductors, organic light emitting diodes (OLED), organic photovoltaics (OPV), organic field effect transistors (OFET), solar cells, liquid crystals.[55]

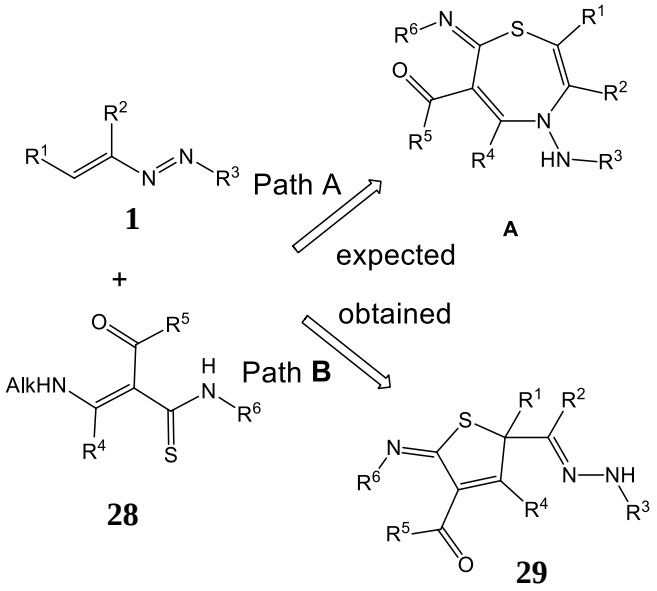
About the preparation of thiophenes, a plethora of synthetic approaches have been reported, some of which can be related to classical Gewald, Fiesselmann, Paal-Knorr, and Hinsberg reactions,[56] and many others examples.[57] However, most of these procedures require multistep protocols with consequent laborious isolation operation.

Also the dihydrothiophene compounds represent a common structural motif of numerous bioactive derivatives and they has shown to be versatile intermediates for synthetic applications.[58] consequently, notable attention has been directed toward new and convenient synthetic procedures to prepare both dihydrothiophenes and thiophenes.

During our ongoing studies[37,59] on the versatility of 1,2-diaza-1,3-dienes,[38] as synthetic starting materials, inspired by the work of Alizadeh,[60] we have investigated the reaction between DDs **1** and 3-alkylamino-2-(carbamothioyl)but-2-enoates **28** (ACTs) to tentatively obtain thiazepines **A** by means of a formal [4+3] cyclization (*Scheme 1, path A*), in analogy to the results previously obtained.[60] Surprisingly, the reaction furnish 5-(phenylimino)-2,5-dihydrothiophenes (DHTs) **29** through an unusual formal [4+1] cyclization,[61] where the DDs contributes in the final heterocyclic structure only with one carbon atom (*Scheme 14, path B*).

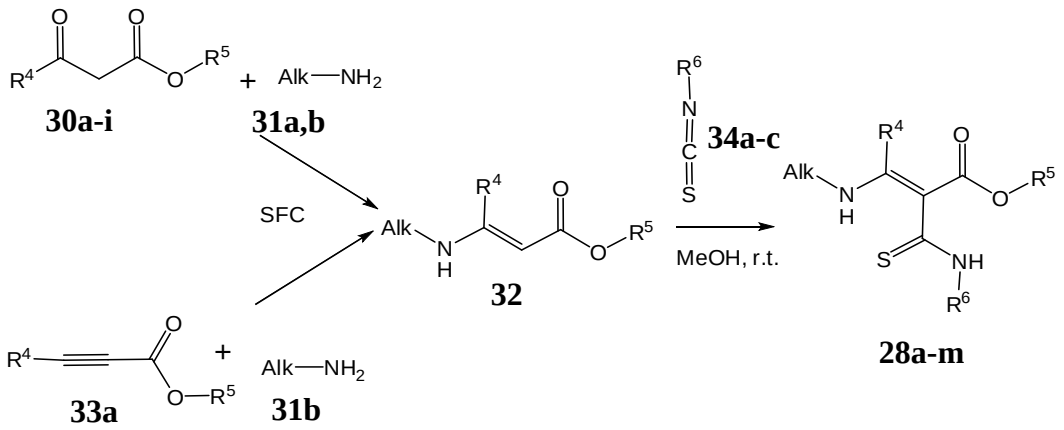
4.2 Results and Discussion

In order to realize a one-pot reaction by subsequent addition of reagents, we began our investigation on the preparation of ACTs **28**. The methodology requires the preliminary formation of enamino ester intermediates,[62] that successively react with aryl-isothiocyanates to produce the desired ACTs **28**. Among all the proposed procedures,[63] Alizadeh describes the most convenient way, where all the steps are performed under solvent free conditions (SFC).[60]



Scheme 14: *Expected and observed behaviour of the reaction between DDs **1** and ACTs **28***

We have observed that conducting the addition of the enamino ester **32** to the aryl isothiocyanates **34a–c** in methanol, the desired ACTs **28** directly precipitate from the reaction medium as pure products with better yields, avoiding any other purification procedures.



Scheme 15: *Synthesis of 3-alkylamino-2-(carbamothioyl)but-2-enoates **28**.*

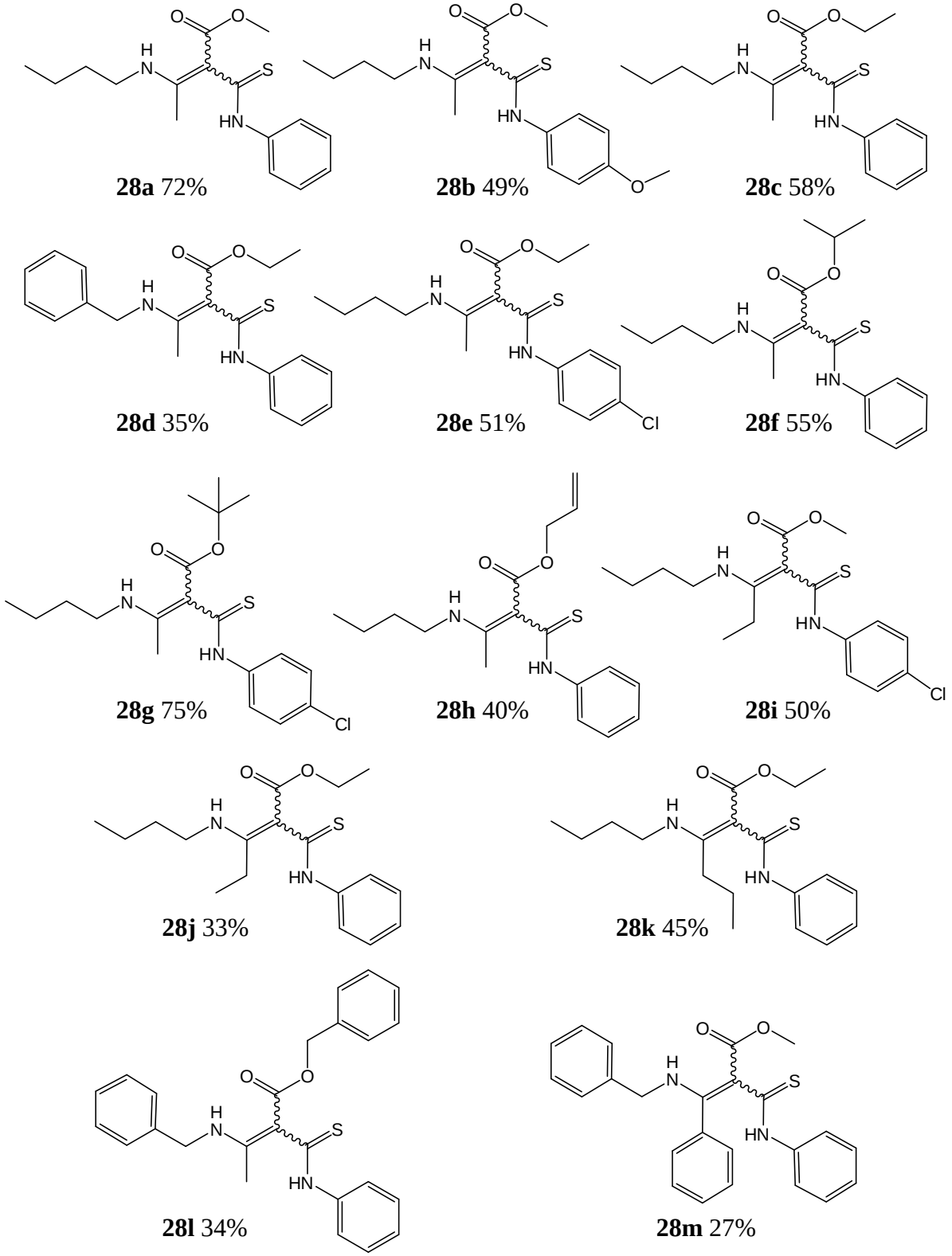


Table 7: *Yields of isolated ACT **28** based on alkyl amines **31***

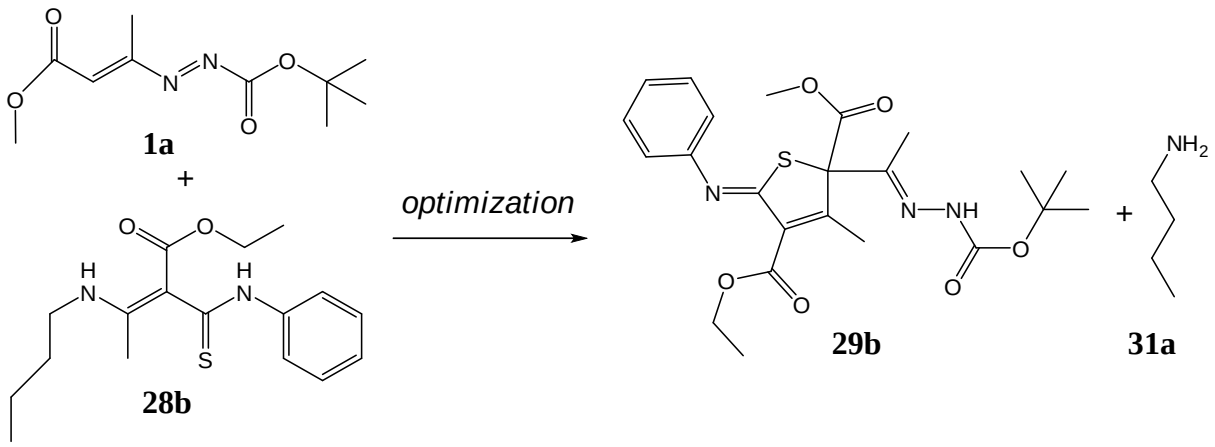
Based on our previous experience,[64] we have tested also the reaction between alkynoates and primary amines to prepare enamino ester derivatives. Only in this case of aza-Michael reaction between ethyl 3-phenylpropiolate **33a** and benzyl amine **31b** conducted in SFC at room temperature, we have quantitatively obtained a waxy solid that was identified as the desired

enamine ester **32a**. The subsequent addition of the phenyl isothiocyanates **34a** to the reaction mixture in SFC produces ACT **28l** only in poor yield, probably because the scarce solubility. Then, various solvents such as dichloromethane (DCM), tetrahydrofuran (THF), ethanol, acetonitrile (ACN), dimethylformamide (DMF), and temperatures were evaluated in the second step (*Table 8*). The best result was obtained in methanol, at room temperature, but unfortunately even in this case the yield was only of 37%.

33	**R^4**	**R^5**	**31**	**Alk**	**34**	**R^6**	**2**	**Solvent**[a]	**Yield (%)**[b]	**Solvent**[c]	**Yield (%)**[b]
a	Ph	Me	**b**	Bn	**a**	Ph	**m**	SFC	13	SFC/MeOH	37
a	Ph	Me	**b**	Bn	**a**	Ph	**m**			SFC/THF	15
a	Ph	Me	**b**	Bn	**a**	Ph	**m**			SFC/ACN	17
a	Ph	Me	**b**	Bn	**a**	Ph	**m**			SFC/DCM	21
a	Ph	Me	**b**	Bn	**a**	Ph	**m**			SFC/DMF	6
a	Ph	Me	**b**	Bn	**a**	Ph	**m**			SFC/MeOH	22[d]
a	Ph	Me	**b**	Bn	**a**	Ph	**m**			SFC/MeOH	18[e]

Table 8: *[a]Reaction conditions: alkyl amines **31** (0.5 mmol) were added to ethyl phenylpropiolate **33a** (0.55 mmol) under solvent-free conditions at room temperature. After 0.5 h aryl isothiocyanates **34a-c** (0.5 mmol) were added and the reactions were stirred until the disappearance of the enamino esters **32** (monitored by TLC). [b] Yields of isolated ACT **2** based on alkyl amines **31**. [c]Reaction conditions: alkyl amines **31** (0.5 mmol) were added to ethyl phenylpropiolate **33a** (0.55 mmol) under solvent-free conditions at room temperature. After 0.5 h aryl isothiocyanates **34a-c** (0.5 mmol) in MeOH (1.5 mL) were added and the reactions were stirred until the disappearance of the enamino esters **32** (monitored by TLC). [d]Reaction conducted at 0 °C. [e]Reaction conducted at 50 °C.*

Thus, a preliminary study of the next step of this sequential reaction was conducted using DD **1a** and ACT **28b** chosen as examples (*Table 9*, *Scheme 16*). In SFC the low solubility affects the reaction; in fact the main spot was isolated with unsatisfactory yield. Spectroscopic studies have revealed that the main product was the corresponding 2,5-dihydrothiophene **29b**. In particular, the signal at 75.4 ppm in the ^{13}C NMR spectra of the quaternary carbon in position two of the thiophene ring is diagnostic. So, different solvents such as DCM, THF, ACN, methanol were tested. Despite the low solubility of the ACTs **28** in MeOH, this solvent has furnished the best result in term of yield. Probably the gradual dissolution and the consequent lower concentration of ACTs **28** favour a better selectivity and promote greater selectivity. While the temperature does not affect the reaction, on the contrary, to an increment of DDs equivalents correspond an appreciable improvement in the final yield. In fact, the primary amine released in the cyclization process gives a nucleophilic attack to the azo-ene system subtracting the DDs **1** from the reaction medium.[65]



Scheme 16: *Reagents used for the optimization of the synthesis of **29b***

Entry	Solvent	T (°C)	molar ratio **1a**/**28b**	**29b** yield (%)[a]
1	SFC	r.t.	1:1	trace
2	SFC	80°C	1:1	trace
3[b]	DCM	r.t.	1:1	5
4[b]	THF	r.t.	1:1	8
5[b]	ACN	r.t.	1:1	33[c]
6[b]	MeOH	r.t.	1:1	43
7[b]	MeOH	50°C	1:1	42
8[b]	**MeOH**	**r.t.**	**2:1**	83
9[b]	EtOH	r.t.	2:1	73
10[b]	EtOH	70°C	2:1	70

Table 8: *Screening of different conditions in the reaction between **DD 1a** and **ACT 28b**.*

.

So, assembling all the information collected on the individual reactions we have finally conducted the one-pot synthesis on a representative model. Butyl amine **31a** (1.0 equivalents) was added to ethyl acetoacetate **30b** (1.1 equivalents) under SFC. A slight excess of β-ketoester **30** is necessary to completely convert the amine **31**, preventing its nitrogen nucleophilic attack to the isothiocyanate **34** that would have led to the formation of the unwanted thiourea.

So, assembling all the information collected on the individual reactions we have finally

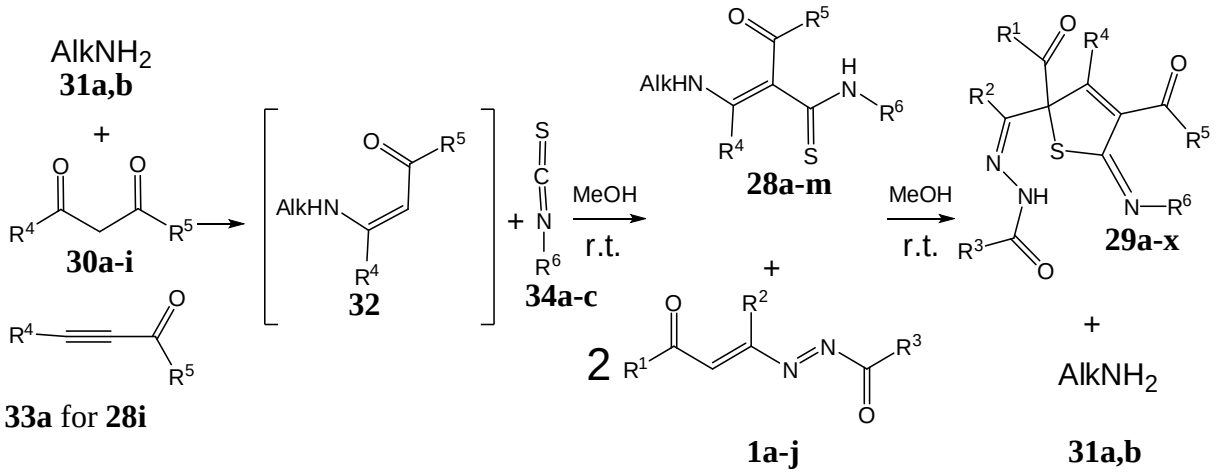
After 0.5 h, a TLC analysis revealed the quantitative formation of the enamino ester **32**. To the reaction medium were then added 3mL of methanol and phenyl isothiocyanates **34a**. The reaction is completed in 6.0 h and ACT **28b** appears as yellow precipitate. After the final addition of two equivalents of DD **1a**, the gradual dissolution of the yellow solid is observed, together with the disappearance of the typical red colour imparted to the solution by the conjugation of the azo-ene system. The reaction ends in 5.0 h furnishing the corresponding 2,5-

dihydrothiophene **29b** in 83 yields (*Scheme 16*, *Table 9*). Based on the success of the one-pot model, various β-ketoesters **30a–i**, isothiocyanates **34a–c**, and DDs **1a–j** were employed and the results are summarized in *Scheme 17*. Generally, the yields are good and it is possible to diversify two, one and three substituents on β-ketoesters, isothiocyanates and DDs respectively, allowing wide molecular diversity. The primary amine employed in the formation of the initial enamine ester derivatives do not influence the efficiency of the process: compound **29b** in fact obtained using both *n*-butyl- and benzylamine with comparable yields.

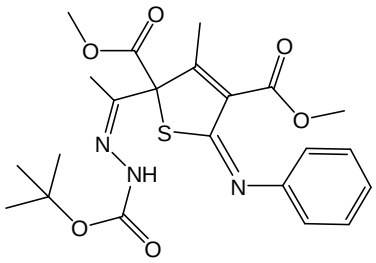
The method well tolerated both moderate electron withdrawing group such as chlorine or strong electron donating moiety as methoxy on the aromatic ring of isothiocyanates.

This procedure was applied also for ethyl phenylpropiolate **33a**, benzyl amine **31b,** and DDs **1b** furnishing only in moderate yields the 2,5-dihydrothiophene **29j**.

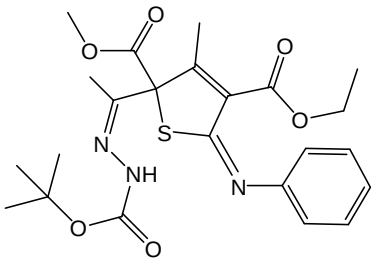
A gram scale synthesis of 2,5-dihydrothiophene **29** was attempted by means of the here reported sequence and the target product was obtained in 63% yield, confirming the effectiveness of our synthetic protocol.



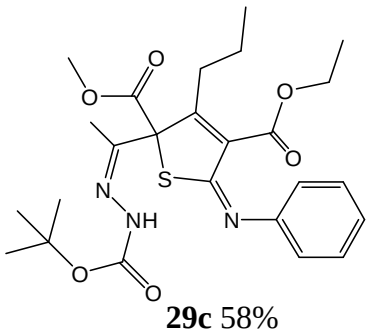
Scheme 17: *Sequential four-component reaction of primary amines **31a,b**, β-ketoesters **32a-i** (or ethyl phenylpropiolate **33a**), aryl isothiocyanates **34a–c**, and DDs **1a-j**: synthesis of 2,5–dihydrothiophenes **29a–x**.*



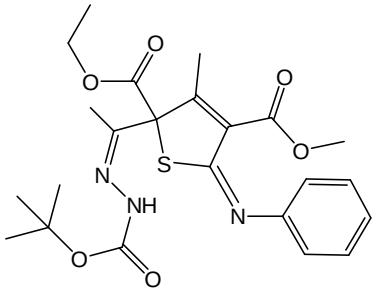
29a 78%



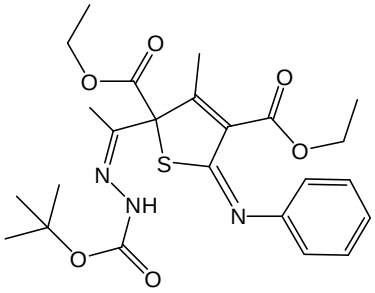
29b 83%



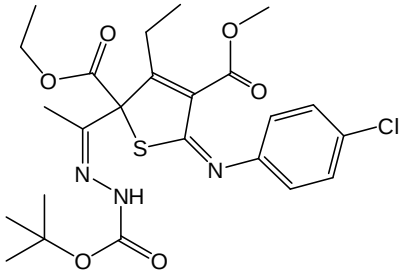
29c 58%



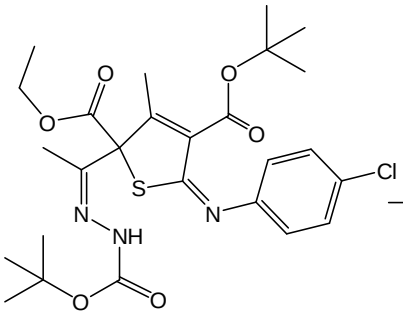
29d 88%



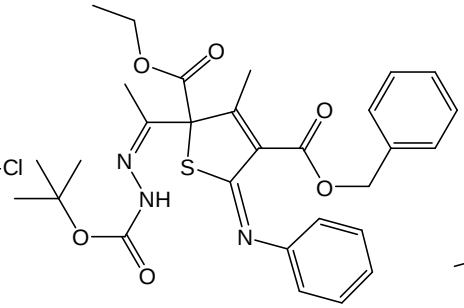
29e 78%



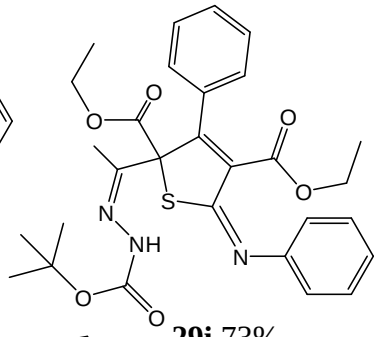
29f 69%



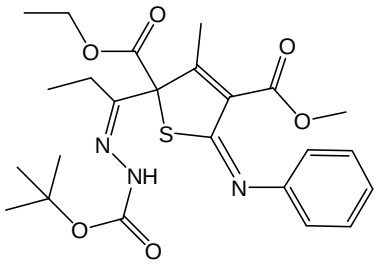
29g 81%



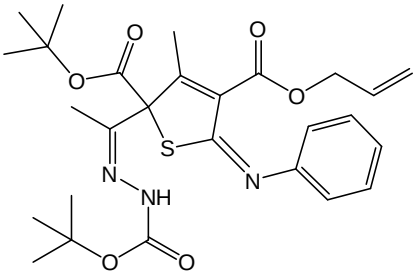
29h 65%



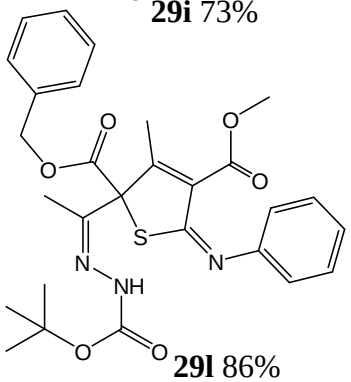
29i 73%



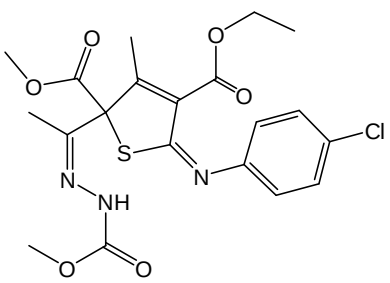
29j 78%



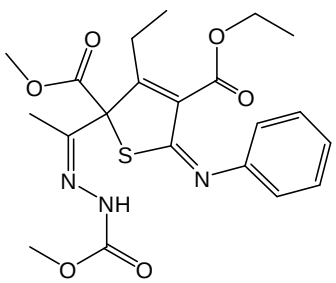
29k 86%



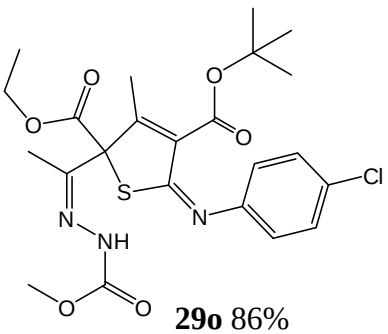
29l 86%



29m 84%



29n 84%



29o 86%

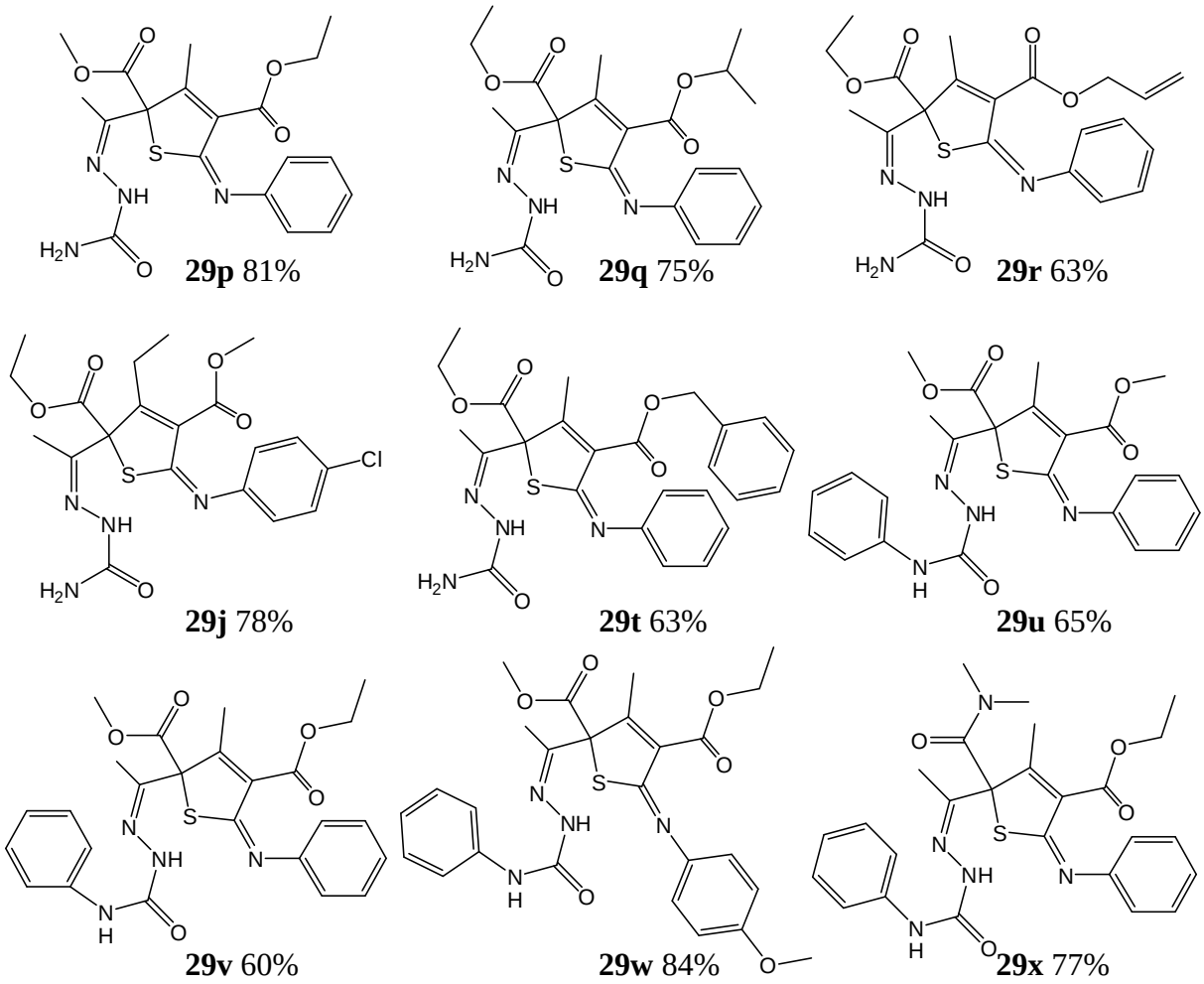
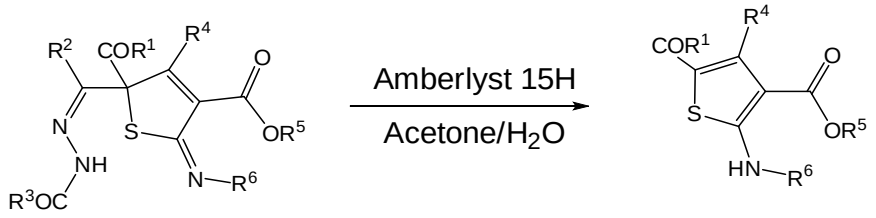


Table 10: *Yields of isolated 2,5-dihydrothiophenes **29a–x** based on compounds **30a–i** or **33a**.*

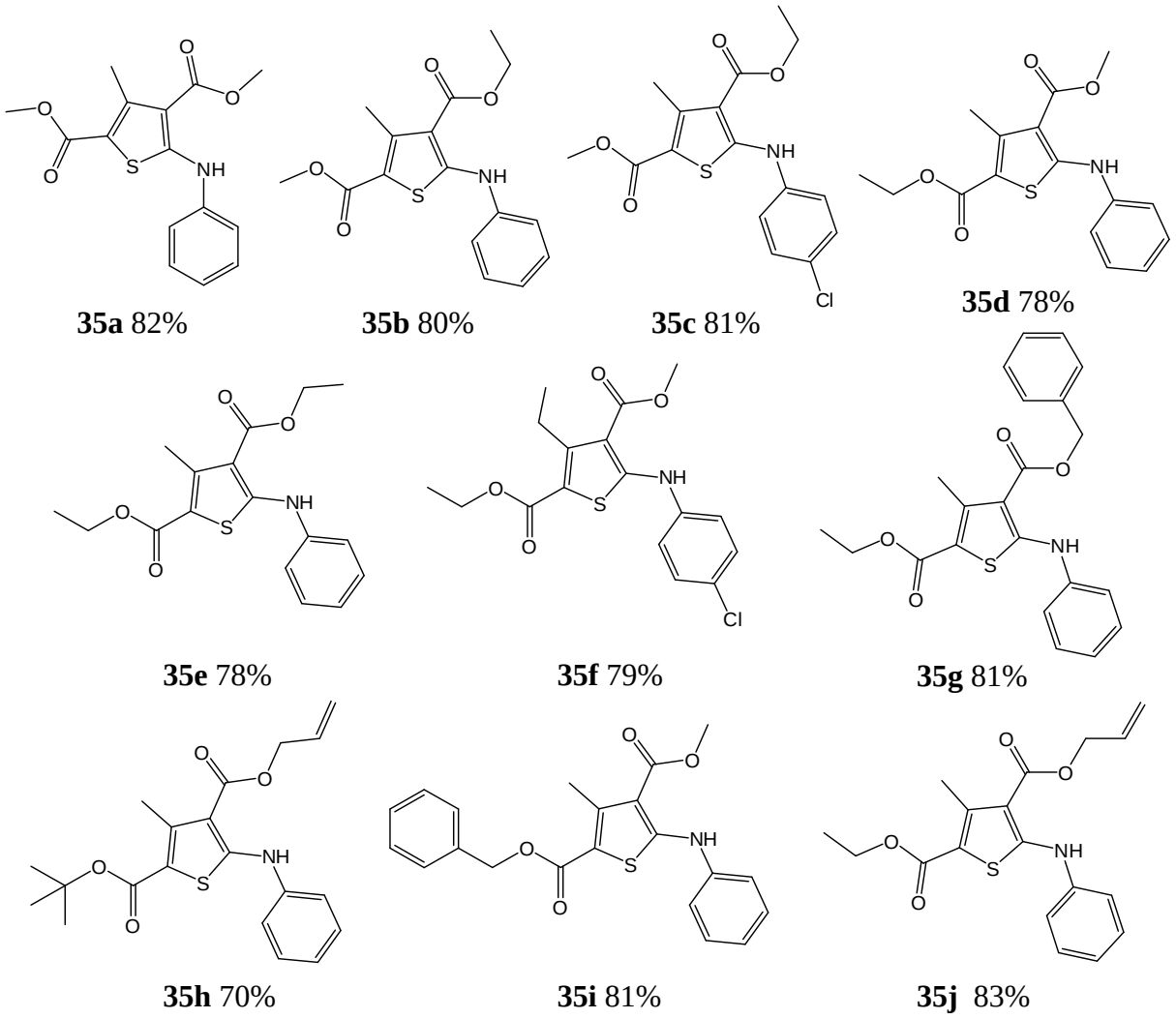
2,5-Dihydrothiophenes **3a–c,e–g,l,m,r,t** were then treated with Amberlyst *15H* in a mixture of acetone/water to tentatively hydrolyse the hydrazone moiety.[66] Surprisingly, instead of the expected 2-acetyl 2,5-dihydrothiophene, the reactions have furnished in good yields the 5-amino thiophene-2,4-dicarboxylates **35a–j** (*Scheme 18, Table 11*). The structures of the aromatic compounds **35** were supported by spectroscopic data and unambiguously confirmed by comparison of diethyl 3-methyl-5-(phenylamino)thiophene-2,4-dicarboxylate **35e** with the same derivative previously synthesized by Kirsch.[67] It is noteworthy that this aqueous treatment is very selective leaving unaltered functional groups that are usually sensitive to acidic aqueous conditions such as esters and in particular BOC (**35k**) or allyl moiety (**35h,j**).



29a-c,e-g,l,m,r,t **35a-j**

Scheme 18: *Synthesis of 5-arylamino thiophenes **35a–j** by acidic treatment of 2,5-dihydrothiophenes **29**.*

On the base of this evidence, a plausible mechanism can involve a double Michael reaction. In facts, probably the sulphur of the ACTs **28** gives a first nucleophilic attack to the terminal carbon atom of the azo-ene system of the DDs **1** with formation of the intermediates **36**. The carbon in α to the hydrazone function is strongly activated and is able to give nucleophilic vinylogous attack at the conjugated system deriving from the starting ACT with consequent formation of the heterocyclic structure **37**. The final elimination of the amino moiety affords the 2,5-dihydrothiophenes **29**. In this formal [4+1] cyclization the carbon atom originally located in position four in the azo-ene system (highlighted in *Scheme 19*) covers a crucial role behaving initially as electrophilic centre and then as nucleophilic site.



35a 82% **35b** 80% **35c** 81% **35d** 78%

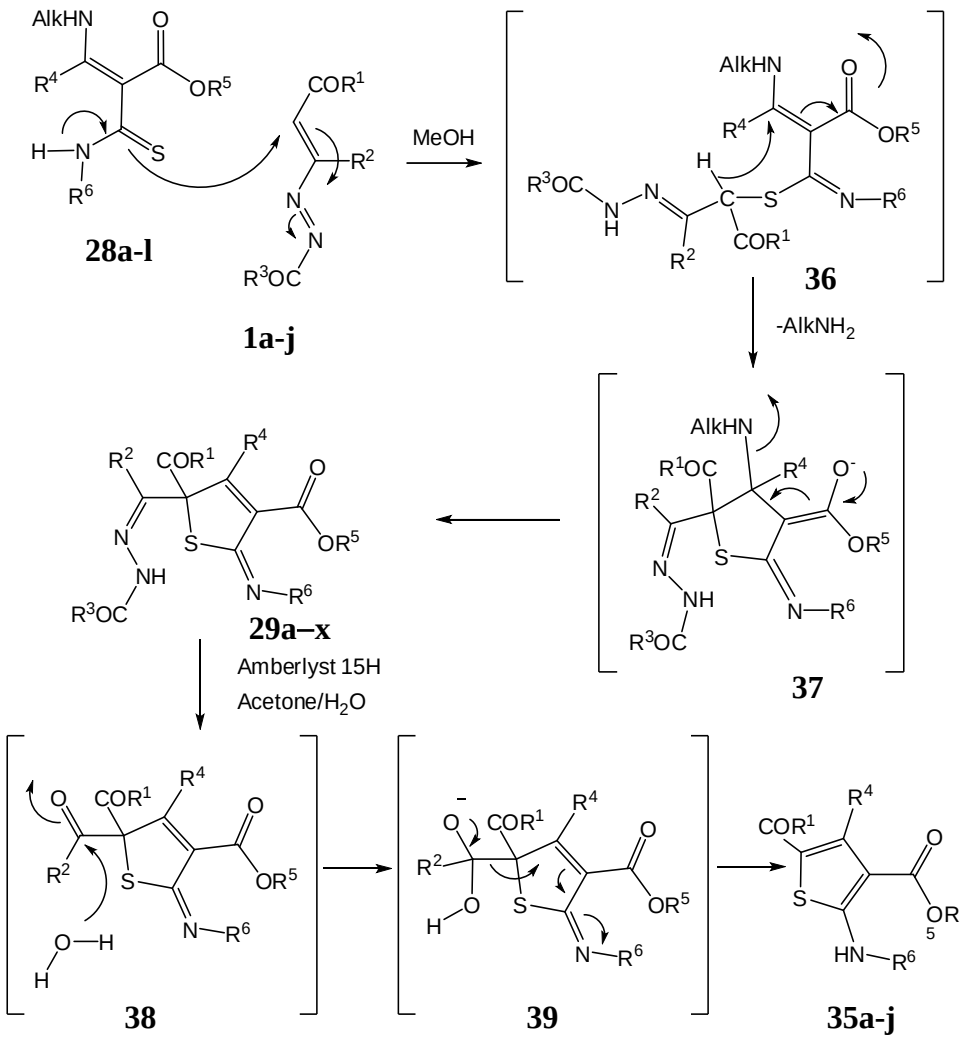
35e 78% **35f** 79% **35g** 81%

35h 70% **35i** 81% **35j** 83%

Table 11: *Yields of isolated 5-arylamino thiophenes **35a–j** based on 2,5-dihydrothiophenes **29**.*

The acidic treatment in a mixture of acetone/water of 2,5-dihydrothiophenes **29** initially probably causes the expected hydrolysis of the hydrazone function producing the non-isolable 2-acyl 2,5-dihydrothiophenes **38**. The reaction conditions favour the addition of a water molecule to the carbonyl moiety producing intermediates **39**. The loss of the carboxylic acid

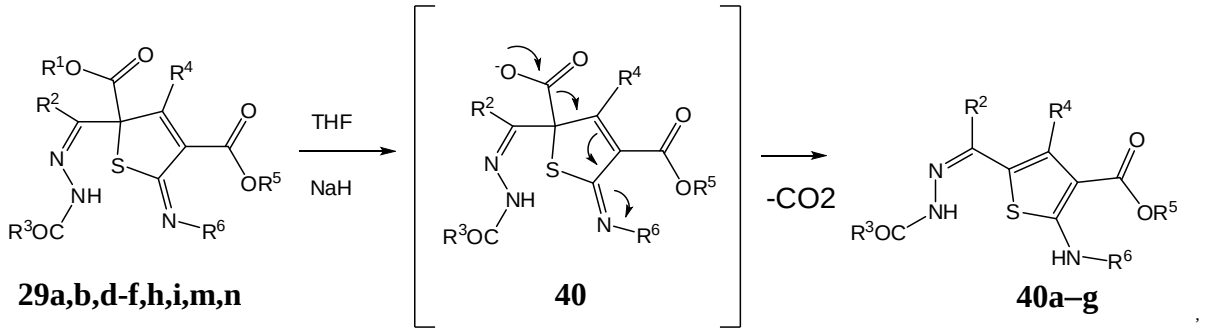
group triggers an electronic shift that produces final 5-arylamino thiophenes **35a–j**. The aromatization is the driving force of this process.



Scheme 19: *Plausible mechanism for the formation of 2,5-dihydrothiophenes **29a–x** and 5-arylamino thiophenes **35a–j**.*

Surprisingly, a different behavior was observed when DHTs **28** are subjected to basic treatment (*Scheme 20*). By adding two equivalents of sodium hydride to a solution of DHTs **8a,b,d–f,h,j,m,n** in THF, at room temperature, novel 2-arylamino 5-hydrazono thiophene-3 carboxylates (AHTs) **41a–g** were obtained in satisfactory yields. The aromatization process, in this case, takes place following the hydrolysis of the ester function. The subsequent decarboxylation process promotes the aromatization of the ring. The two distinct regioselectivities observed in the formation of ATDs **35** or AHTs **41**, respectively, are probably ascribable to the different electrophilicity of the substituents in position 2 of DHTs **29**. In acidic conditions, the hydrolysis of the hydrazone generates the ketonic function, that it is notoriously

more electrophile with respect to the ester function. In basic medium, instead, the hydrolysis involves the ester function in position 2 of DHT, promoting a different aromatization process.



Scheme 20**:* *Synthesis of 2-arylamino 5-hydrazono thiophene-3 carboxylates (AHTs) **41a–g** by basic treatment of DHT **29

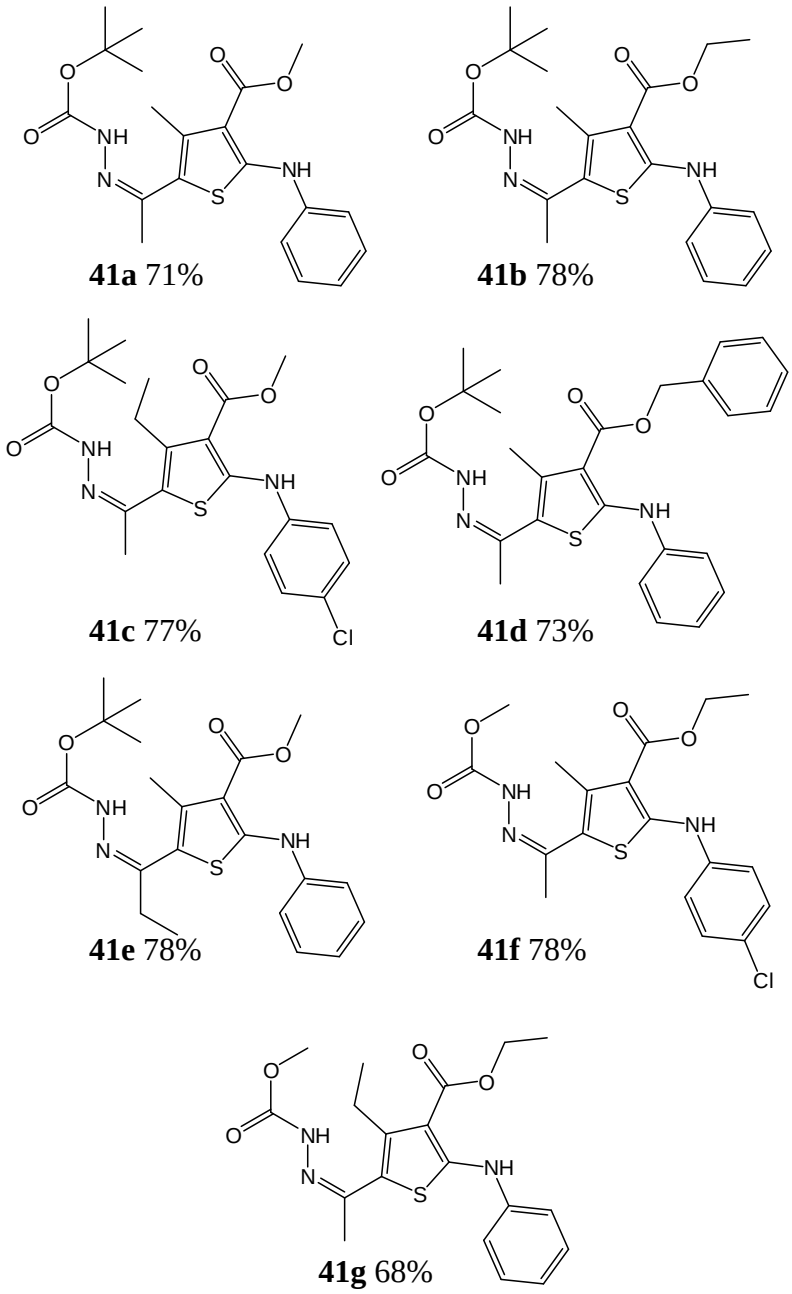
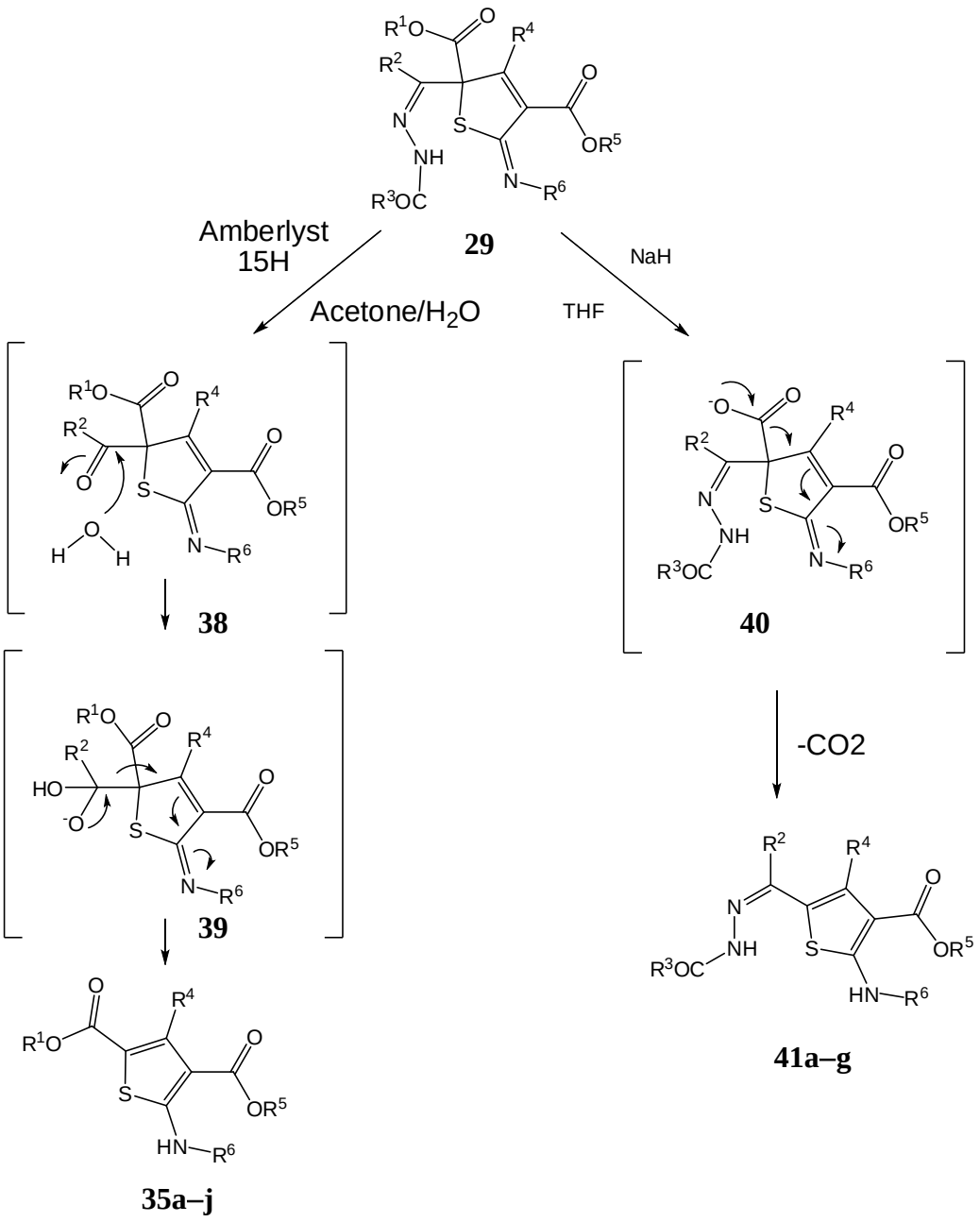


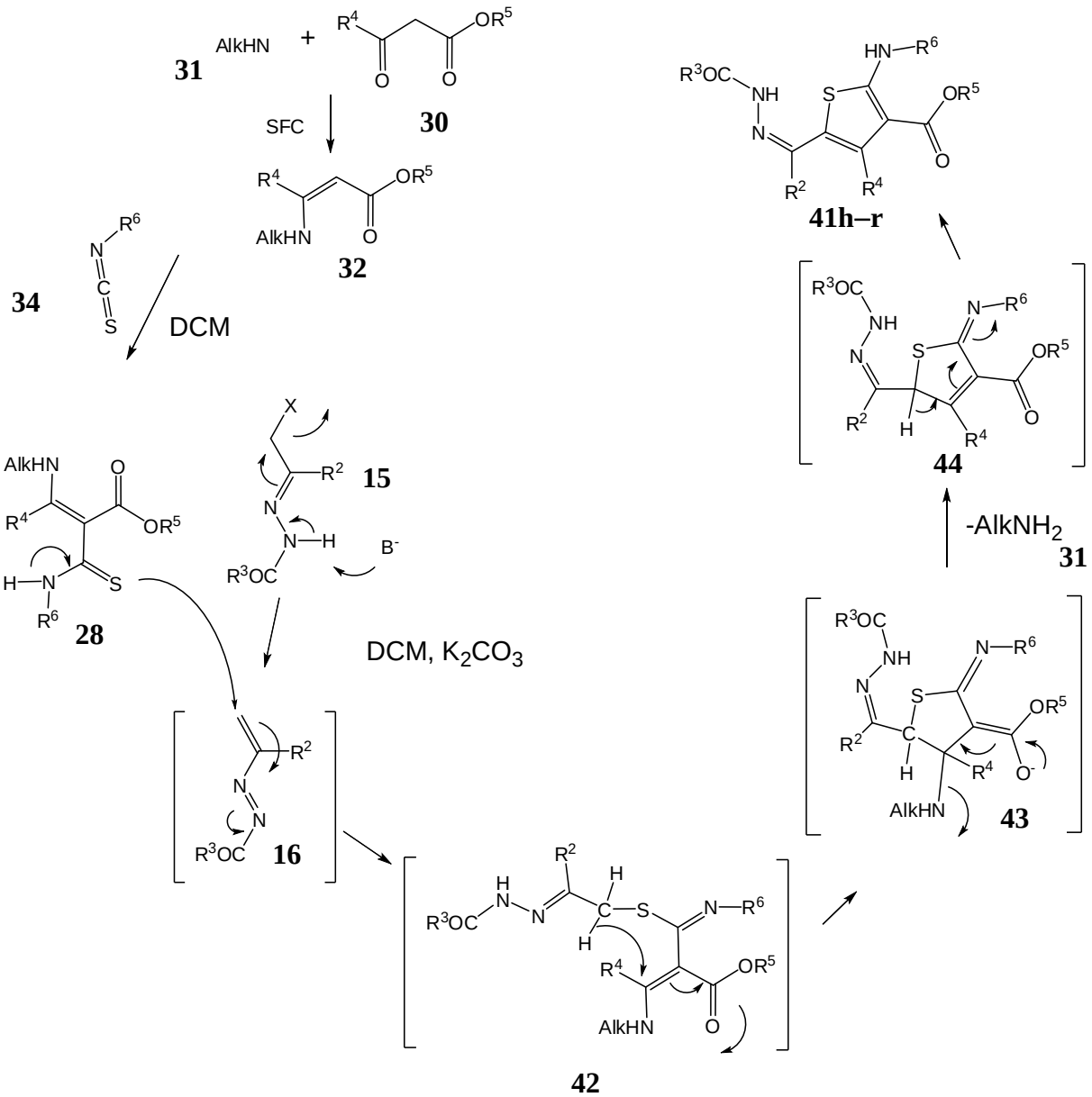
Table 12**:* *Yields of isolated AHTs **41a–g** based on DHT **29



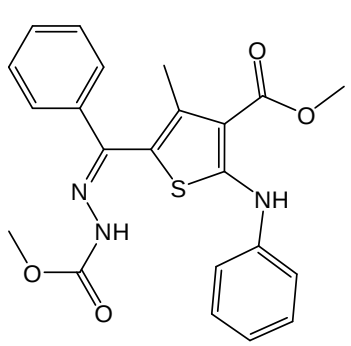
Scheme 21: *Different ways in the treatment of DHTs **29***

From the conversion of DHTs **29** in to ATDs **35** or in to AHTs **41** it emerges that the elimination of one substituent in position two triggers an aromatization process. To realize the direct synthesis of aromatic 2-amino thiophenes through a sequential multi component synthesis, similar to that described above, we have planned to insert an easily removable hydrogen in position 2 of the DHTs **29**. The carbon in position two of DHT **29** derives from the DDs, **1** and originally is located in position four of the azo-ene system. Then, employing 4-unsubstituted DDs we should achieve the proposed target. As these substrates showed poor stability, they are usually generated in situ by basic treatment of the corresponding α-alo hydrazones **15a–g**.[38,68] This circumstance requires an adjustment of the reaction conditions previously employed. As

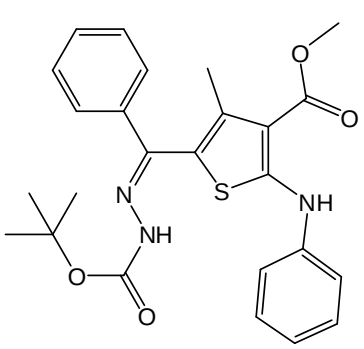
above described, initially we prepared the enamino ester intermediates **32** under solvent free conditions and successively the aryl isothiocyanates **33a–c** was added furnishing the desired 3-alkylamino-2-(carbamothioyl)but-2-enoates (ACTs) **28a–m**. To the reaction mixture were added the α-halo hydrazones **14** were added and four equivalents of potassium carbonate were needed for the conversion of the hydrazones in the 4-unsubstituted DDs. The methanol, previously used as solvent, reacts with derivatives **14** giving the substitution of the halogens. Some other solvents were then tested, and the best results were obtained using dichloromethane (*Scheme 22*).



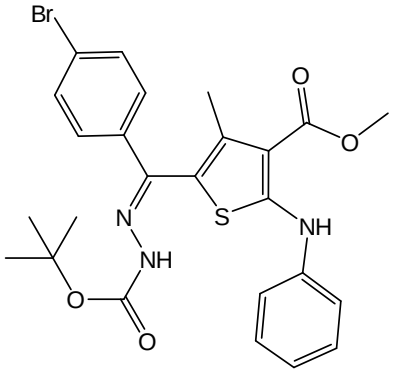
Scheme 22: *One pot sequential synthesis of 2-arylamino 5-hydrazono thiophene-3 carboxylates (AHTs)* ***41h–r*** *starting from in situ generated 4-unsubstuited DDs.*



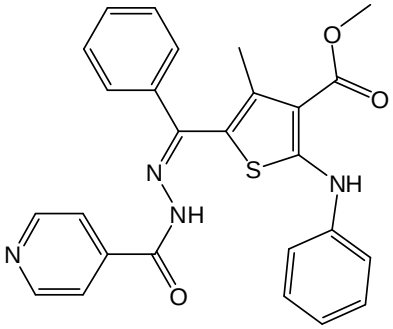
41h 80%



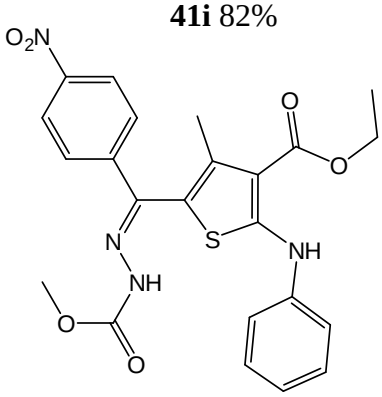
41i 82%



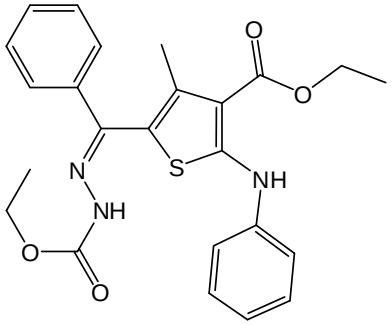
41j 87%



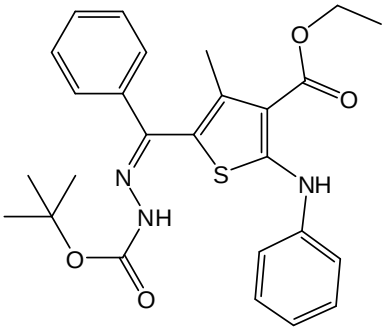
41k 53%



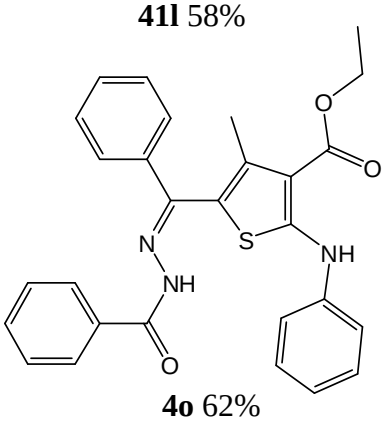
41l 58%



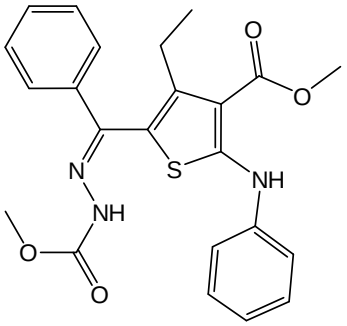
41m 80%



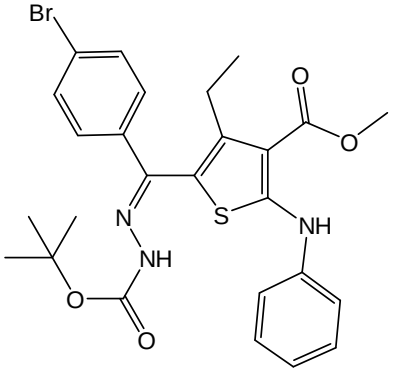
41n 85%



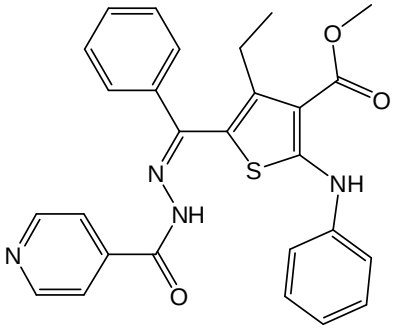
4o 62%



41p 87%



41q 91%



41r 74%

Table 13: *Yields of isolated AHTs **41h–r** based on β-ketoesters **32a**.*

On the basis of this evidence a plausible mechanism can involve a sulphur nucleophilic attack of ACTs **28** to the terminal carbon atom of the azo-ene system of the 4-unsubstitued DDs **15**. The basic medium favors the loss of the first acid proton activating the carbon nucleophilic vinylogous attack at the conjugated system deriving from the starting ACT **28**. By means of this annulation, the first sulphur-heterocyclic structure of the tetrahydrothiophene intermediates was created. The conjugated elimination of the amino moieties affords the corresponding 2,5-dihydrothiophene intermediates. The loss of the second proton promotes the aromatization of the ring. In summary, when there are two hydrogens at the terminal carbon atom of the azo-ene of 4-unsubstituted DDs **15**, the aromatization process can be easily incorporated in the synthetic sequence, obtaining directly the 2-arylamino 5-hydrazono thiophene-3 carboxylates **41h–r**. On the other hand, employing 4-substytuted DDs **1a–j**, lacking an easily removable group, the final aromatization is not possible, and the sequence stops at the formation of DHTs **29a–x** (*Table 13*).

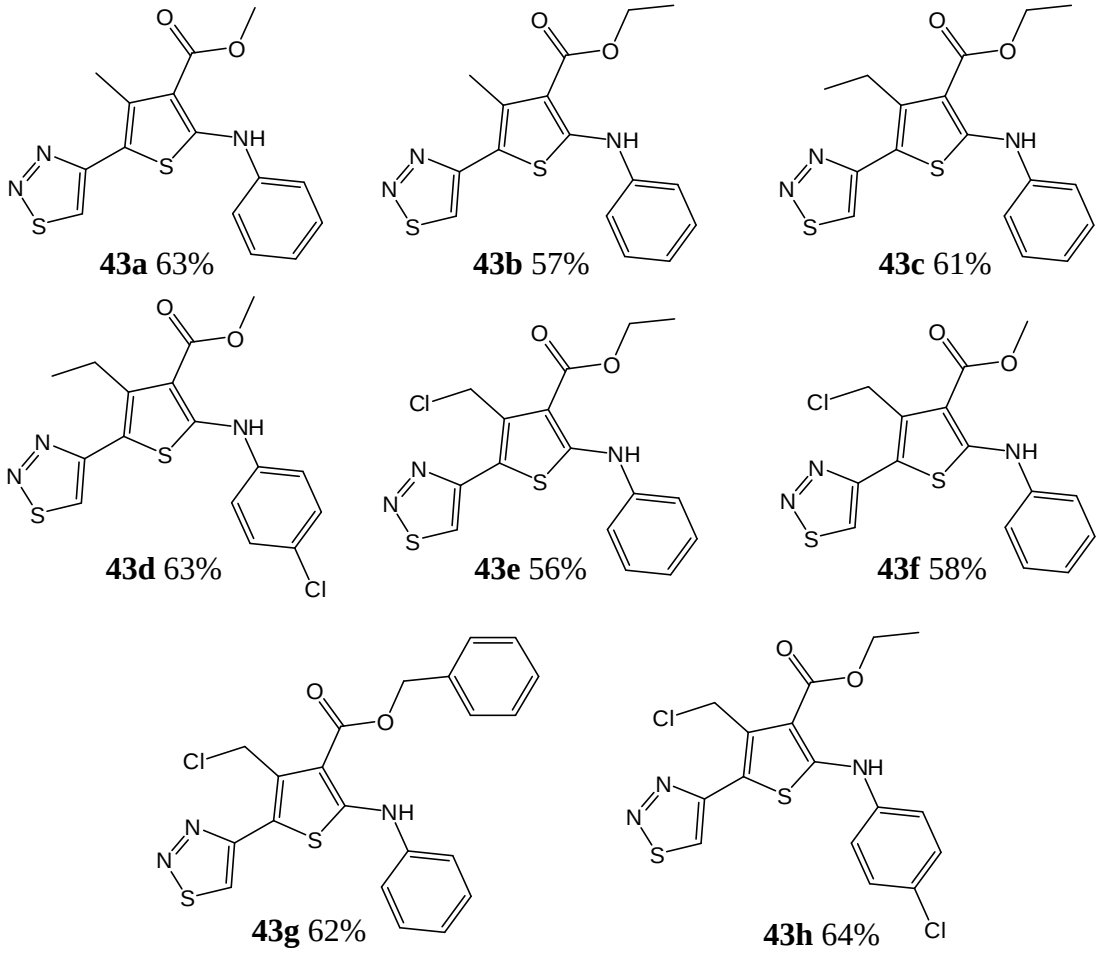
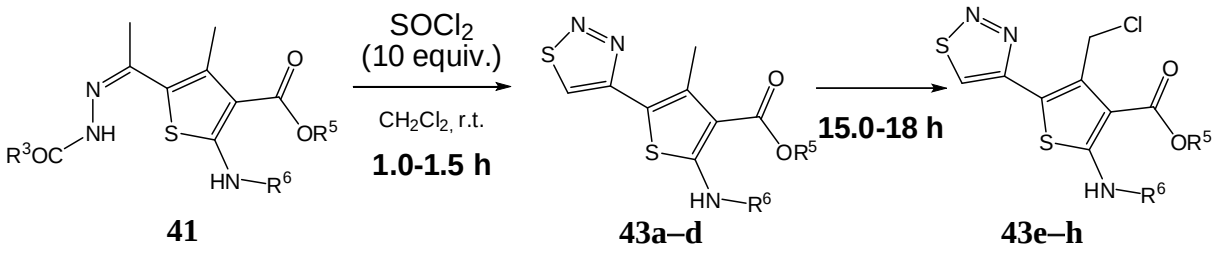


Table 14: *Yields of isolated AHTs **43a-g** based on AHT **41**.*

2-Arylamino 5-hydrazono thiophene-3 carboxylates (AHT) **41** are valuable starting materials to create a second sulphur-containing-heterocycle direct linked to the thiophene ring. In fact, the treatment of AHTs **41a–g** containing a methyl substituted hydrazone moiety with thionyl chloride, according to a typical Hurd-Mori conditions, produces new 2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylates (ATTs) **43a–d**.



Scheme 23: *Progress of the Hurd-Mori reaction.*

Initially, the reactions were conducted on AHTs **41a** (0.5 mmol) chosen as representative compound at room temperature in dichloromethane (2.0 mL) testing different molar ratios of thionyl chloride. With one or two equivalents the reactions do not come to completeness, while with five equiv. the reaction proceeds slowly (36 h, monitored by TLC) and together with the desired ATT **43a** (26% yield) also the corresponding methyl 4-(chloromethyl)-2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylate **43e** (17% yield) was produced.

The best result was achieved employing ten equivalents of $SOCl_2$. In fact, under this condition the desired ATT **43a** was formed in 63% yield in 1.5 h together with traces of the corresponding 4-chloromethyl ATT **43e** (monitored by TLC). On the other hand, the introduction of an halogen makes this latter compound **43e** interesting reaction intermediate that can be further functionalized through simple nucleophilic substitution reactions. For this reason, we have also developed a method for its preparation. Prolonging the reaction time to 15 hours, under the same reaction conditions previously employed, the 4-chloromethyl ATT **43e** was produced in 56%yield, and no traces of the corresponding ATT **43a** were detected. On the basis of these evidences, it can be deduced that the chlorination proceeds slowly, and prolonging the reaction time the 4-chloromethyl ATT **43e** becomes predominant. Using 5.0 equiv. of thionyl chloride, the Hurd-Mori reaction proceeds slowly and the chlorination reaction also occurs, while employing 10.0 equiv. Hurd-Mori reaction is faster minimizing the formation of the compound **43e**.

4.3 Conclusion

In conclusion, we have developed with success a sequential MCR that produces 2,5-dihydrothiophene derivatives by reaction of DDs and ACTs. Importantly, this protocol provides wide and flexible substitution patterns in the 2,5-dihydrothiophenes allowing the planning "*ab initio*" of six different substituents by appropriately selecting the starting β-ketoesters, isothiocyanates or DDs. This opportunity, together with mild and simple reaction conditions (no catalysts, or dry solvents, or inert atmosphere), make this sequential MCR well suitable for the easy creation of broad libraries.

A careful selection of the starting materials enables the choice up to six different variation in the architecture of the final products. Employing the *in situ* generated 4-unsubstituted DDs the same methodology produces directly the 2-arylamino 5-hydrazono thiophene-3 carboxylates, incorporating the aromatization process in the sequence. Also the 2,5-dihydrothiophenes are valuable intermediates producing in turn regioselectively the corresponding 5-amino thiophene-2,4-dicarboxylates or 2-arylamino 5-hydrazono thiophene-3 carboxylates simply by acid or basic treatment. All these aspects highlight the versatility of this sequential multicomponent reaction that under mild conditions can produce a broad variety of thiophene derivatives. And last but not least, the synthesis of unknown new 2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylates further enriches the range of sulfuric heterocycles obtainable, combining in this latter case the properties of both thiophene and 1,2,3-thiazoles.

5. *N*-ACYLIMINIUM IONS AS NOVEL MANNICH-TYPE ACCEPTORS IN 1,4 ADDITIONS OF NUCLEOPHILES

CHAPTER 5

G. Mari, L. De Crescentini, F. Mantellini, S. Santeusanio, G. Favi
Org. Chem. Front., **2018**, *accepted manuscript*

5.1 Introduction

Iminium ions play a significant role in a range of enzyme-catalyzed processes[69] and synthetic transformations as key (reactive) intermediates[70] for the construction of C-C and C-Het bonds. For example, nature utilizes type I aldolase enzymes to catalyze an aldol process that operates via an iminium cation under physiological conditions.

In the field of organocatalysis[71] and in the emerging photoredox catalysis,[72] a plethora of stereo- and enantioselective methods have been achieved that rely on the use of chiral iminium ions.[73] Within the iminium species, *N*-acyliminium ions[74] (NAIs) are recognized to be much more reactive as electrophiles than *N*-alkyliminium counterparts due to the electron-attracting properties of the carbonyl group on nitrogen. Among different named reactions involving NAIs, the two most-studied types, Mannich[75] and Pictet−Spengler[76] reactions, have been extensively employed for the assembly of alkaloids, natural-product-like compounds, and biologically active molecules. The generation of these highly unstable (effective reactive or transient electrophilic) species, which typically require acidic conditions, is followed by in situ trapping by nucleophiles or dienes in both addition and cycloaddition reactions, respectively. Despite significant advances in the nucleophilic addition of NAIs,[77] reactions that involve the intermolecular addition of conjugated *N*-acyliminium acceptors still remain elusive due to the challenging issue of regioselectivity (*Figure 10*). To date, (to the best of our knowledge) only rare examples have demonstrated the feasibility of the γ-selective addition of nucleophiles to cyclic conjugated *N*-acyliminium ions.[78] Although these procedure can furnish the γ-adduct in a 1,4-addition fashion, they are often complicated by the formation of mixture of α- and γ-regioisomers as well as limited to a few dicarbonyl nucleophiles only. In addition, these reactions provide functionalization at the C4-position of the azaheterocyclic (e.g. piperidine) skeleton since an endocyclic double bond results in olefin *N*-acyliminium ion structures of type **A**. On the other hand, heterocyclic architectures featuring an iminium cation flanked by an exocyclic double bond of type **B** (e.g. 5-methylene *N*-acyl dihydropyridazinium ions) have not yet reported. This is surprising, because they could be used to effect direct functionalization at the γ' position, a transformation that would permit to introduce nucleophiles onto *exo* methylene group of the ring (olefin terminus).

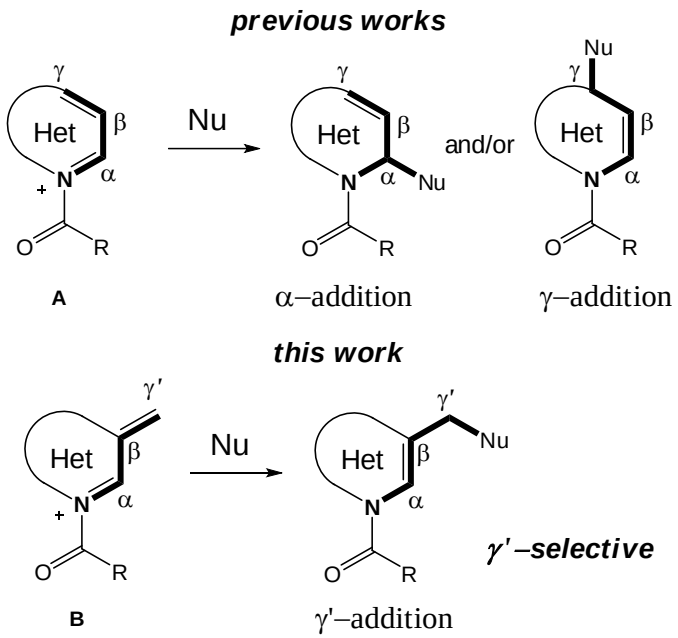


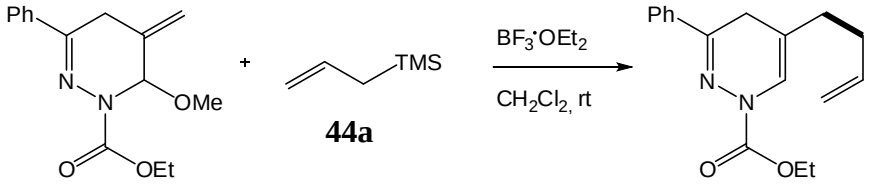
Figure 10: *Nucleophilic Addition to Conjugated Cyclic N-Acyliminium ions.*

The challenge and opportunity of controlling regioselectivity as well as the possibility of adding a variety of nucleophiles have prompted us to investigate the reactivity of these intriguing acceptors in addition reactions. Along these lines, our interest in the chemistry of the azaheterocycles led us to consider 5-methylene-6-methoxy-1,4,5,6-tetrahydropyridazines (*N*,*O*-acetals) easily prepared via a regioselective inverse-electron-demand hetero-Diels-Alder reaction of in situ generated 1,2-diaza-1,3-dienes with methoxyallene.[8] For this purpose, the most widely applied method for NAI formation that involves the release of a leaving group from the α-position of the acyl nitrogen under acidic reaction conditions has been used to assist the formation of transient electrophilic species. Following this strategy, a varied repertoire of adducts featuring nontraditional dihydropyridazine-based skeleton connections molecules or difficult-to-access 5-methylenyl-substituted 1,4-dihydropyridazines can be assembled in high yield and excellent regioselectivity in the first example of a direct, Michael-type addition reaction of nucleophiles to in situ generated cyclic 5-methylene *N*-acyl dihydropyridazinium ions (*β*-methylene *N*-acyliminium ions or exocyclic ene conjugated iminium ions).

5.2 Results and discussion

It well-known that various Brønsted or Lewis acids such as $TiCl_4$, $BF_3{\cdot}OEt_2$, $SnCl_4$, $InCl_3$, $NbCl_5$, $Zn(OTf)_2$, $HNTf_2$, $Zn(NTf_2)_2$, TMSOTf[74,75] are effective for the Mannich-type additions of *N*-acyliminium ions. Among the different Lewis acids tested in our preliminary experiments ($BF_3{\cdot}OEt_2$, CuI, $Cu(OTf)_2$, $ZnBr_2$, $Zn(OTf)_2$, $Y(OTf)_3$, $InBr_3$, $LiClO_4$), only indium tribromide (40%) and boron trifluoride etherate (73%) were found to be productive. The superiority of

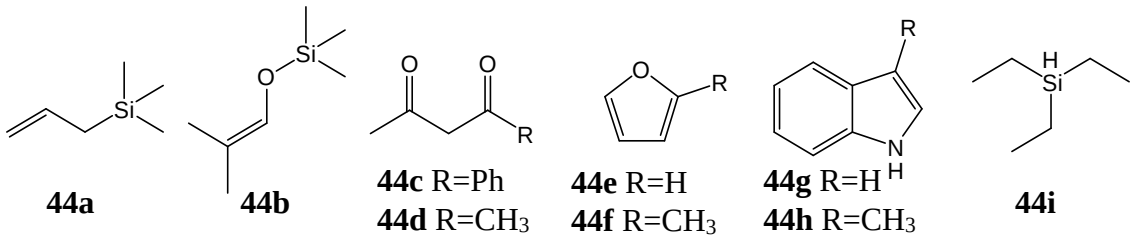
$BF_3 \cdot OEt_2$ to promote the generation of cyclic NAIs, followed by the addition of allyl silane **44a** was evaluated with *β*-methylene tetrahydropyridazine *N*,*O*-acetals **18a** at room temperature in CH_2Cl_2 (*Scheme 24*).



18a **44a** **45a**

Scheme 24: *BF_3•OEt_2-mediated conjugated addition of allyltrimethylsilane 44a to N-acyliminium ions derived from **18a***

To our delight, under these reaction conditions, a complete γ'-selectivity was observed and the exclusive corresponding γ' adduct **45a** was isolated in 73% yield after 10 min. Lowering the reaction temperature to 0 °C did not provide any obvious beneficial effect (had not influence on the reaction) (72%).

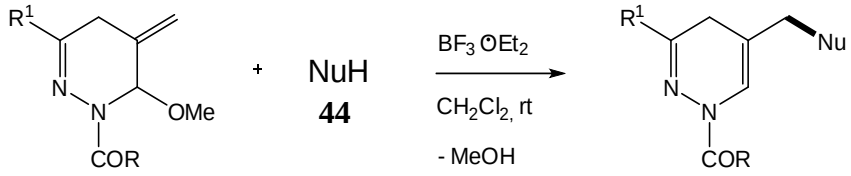


44a **44b** **44c** R=Ph **44d** R=CH_3 **44e** R=H **44f** R=CH_3 **44g** R=H **44h** R=CH_3 **44i**

Figure 11: *Representative nucleophile used to valid thee method.*

The scope of this transformation was next evaluated with different 5-methylene-6-methoxy-1,4,5,6-tetrahydropyridazine substrates **18a−g** and a series of representative nucleophiles **44a−i**. The allyl silane **44a** acted as a good nucleophile with **18a−f** to give the corresponding β-homoallyl substituted dihydropyridazines **45a−f** with better results being osserved with **45a−d** when compared to **45e** and **45f**. Also, silyl enol ether **44b** reacted with **18c** gave **45g** in 65% yield. β-Diketones **44c** and **44d** reacted with electrophiles **18a−c** to give the corresponding adducts **45g−i**. Good results were observed in the addition of heterocyclic nucleophiles (Friedel Crafts alkylation), such as furans and indoles. Interestingly, while furan **44e** was employed as substrate, the formation of the expected monoalkylation product **45k** (56%) was accompanied by the presence of the dialkylation product bis-adduct **45l** (34%) deriving from a further addition of **45k** on **18a**.[79]

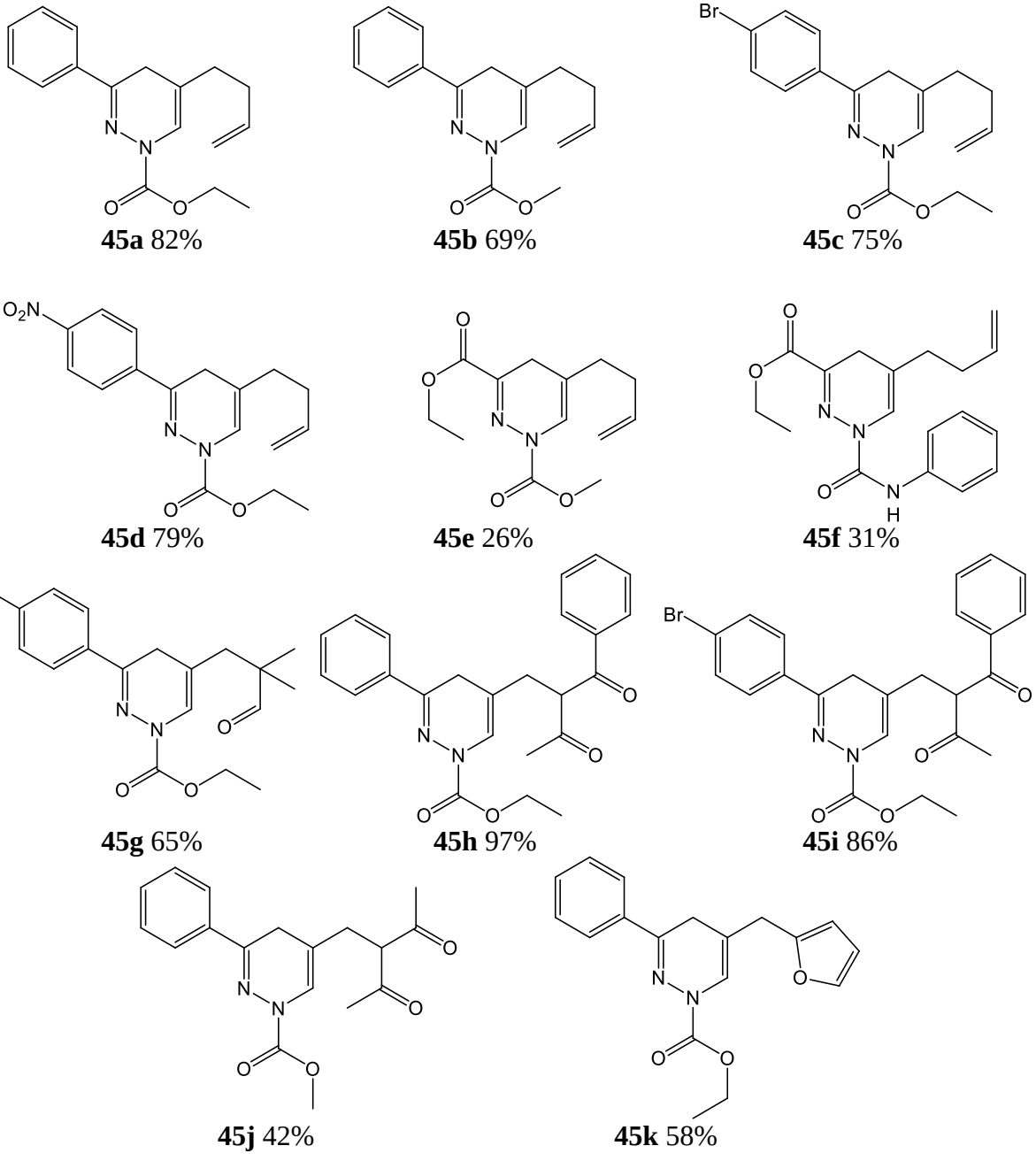
When 2-methyl furan **44f** was used, the sole products **45m,n** were afforded in 30% and 94% yields, respectively. Also *N*H-indole **44g** and *N*-methyl indole **44h** reacted with **18a−c,g** to give the relative adducts. Finally, reduction of **18b** with Et_3SiH (**44i**) successfully furnished the desired product **45s** (53%).



18 **45**

Scheme 25: *Novel iminium ion activated nucleophilic addition.*

Although the explanation for these results is not clear at the present time, the Mannich-type additions of a variety of nucleophiles to exocyclic ene conjugated iminium ions afforded exclusive 1,4-adducts with only trace amounts of 1,2-regioisomers being detected in a few cases.



45a 82% **45b** 69% **45c** 75%

45d 79% **45e** 26% **45f** 31%

45g 65% **45h** 97% **45i** 86%

45j 42% **45k** 58%

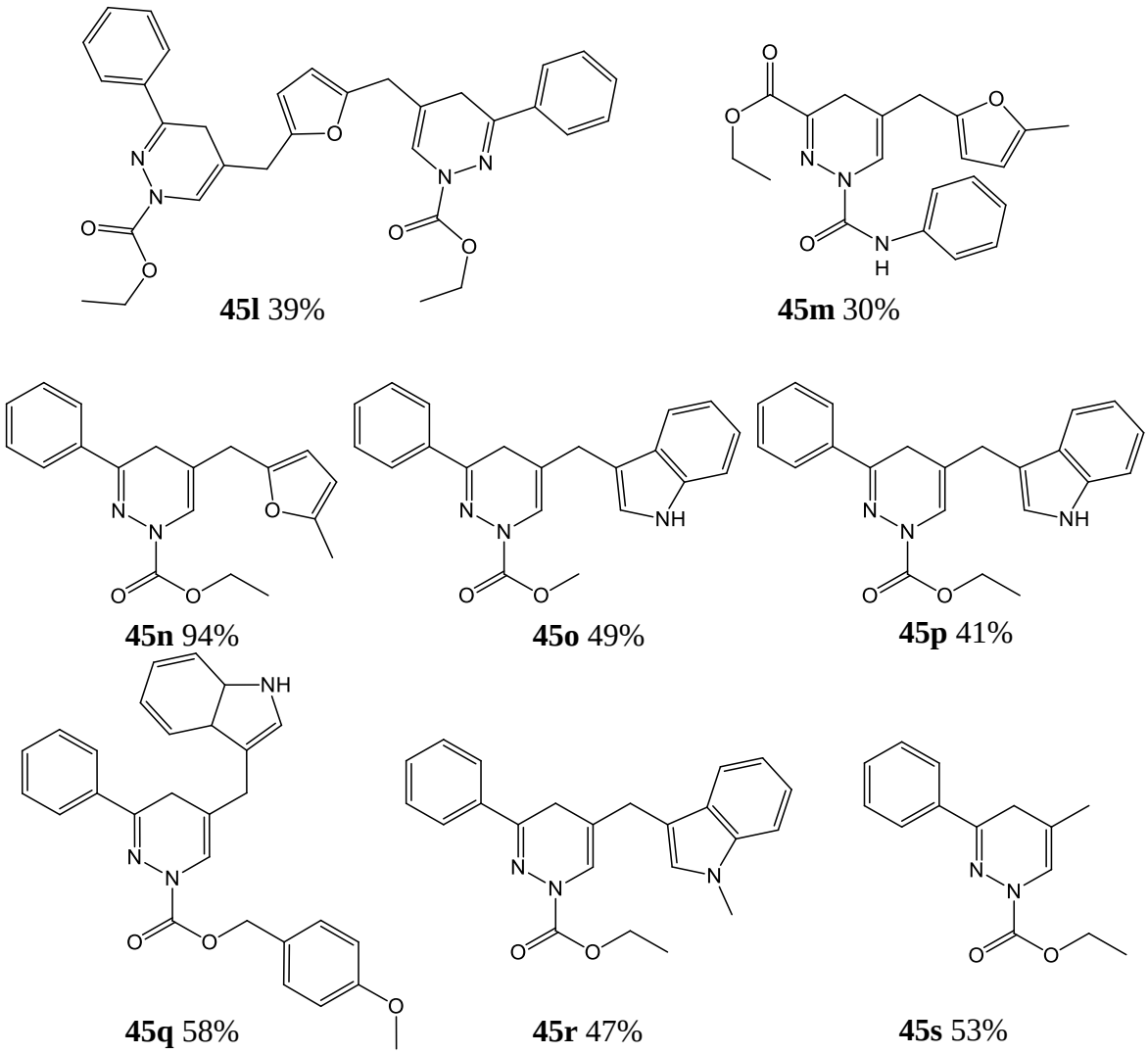


Table 15: *Yields of isolated γ' adducts **45a–s** based on **43**.*

The pyridazine and perhydropyridazine frameworks are often encountered as structural components of compounds possessing biological activity since both nitrogen atoms can be involved in interactions with the protein target. In particular, the 1,4-dihydropyridazine rings are important synthetic targets due to their significance in pharmacological activity[80] whereby they have found used as vasodilators and coronary therapeutic agents, or as spasmolytic agents.[81] By exploiting the inherent reactivity of conjugated *N*-acyliminium ion intermediates it was possible to develop an unprecedented method that allowed the direct incorporation of a variety of groups/substituents at exocyclic γ' position of the 1,4-dihydropyridazine rings. Notably, the installed plural functionalities are versatile handles for further synthetic manipulations. In view of the importance of these target molecules (partially saturated pyridazines/privileged structural motifs) and their rare representation in literature, we hope this methodology may be of value for further studies/applications.

5.3 Conclusion

In conclusion, we have developed a procedure for the preparation of otherwise inaccessible 1,4-dihydropyridazine derivatives (diversely substituted 1,4-dihydropyridazines, uniquely multifunctionalized scaffolds or small dihydropyridazine-based library) by Lewis acid-mediated Mannich-type addition of nucleophiles to 5-methylene-6-methoxy-1,4,5,6-tetrahydropyridazines, which is easily realized via iminium chemistry. From a synthetic perspective, the chemistry here reported is relevant since it provides a straightforward method for the direct installation of diverse σ- and π-nucleophiles at the methylene (γ') position of heterorings, a transformation for which there are no (only sporadic) precedents. These reactions proceed under simple, mild conditions in high yields and with a high level of regioselectivity.

6. SINTHESYS AND BIOLOGICAL EVALUATION OF NEW BERBERINO DERIVATIVES

CHAPTER 6

G. Mari, L. De Crescentini, G. Favi, P. Lombardi, G. Fiorillo, G. Giorgi, F. Mantellini
Asian J. Org. Chem., **2017**, *6*, 720–727

6.1 Introduction

One of the main interests of the organic chemists is directed to the development of simple, viable strategies to access structurally complex polyheterocyclic cores, by assembling ready available building blocks.[82,83]

Among these polyheterocycles, berberine (BB) (*Figure 12*) and its derivatives are of considerable interest because they are known to possess several healthy properties.[84–88]

Berberine is an isoquinoline alkaloid extracted from rhizomes and roots of several species of plants, such as *Coptis* (*Coptis chinensis* and *Coptis japonica*), *Hydrastis* and *Berberis* (*Berberis vulgaris* and *Berberis croatica*). These plants have long been used in traditional Chinese, Ayurvedic and Native American medicine for the treatment of different maladies. BB is known to possess a variety of pharmacological and therapeutic activities and it was employed to treat diarrhea,[84] stomatitis[84] and hepatitis,[84] due to its antiprotozoal,[84,85] antimicrobial[84] and anti-inflammatory properties.[84] Recently, BB has been identified as a good candidate for the treatment of type 2 diabetes mellitus[84] and of cardiovascular diseases.[84] Numerous studies also reported its activity as anticancer agent, in different stages of cancer development (proliferation, growth, metastasis).[84,86,87] This effect is ascribable to the antiproliferative activity of berberine and its derivatives against several cancer cellular lines.[87]

Tetrahydroberberine (THB) (*Figure 12*), the fully reduced form of BB, is a naturally occurring alkaloid mainly extracted from *Corydalis ambigua* and it is known to have significant pharmacological properties that differ from that of the parent berberine.[88] In fact, THB shows little antiproliferative activity toward several lines of cells, but instead it is effective as an antioxidant[89a] and a Ca2+ channel blocker.[89b]

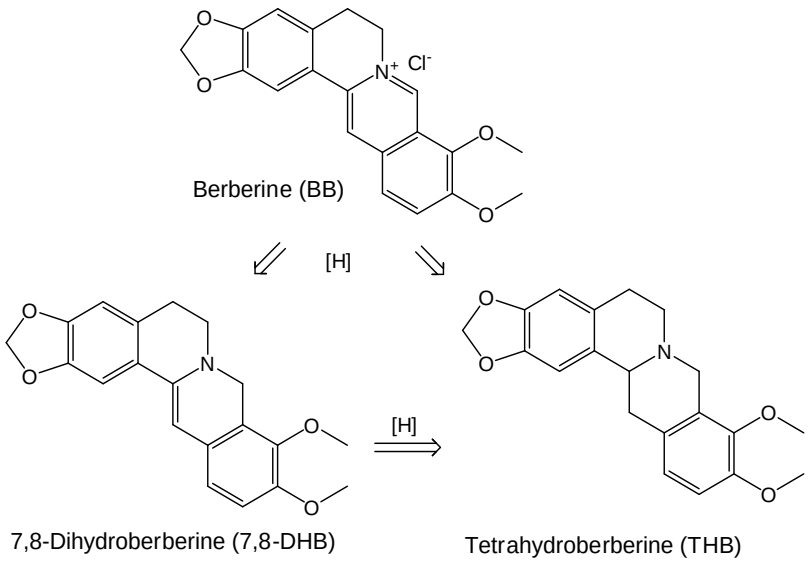
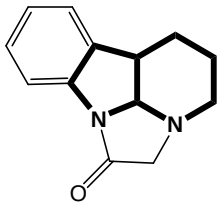


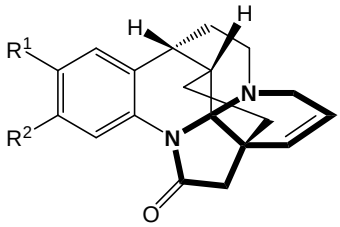
Figure 12: *Berberine (BB) and its reduced forms dihydroberberine (DHB) and 7,8-tetrahydroberberine (THB).*

Additionally, THB has also been observed to block ATP-sensitive K^+ ion channels involved in the pathogenesis of Parkinson's disease,[89c] indicating an important neuroprotective role.

As part of our program aimed at focusing new strategies for the construction of potential biologically active compounds, we devised a strategy aiming at the synthesis of structurally complex tetrahydroberberine analogues containing the pyrrolo[*2,3-b*]pyridine system. The pyrrolo[*2,3-b*]pyridine core is structurally important because, for example, it is related to the alkaloids of the chaetominine and kapakahine families (*Figure 13*)[82,90] and it has been found in several pharmaceutical active molecules and drug candidates.[91] The same nucleus can be found in isoschizogamine and isoschizogaline (*Figure 13*), belonging to the family of schizozygane alkaloids, that exhibit antimicrobial and antifungal activities at low micromolar concentrations.[83]



pyrrolo[*2,3-b*]pyridine core
of chaetominines and kapakahines



pyrrolo[*2,3-b*]pyridine core of
isoschizogamine (R^1, R^2 = OMe) and
isoschizogaline (R^1 = H, R^2 = OMe)

Figure 13: *Pyrrolo[2,3-b]pyridine core of chaetominines and kapakahines and of isoschizogamine and isoschizogaline.*

The retrosynthetic analysis of this fused-bicyclic structure emphasizes two strategic disconnections of the pyrrole ring: the first one is along the N(1)-C(7a) and the second one along C(3)-C(3a) bonds. This reveals two subunits that trace the left half back to the zwitterionic amine **A** and the right half to the pertinent heterocyclic zwitterion **B** (*Scheme 26*). Fragment **A** can be correlated to the azo-ene system of 1,2-diaza-1,3-dienes, that represent the main field of our research for the past several years.[92] The fragment **B** can be related to the pertinent dihydro-pyridine **D**, that is contained in the structure of 7,8-dihydroberberine (7,8-DHB), the partially reduced form of berberine (*Figure 12, Scheme 26*).

In fact, 7,8-DHB contains an enamine moiety that could react with the azoene system of DDs, as previously reported in literature by several authors[93] and also by some of our group.[94]

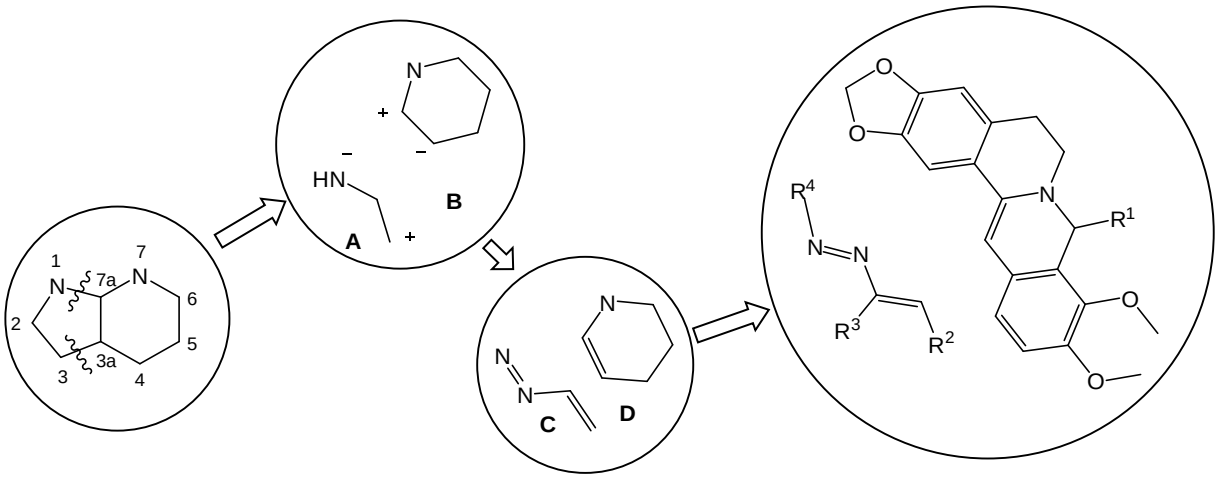
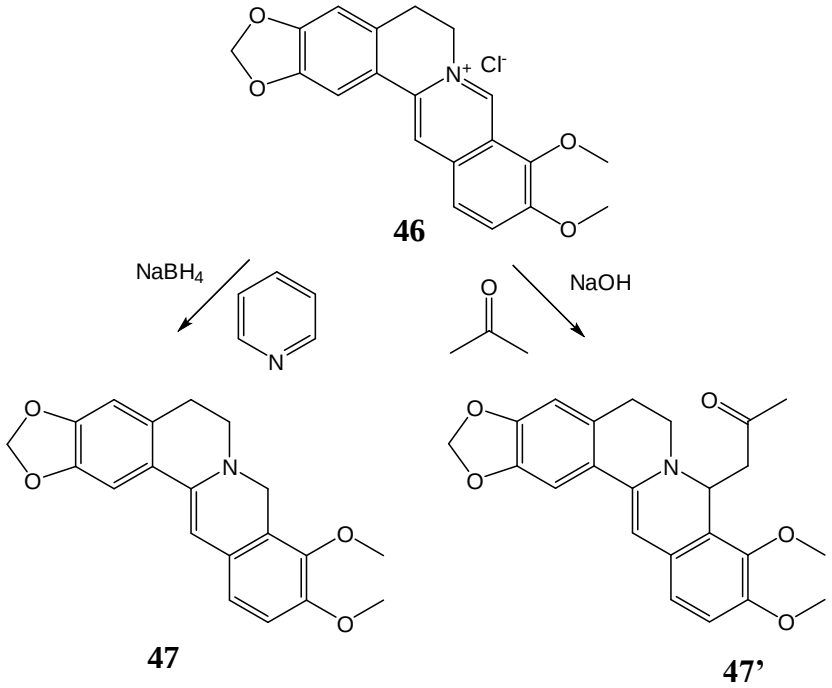


Figure 14: *Retrosynthetic analysis of pyrrolo[2,3-b]pyridine systems.*

6.2 Results and Discussion

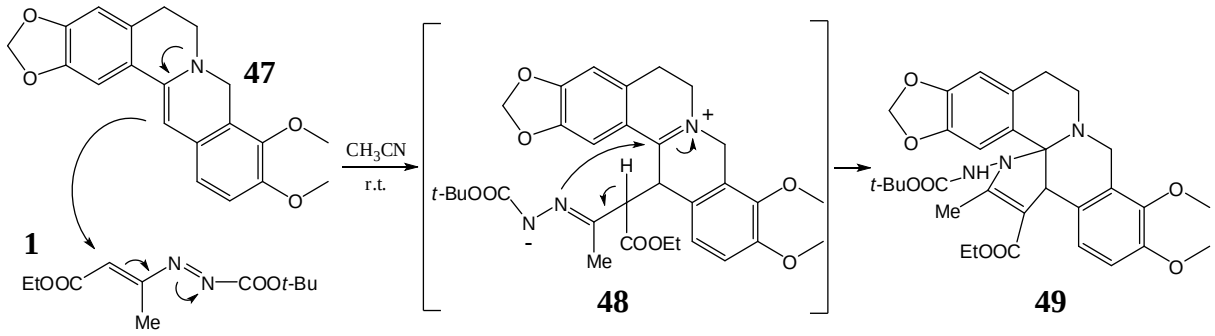
Following known procedures, we prepared 7,8-dihydroberberine **47**[95] and 8-acetonyl-7,8-dihydroberberine **47'**[96] (*Scheme 26*). Both 7,8-dihydroberberines **47** and **47'** possess the enamino function, required for the nucleophilic attack on the DDs.



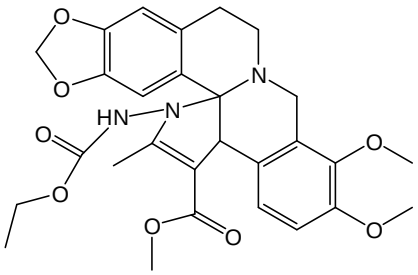
Scheme 26: *Synthesis of 7,8-dihydroberberine **47** [95] and of 8-acetonyl-7,8-dihydroberberine **47'**,[96] starting from commercial berberine chloride **46**.*

By choosing DD **1e** and 7,8-DHB **47** as representative examples, we investigated the reaction in different conditions of solvents (dichloromethane, tetrahydrofuran, diethyl ether, ethyl acetate and acetonitrile) and temperatures (r.t., 0 °C and -15 °C) (*Scheme 27*). In all the conditions tested, the reaction took place with the formation of the corresponding fused pyrrolino-THB derivative **49e** in good yields. By using acetonitrile, the reaction proceeds at room temperature and **49e** directly precipitates in 87% yield from the reaction medium.

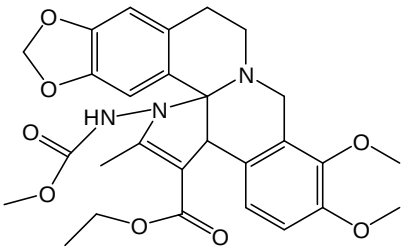
Having optimized the conditions, we explored the reactions of various DDs **1a–n** with 7,8-DHB derivatives **47** and **47'**. The berberberino derivatives **49a–n** were obtained in good to excellent yields (49–92%), with the exception of **49g** (22%), and all the reactions were completed in 0.25 h (*Table 16*).



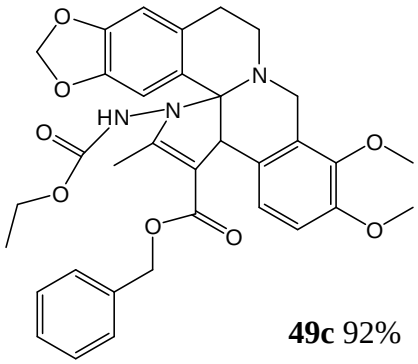
Scheme 27: *Mechanism for the synthesis berberino derivatives **49a–n.***



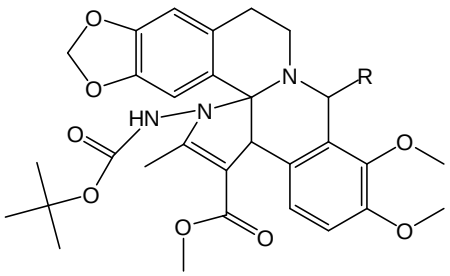
49a 61%



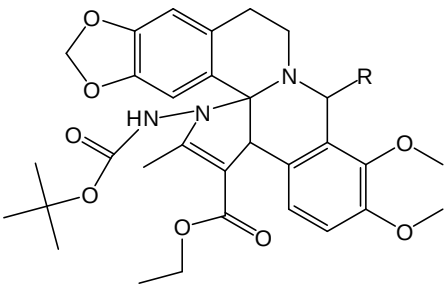
49b 83%



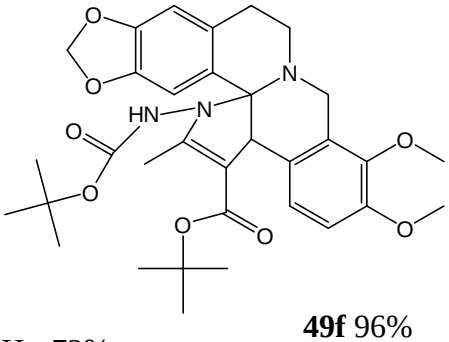
49c 92%



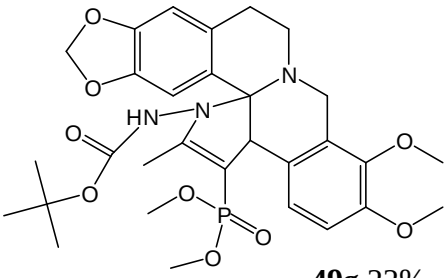
49d R=H 60%
49d' R=CH_3COCH_3 49%



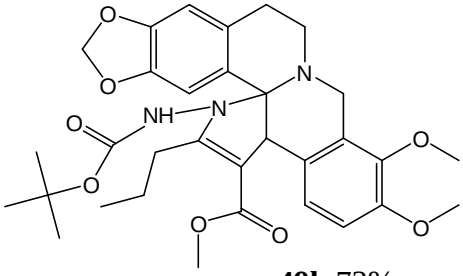
49e R=H 87%
49e' R=CH_3COCH_3 72%



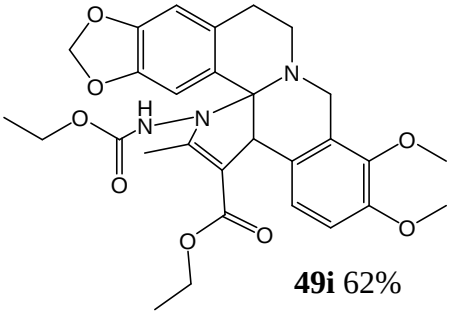
49f 96%



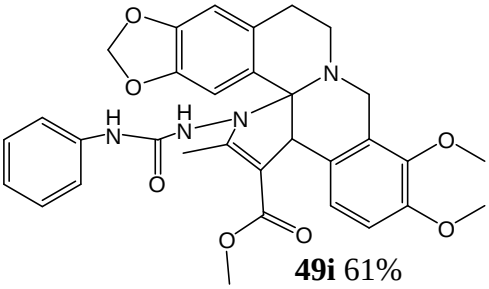
49g 22%



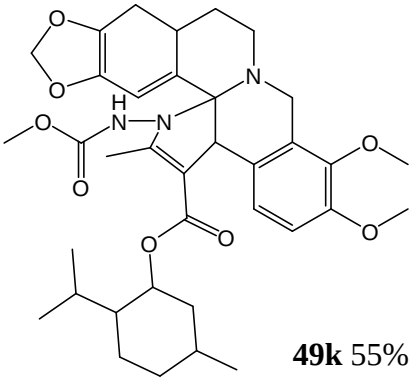
49h 73%



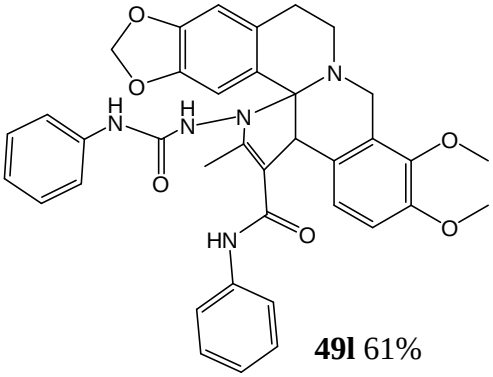
49i 62%



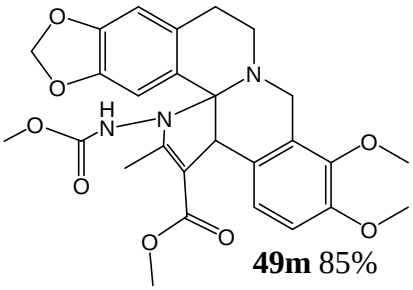
49i 61%



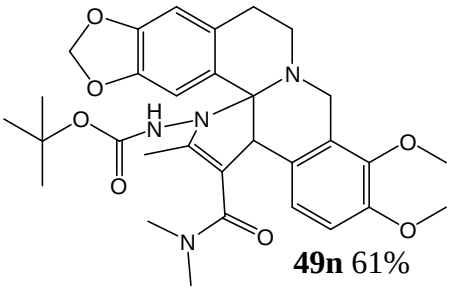
49k 55%



49l 61%



49m 85%



49n 61%

Table 15: *Yields for the synthesis of berberino derivatives **49a–n** referred to **1***

Compounds **49** possess an intriguing fused esacyclic new system and three of the rings are arrayed around the aminal carbon. Their formation occurs through a formal [3+2] cycloaddition and the plausible mechanism could involve a preliminary regioselective Michael type addition of the β-carbon atom of the enamino function in position 13 of 7,8-DHBs **47,47'** to the electrophilic terminal carbon of the azo-ene system of DDs **1** producing the non-isolable zwitterionic hydrazone intermediates **48**. The loss of the hydrogen in α-position to the hydrazine moiety of **48** could promote the nitrogen intramolecular nucleophilic attack onto the iminium function, determining the formation of the dihydropyrrole ring of **49** (*Scheme 27*). To the best of our knowledge, only one other example of Michael-type addition involving DHB derivatives was reported.[97]

The structures of compounds **49** were determined by mono and bidimensional NMR measurements. Additionally, X-ray diffraction studies of **49e**, derived from DD **1e** and 7,8-DHB **47**, and of **49e'**, derived from the same DD **1e** and 8-acetonyl-7,8-DHB **47'**, were performed and they unequivocally confirmed the proposed structures (*Figure*s 15 and 16).[98]

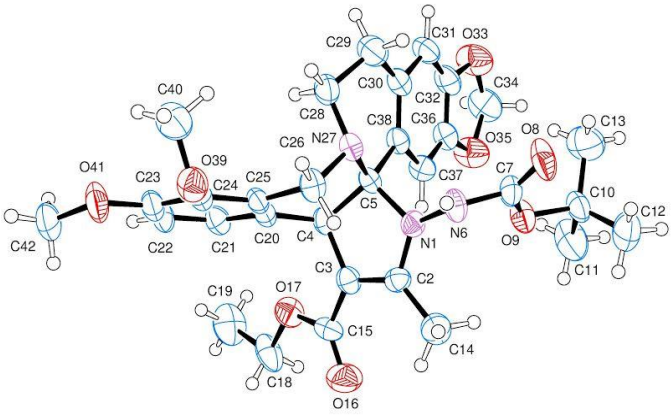


Figure 15: *X-ray structure of compound **49e***

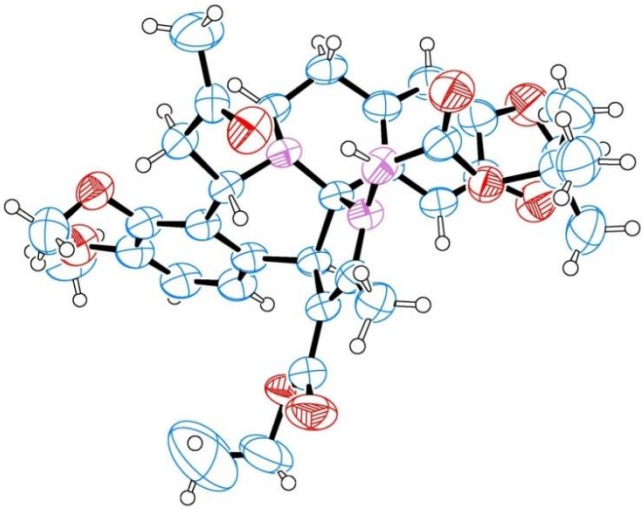
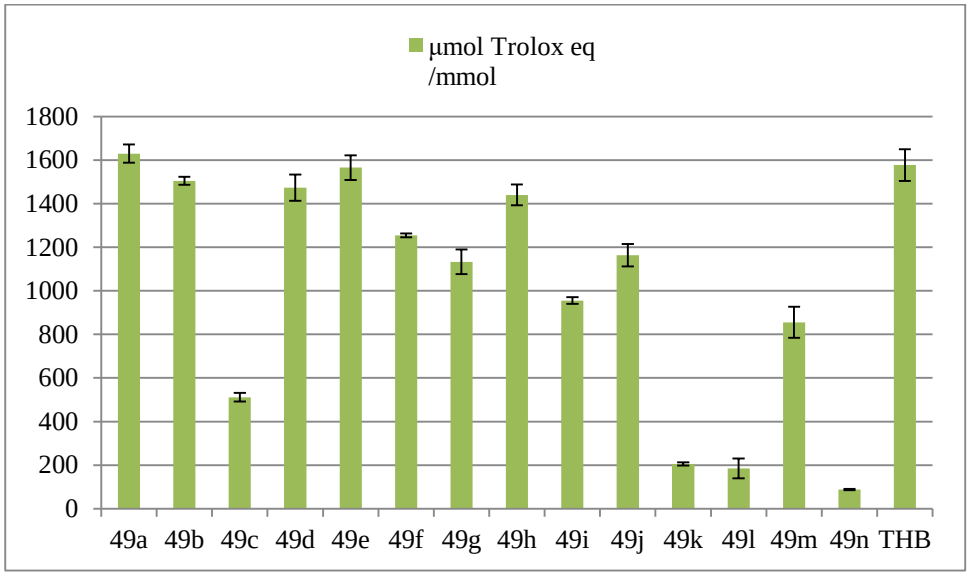


Figure 16: *X-ray structure of compound **49e'***

6.3 Antioxidant properties:

The novel THB derivatives **49a–n** were found to exhibit antioxidant properties at various degrees, by using the ORAC (Oxygen radical absorbance capacity) method.[100] (*Table 16 and Graphic 1*). In particular, ORAC values of **49a**, **49b** and **49e** were comparable to those of THB. A slight ORAC decay (10%) was observed for the compounds **49d** and **49h**, followed by **49f** (20%). Instead, compounds **49g**, **49i** and **49l** showed 30% antioxidant capacity decay as compared to that of THB. The analogues **49c**, **49j**, **49k**, **49m** and **49n** showed the lowest ORAC values.



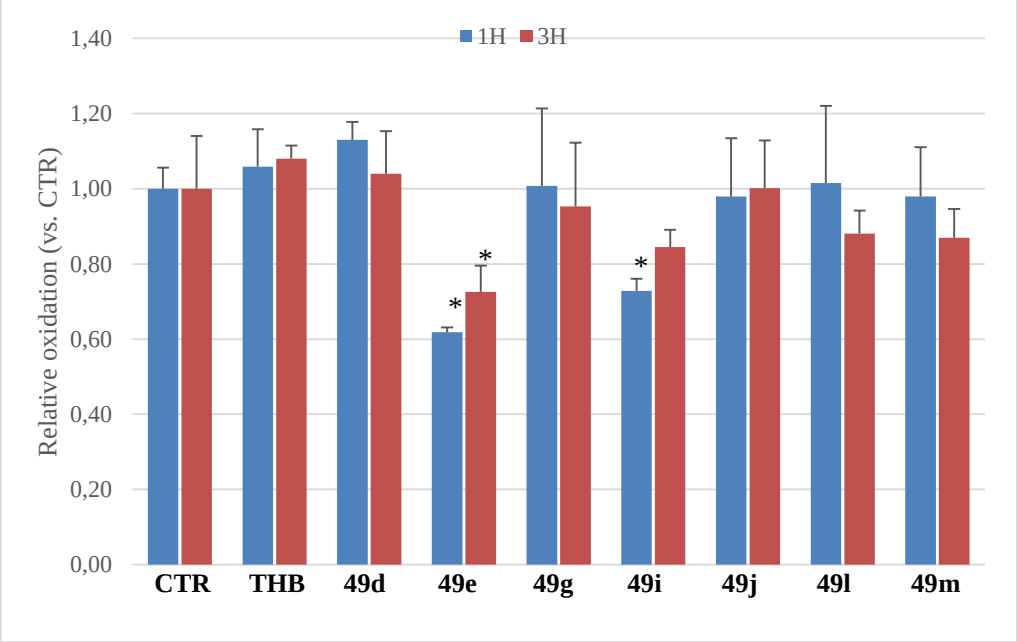
Graphic 1: *Antioxidant capacity of THB and its analogues **49a–n** detected by the ORAC method. ($p<0.05$, one-way ANOVA).*

SAMPLES	ORAC values (µmol TE/mmol)
THB	1577±72
49a	1629±42
49e	1565±56
49b	1504±18
49d	1474±60
49h	1440±49
49f	1255±9
49j	1164±51
49g	1133±57
49i	955±15
49m	855±71
49c	512±19
49k	206±7
49l	185±46
49n	87±3

Table 16: *Antioxidant capacity value of THB and its analogues **49a–n***

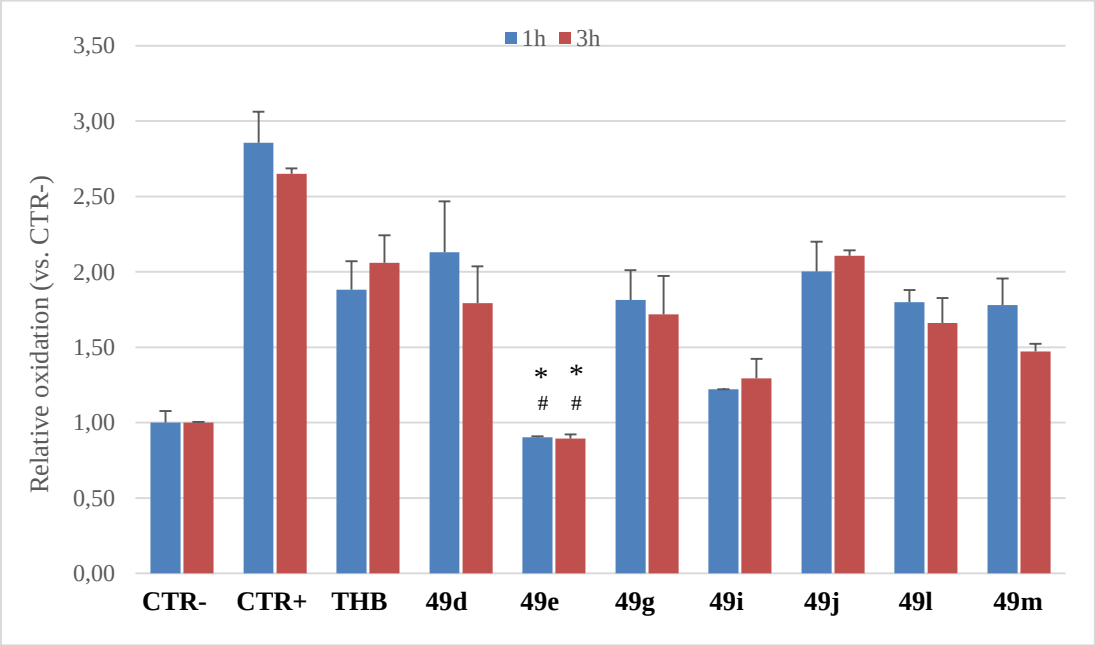
Based on synthesis procedures and ORAC values, 7 out of 14 THB derivatives were selected for further in vitro antioxidant assays: 2 with high ORAC values (**49d** and **49e**), 2 with medium/high values (**49g** and **49j**), 2 with medium values (**49i** and **49m**) and 1 with a low ORAC value (**49l**).

Referring to the DCFH-DA assay, experiments were conducted in NCTC-2544 cells both in the absence (basal condition) and in presence (oxidative condition) of the oxidant molecule H_2O_2. In **basal conditions**, cells were incubated with the selected THB derivatives up to 3h, and then the intracellular oxidation levels were evaluated by recording DCFH-DA fluorescence emission. As reported in *Graphic 2*, NCTC-2544 cells incubated for 1h with compounds **49e** and **49i** presented significantly lower oxidation levels as compared to untreated control cells ($p<0.05$ vs. CTR). The same trend was observed for **49e** even after 3 h of cell incubation with the test molecule.



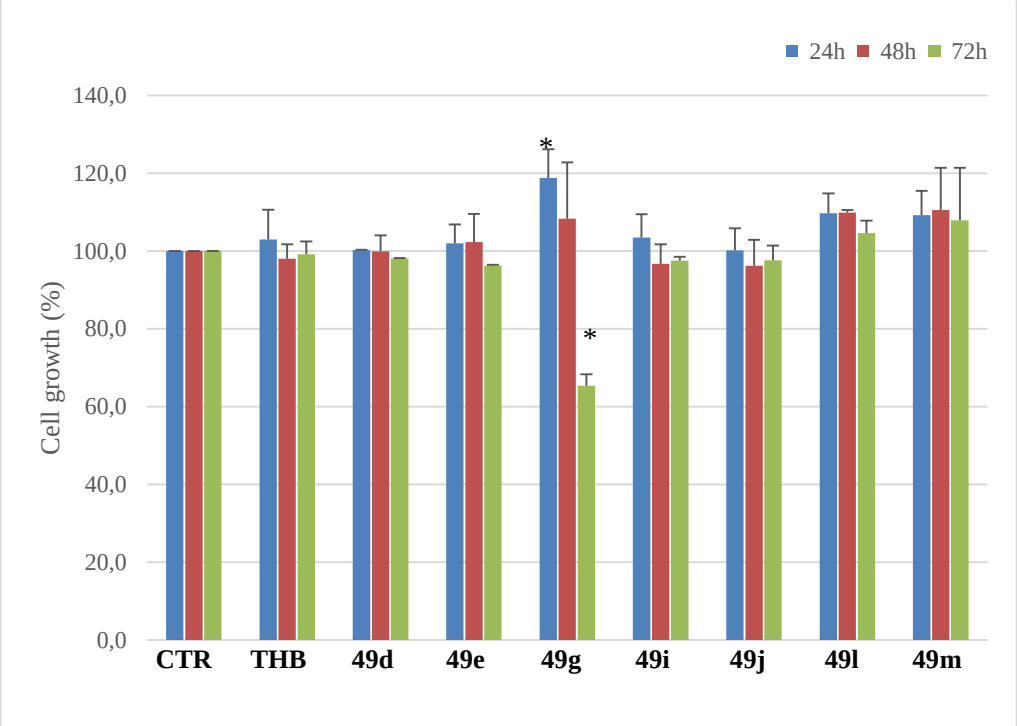
Graphic 2: *Relative intracellular oxidation levels in NCTC-2544 cells incubated for 1 and 3h with THB derivatives (30 µM) or vehicle (0.1% DMSO, CTR). Data are expressed as mean ± SD (n=3). *p<0.05 vs. untreated cells (CTR).*

In **oxidative conditions**, NCTC-2544 cells were pre-incubated with the selected THB derivatives up to 3 h and then oxidized with H_2O_2. DCFH-DA fluorescence emission was recorded for 30 min after H_2O_2 administration. As shown in graphic 3, both THB and its derivatives significantly reduced H_2O_2-induced intracellular oxidation ($p<0.05$ vs CTR+). Among THB derivatives, **49e** and **49i** presented the highest antioxidant protection against H_2O_2. In particular, **49e** exhibited an antioxidant capacity significantly different from THB as well as from the negative control (non-oxidized cells).



Graphic 3: *Relative intracellular oxidation levels after H_2O_2 administration (100 µM) to NCTC-2544 cells pre-incubated for 1 and 3 h with THB derivatives (30 µM). CTR-: non-oxidized cells (negative control, reference value). CTR+: H_2O_2-treated cells (positive control, maximum oxidation). Data are expressed as mean ± SD (n=3). *p<0.05 vs. CTR-. #p<0.05 vs. THB.*

The effects of the selected THB derivatives on cell viability were also explored. As reported in *Graphic 3*, compounds **49d**, **49e**, **49i** and **49j** had no significant effects on NCTC-2544 cell growth when compared to untreated cells (CTR). Compounds **49l** and **49m** showed a bland stimulatory effect on cell growth ($p>0.05$ vs. CTR), while **49g**, after an initial stimulatory effect ($p<0.05$ vs. CTR), exhibited a late inhibitory action (cell growth decrement by 35% at 72h as compared to CTR, $p<0.05$).



Graphic 4: *Effect of THB derivatives (30 µM) on NCTC-2544 cell growth after 24, 48, and 72 h of incubation. Data are expressed as mean ± SD (n=3). *p<0.05 vs. untreated cells (CTR).*

6.4 Conclusion

In conclusion, starting from easily available starting materials, such as DDs and dihydroberberines, we developed a facile procedure for the synthesis of novel unprecedented pyrrolino-tetrahydroberberine derivatives through a formal [3+2] cycloaddition. These compounds are fascinating in virtue of the fact that they possess a complex fused esacyclic new system and three of these rings are arrayed around an aminal carbon. Besides, the pyrrolino-tetrahydroberberines synthesized have shown a promising antioxidant activity, as a study carried out with the ORAC method and NCTC-2544 cell line text have been demonstrated.

7. GENERAL CONCLUSION

CHAPTER 7

The results reported in this thesis demonstrate the usefulness of DDs as powerful Michael acceptors. DDs allow various functionalizations of the carbon atom adjacent to the masked carbonyl moiety that permit the construction of many types of five- and six-membered heterocycles.

Most of the reactions surveyed herein occur with good yields under very mild conditions, usually at room temperature especially in the first step of the 1,4-addition of the nucleophilic agents to the azo-ene systems. This step, as well as the heterocycle formation are often spontaneous, and, at times, may be favoured by acid or basic catalysis. Rarely, the eterocyclization process is affected by temperature. Although most of the reactions described formally proceed through many chemical steps, in practice they can frequently be executed in one pot and in no case, require anhydrous solvent or inert atmosphere. Finally, the equipment (generally magnetic stirring) and work-up procedures (normally extraction with water, purification by column chromatography and/or crystallization) are very simple.

The creation of a new library of several compounds with a large scaffold diversity, highlights the utility of these compound as valuable products and key intermediates in organic and pharmaceutical chemistry.

In this regard, an example is provided by the new berberino derivatives, that have shown a significant antioxidant activity both in chemical and biological experiments.

8. EXPERIMENTAL SESSION

CHAPTER 8

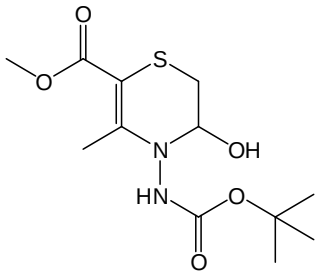
CHAPTER 2

General information

All chemicals and solvents were purchased from commercial suppliers and used as received. 1,2-Diaza-1,3-dienes were prepared as reported[99] and used as *EE/EZ* isomer mixtures. Melting points were determined in open capillary tubes and are uncorrected. FTIR spectra were obtained as Nujol mulls. All ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Proton and carbon spectra were referenced internally to solvent signals, using values of δ = 2.50 ppm for proton (middle peak) and δ = 39.50 ppm for carbon (middle peak) in DMSO-d6 and δ = 7.27 ppm for proton and δ = 77.00 ppm for carbon (middle peak) in $CDCl_3$. All coupling constants (*J*) are given in Hz. All the NH and OH exchanged with D_2O. Precoated silica gel plates 0.25 mm was employed for analytical thin layer chromatography. All new compounds showed satisfactory elemental analysis. Mass spectra were recorded in the EI mode (70eV). The nomenclature was generated using ACD/IUPAC Name (version 3.50, 5 Apr. 1998), Advanced Chemistry Development Inc., Toronto, ON (Canada).

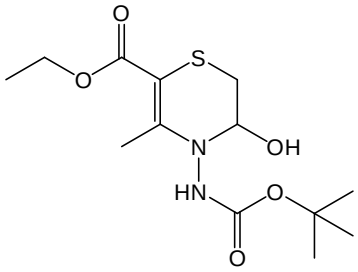
General Procedure for the Synthesis of 3-hydroxy-3,4-dihydro-2*H*-1,4-thiazine 21a–k.

To a solution of dithiane-2,5-diol **1** (0.5 mmol, 1.0 equiv.) and DDs **1a–k** (1.0 mmol, 1.0 equiv.)[99] in diethyl ether (2.0 mL), at room temperature, *N*,*N*-dimethylethylamine (0.2 mmol, 0.2 equiv.) was added under magnetic stirring. At the disappearance of the starting materials (visual and TLC monitoring), the solvent and the *N*,*N*-dimethylethylamine were evaporated under vacuum at room temperature obtaining the corresponding crude pure 3-hydroxy-3,4-dihydro-2*H*-1,4-thiazines **21a–k**.



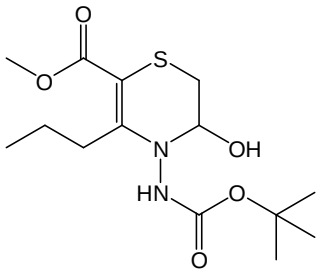
Methyl 4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-5-methyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21a.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.32 (s, 9H), 2.24 (s, 3H), 2.60-2.80 (m, 2H), 3.56 (s, 3H), 4.25 (brs, 1H), 4.97 (s, 1H), 7.85 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.0 (q), 28.1 (q), 31.8 (t), 51.6 (q), 80.6 (d), 81.6 (s), 91.3 (s), 152.1 (s), 154.7 (s), 165.9 (s).; IR (nujol): ν_{max} = 3462, 3292, 1762, 1723 cm^{-1}; MS *m/z* (%): 304 (M^+) (25), 300 (7), 247 (54), 231 (79), 203 (35), 172 (53), 144, (38), 111 (90); anal. calcd. for $C_{12}H_{20}N_2O_5S$ (304.3637): C 47.35, H 6.62, N 9.20; found: C 47.19, H 6.59, N 9.37.



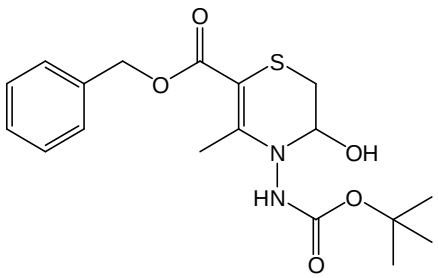
Ethyl 4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-5-methyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21b.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.23 (t, 3H, *J* = 7.2 Hz), 1.41 (s, 9H), 2.33 (s, 3H), 2.63-2.86 (m, 2H), 3.72 (brs, 1H), 4.12 (q, 2H, *J* = 7.2Hz), 5.04 (s, 1H), 7.51 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.3 (q), 16.1 (q), 28.2 (q), 32.0 (t), 60.6 (t), 80.5 (d), 81.8 (s), 91.9 (s), 152.2 (s), 154.6 (s), 165.5 (s); IR (nujol): ν_{max} = 3452, 3286, 1742, 1706 cm^{-1}; MS *m/z* (%): 318 (M^+) (25), 300 (14), 261 (76), 245 (43), 230 (76), 188 (35); anal. calcd. for $C_{13}H_{22}N_2O_5S$ (318.3903): C 49.04, H 6.96, N 8.80; found: C 48.92, H 6.93, N 8.94.



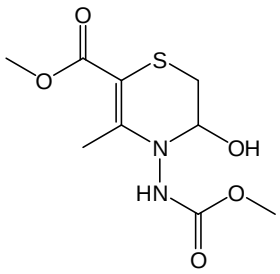
Ethyl 4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-5-propyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21c.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 (t, 3H, *J* = 8.0 Hz), 1.24 (t, 3H, *J* = 8.0 Hz), 1.39-1.43 (m, 11H), 2.55-2.91 (m, 4H), 3.70 (brs, 1H), 4.12 (q, 2H), 5.04 (brs, 1H), 7.37 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.3 (q), 22.3 (q), 28.1 (q), 31.4 (t), 31.9 (t), 37.6 (t), 60.5 (t), 80.0 (d), 81.9 (s), 91.9 (s), 154.4 (s), 155.7 (s), 165.0 (s); IR (nujol): ν_{max} = 3465, 3274, 1759, 1704 cm^{-1}; MS *m/z* (%): 346 (M^+) (5), 290 (11), 247 (9), 216 (21), 167 (53), 149 (100); anal. calcd. for $C_{15}H_{26}N_2O_5S$ (346.4434): C 52.00, H 7.56, N 8.09; found: C 52.11, H 7.59, N 7.98.



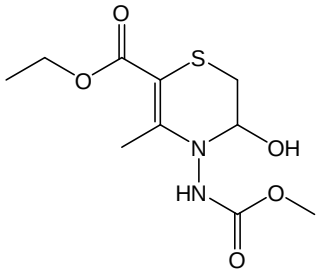
Benzyl 4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-5-methyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21d.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.44 (s, 9H), 2.39 (s, 3H), 2.66-2.91 (m, 2H), 4.03 (brs, 1H), 5.07 (s, 1H), 5.15 (s, 2H), 7.19-7.41(m, 5H), 7.69 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.2 (q), 28.2(q), 32.0 (t), 66.2 (t), 80.6 (d), 81.9 (s), 91.5 (s). 127.7 (d), 127.9 (d), 128.5 (d), 136.4 (s), 152.5 (s), 154.7 (s), 165.3 (s); IR (nujol): ν_{max} = 3467, 3235, 1752, 1701 cm^{-1}; MS *m/z* (%): 380 (M^+) (37), 362 (8), 324 (100), 306 (68), 289 (12), 281 (10), 250 (82), 234 (30); anal. calcd. for $C_{18}H_{24}N_2O_5S$ (380.4596): C 56.82, H 6.36, N 7.36; found: C 56.66, H 6.32, N 7.53.



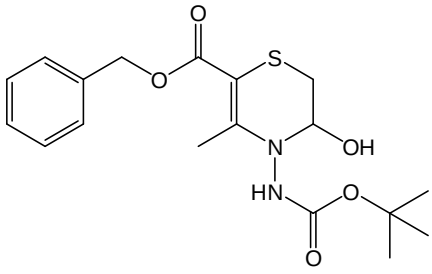
Methyl 3-hydroxy-4-[(methoxycarbonyl)amino]-5-methyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21e.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.30 (s, 3H), 2.61-2.86 (m, 2H), 3.62 (s, 3H), 3.65 (m, 3H), 5.05 (brs, 2H), 8.30 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.4 (q), 31.9 (t), 52. 0 (q), 53.1 (q), 80.4 (d), 92.1 (s), 152.1 (s), 156.7 (s), 166.2 (s); IR (nujol): ν_{max} = 3468, 3251, 1758, 1708 cm^{-1}; MS *m/z* (%): 262 (M^+) (34), 244 (14), 231 (11), 188 (52), 159 (100); anal. calcd. for $C_9H_{14}N_2O_5S$ (262.2839): C 41.21, H 5.38, N 10.68; found: C 41.37, H 5.41, N 10.54.



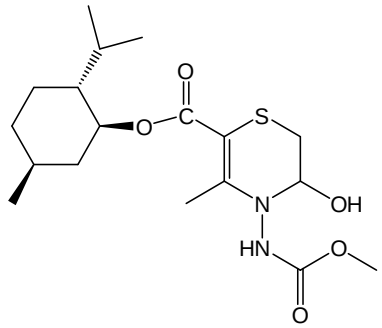
Ethyl 3-hydroxy-4-[(methoxycarbonyl)amino]-5-methyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21f.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 (t, 3H, *J* = 8.0 Hz), 2.28 (s, 3H), 2.61-2.83 (m, 2H), 3.64 (s, 3H), 4.07 (q, 2H, *J* = 8.0 Hz), 4.50 (brs, 1H), 5.03 (s, 1H), 8.20 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.5 (q), 16.4 (q), 32.0 (q), 53. 2 (q), 60.8 (t), 80.5 (d), 92.4 (s), 151.7 (s), 156.6 (s), 165.8 (s); IR (nujol): ν_{max} = 3463, 3302, 1773, 1714 cm^{-1}; MS *m/z* (%): 276 (M^+) (20), 258 (7), 233 (20), 173 (15); anal. calcd. for $C_{10}H_{16}N_2O_5S$ (276.3105): C 43.47, H 5.84, N 10.14; found: C 43.65, H 5.88, N 9.98.



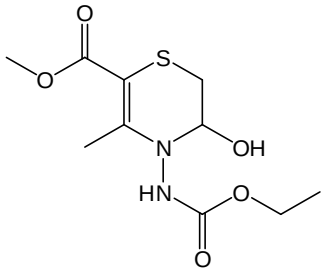
Benzyl 3-hydroxy-4-[(methoxycarbonyl)amino]-5-methyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21g.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.34 (s, 3H), 2.68-2.87 (m, 2H), 3.67 (s, 3H), 4.23 (s, 1H), 5.07 (s, 1H), 5.12 (s, 2H), 7.24-7.34 (m, 5H), 7.96 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.5 (q), 32.3 (t), 52.1 (q), 68.1 (t), 80.6 (d), 92.2 (s), 128.5 (d), 128.8 (d), 128.9 (d), 135.6 (s), 152.1 (s), 155.9 (s), 166.1 (s); IR (nujol): ν_{max} = 3431, 3261, 1758, 1704 cm^{-1}; MS *m/z* (%): 338 (M^+) (75), 320 (23), 307 (6), 279 (6), 264 (53), 247 (5), 171 (52), 159 (100); anal. calcd. for $C_{15}H_{18}N_2O_5S$ (338. 3799): C 53.24, H 5.36, N 8.28; found: C 53.09, H 5.32, N 8.42.



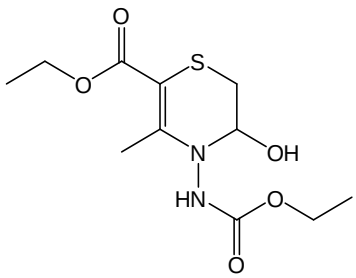
(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 3-hydroxy-4-[(methoxycarbonyl)amino]-5-methyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21h.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.70 (d, 3H, *J* = 8.0 Hz), 0.83 (d, 6H, *J* = 8.0 Hz), 1.00-1.07 (m, 1H), 1.29-1.63 (m, 4H), 1.82-1.98 (m, 3H), 2.06-2.12 (m, 1H), 2.31 (s, 3H), 2.59-2.91 (m, 2H), 3.31 (t, 1H, *J* = 8.0 Hz), 3.67 (s, 3H), 4.56-4.64 (m, 1H), 5.01 (brs, 1H), 8.12 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.2 (q), 21.2 (q), 22.4 (q), 23.2 (t), 25.9 (t), 31.8 (t), 34.7 (d), 41.2 (d), 45.1 (t), 50.2 (d), 53.1 (q), 71.6 (d), 80.4 (s), 93.1 (s), 151.1 (s), 157.0 (s), 165.3 (s); IR (nujol): ν_{max} = 3477, 3275, 1762, 1705 cm^{-1}; MS *m/z* (%): 386 (M^+) (24), 368 (11), 355 (18), 327 (47), 247 (25), 231 (37), 144 (64); anal. calcd. for $C_{18}H_{30}N_2O_5S$ (386.5073): C 55.93, H 7.82, N 7.25; found: C 56.09, H 7.86, N 7.11.



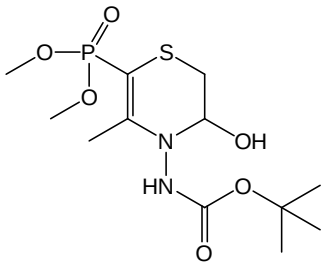
Methyl 4-[(etoxycarbonyl)amino]-3-hydroxy-5-methyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21i

. White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.25 (t, 3H, *J* = 8.0 Hz), 2.38 (s, 3H), 2.65-2.87 (m, 2H), 3.70 (s, 3H), 4.11-4.19 (m, 3H), 5.09 (s, 1H), 7.61 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.7 (q), 16.5 (q), 32.2 (t), 52.0 (q), 62.5 (t), 80.6 (d), 92.2 (s), 152.1 (s), 156.0 (s), 166.1(s); IR (nujol): ν_{max} = 3470, 3301, 1758, 1698 cm^{-1}; MS *m/z* (%): 276 (M^+) (40), 258 (15), 245 (7), 233 (40), 202 (55), 173 (75),111 (100); anal. calcd. for $C_{10}H_{16}N_2O_5S$ (276.3105): C 43.47, H 5.84, N 10.14; found: C 43.62, H 5.87, N 9.97.



Ethyl 4-[(etoxycarbonyl)amino]-3-hydroxy-5-methyl-3,4-dihydro-2*H*-1,4-thiazine-6-carboxylate 21j.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.05-1.26 (m, 6H), 2.28 (s, 3H), 2.61-2.71 (m, 2H), 4.01-4.11 (m, 5H), 5.02 (brs, 1H), 8.02 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.2 (q), 14.4 (q), 16.1 (q), 31.8 (t), 60.6 (t), 62.1 (t), 80.5 (d), 92.0 (s), 151.5 (s), 155. 9 (s), 165.6 (s); IR (nujol): ν_{max} = 3465, 3276, 1764, 1712 cm^{-1}; MS *m/z* (%): 290 (M^+) (32), 272 (15), 247 (32), 227 (7), 216(45), 173 (90), 156 (35), 129 (65), 111 (100); anal. calcd. for $C_{11}H_{18}N_2O_5S$ (290.3371): C 45.50, H 6.25, N 9.65; found: C 45.36, H 6.22, N 9.79.



Dimethyl {4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-5-methyl-3,4-dihydro-2*H*-1,4-thiazin-6-yl}phosphonate 21k.

White foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.36 (s, 9H), 2.15 (s, 3H), 2.68 (brs, 1H), 3.60 (s, 3H), 3.63 (s, 3H), 4.25 (brs, 1H), 3.66-3.77 (m, 2H), 5.06 (s, 1H), 8.06 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 17.2 (q), 28.1 (q), 32.4 (t), 52.6 (q, $^{2}J_{CP}$ = 5.4 Hz), 52.8 (q, $^{2}J_{CP}$ = 5.1 Hz),81.0 (d), 81.4 (s), 100.5 (s, $^{1}J_{CP}$ = 130.8 Hz), 153.1 (s), 154.8 (s); IR (nujol): ν_{max} = 3478, 3281, 1762 cm^{-1}; MS *m/z* (%): 354 (M^{+}) (5), 281 (17), 253 (34), 245 (23), 144 (85),; anal. calcd. for $C_{12}H_{23}N_2O_6PS$ (354.3607): C 40.67, H 6.54, N 7.91; found: C 40.86, H 6.59, N 7.65.

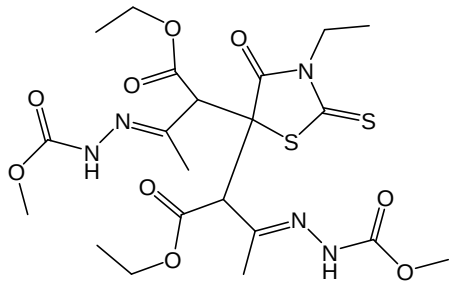
CHAPTER 3

General information

All chemicals and solvents were purchased from commercial suppliers and used as received. Rhodanines **22a–d** were obtained as follows, according to the procedure reported in literature:[43a] a mixture of thioglycolic acid (0.92 g, 10 mmol) and ethyl, phenyl, 4-chlorophenyl or 4-methoxyphenyl isothiocyanates (12 mmol) in methanol (8 ml) and water (100 ml) was heated in an oil bath for 4 h at 100 °C. 1,2-Diaza-1,3-dienes **1a–g** were prepared as reported[99] and used as *EE*/*EZ* isomers mixtures. Melting points were determined in open capillary tubes and are uncorrected. FTIR spectra were obtained as Nujol mulls. All ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Proton and carbon spectra were referenced internally to solvent signals, using values of δ = 2.50 ppm for proton (middle peak) and δ = 39.50 ppm for carbon (middle peak) in $DMSO_{d6}$ and δ = 7.27 ppm for proton and δ = 77.00 ppm for carbon (middle peak) in $CDCl_3$. All coupling constants (*J*) are given in Hz. All the NH exchanged with D_2O. Precoated aluminium oxide plates 0.25 mm were employed for analytical thin layer chromatography. All new compounds showed satisfactory elemental analysis. Mass spectra were recorded in the ESI mode. The nomenclature was generated using ACD/IUPAC Name (version 3.50, 5 Apr. 1998), Advanced Chemistry Development Inc., Toronto, ON (Canada).

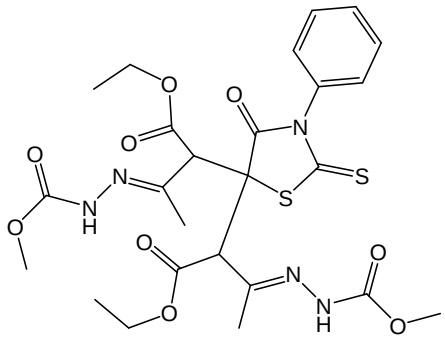
General procedure for synthesis of dialkyl 2,2'-((3-substituted-4-oxo-2-thioxothiazolidine-5,5-diyl)bis(4-alkoxy-4-oxobutan-3-yl-2-ylidene))bis(hydrazine-carboxylates) (24a–f).

To a magnetically stirred solution of 1,2-diaza-1,3-dienes **1a,c,d** (4.2 mmol) and 3-ethyl or 3-phenyl or 3-(4-chlorophenyl) or 3-(4-methoxyphenyl)-2-thioxothiazolidin-4-ones **22a–d** (2.0 mmol) in THF (10 mL) at room temperature, potassium carbonate (2.0 mmol) was added. After the disappearance of the reagents (0.1–0.5 h) (TLC monitoring), K_2CO_3 was filtered, the reaction solvent was evaporated under reduced pressure and the crude mixture was purified by column chromatography on silica gel (cyclohexane : ethyl acetate mixtures) to afford the corresponding bis-hydrazone-functionalized rhodanines **24a–f**, that were crystallized from diethyl ether-petroleum ether (b.p. 40–60 °C).



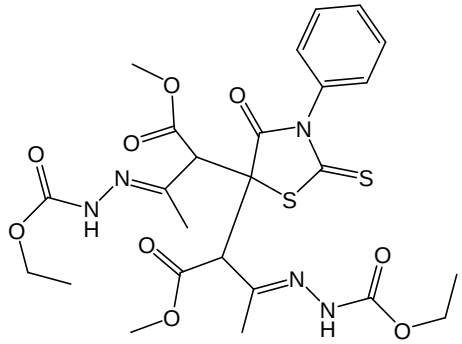
Dimethyl 2,2'-((3-ethyl-4-oxo-2-thioxothiazolidine-5,5-diyl)bis(4-ethoxy-4-oxobutan-3-yl-2-ylidene))bis(hydrazinecar-boxylate) (24a).

White powder (887.2 mg, 79% yield); mp: 141–143 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.13–1.23 (m, 9H, 2OCH$_2$*CH$_3$*, NCH$_2$*CH$_3$*), 1.87 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.02–4.12 (m, 7H, CH, 2O*CH$_2$*CH$_3$, N*CH$_2$*CH$_3$), 4.51 (brs, 1H, CH), 8.24 (brs, 2H, 2NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 11.0 (q), 13.8 (q), 13.9 (q), 14.8 (q), 17.2 (q), 39.6 (t), 52.8 (q), 58.2 (s), 58.7 (d),61.0 (d), 61.8 (t), 62.1 (t), 146.0 (s), 146.3 (s), 154.1 (s), 154.7 (s), 167.5 (s), 168.5 (s), 177.0 (s), 203.4 (s);); IR (nujol): ν_{max} = 3239, 1737, 1715, 1666 cm^{-1}; MS m/z (ESI): 562.70 (M + H$^+$); anal. calcd. for $C_{21}H_{31}N_5O_9S_2$ (561.6289): C 44.91, H 5.56, N 12.47; found: C 45.04, H 5.59, N 12.35.



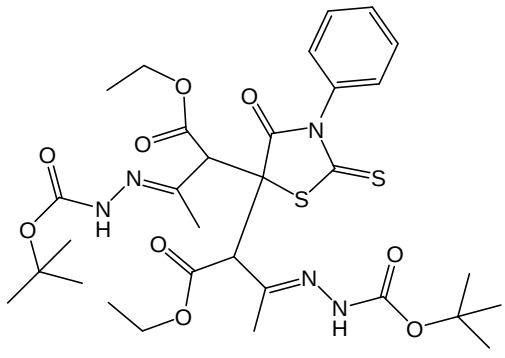
Dimethyl 2,2'-((4-oxo-3-phenyl-2-thioxothiazolidine-5,5-diyl)bis(4-ethoxy-4-oxobutan-3-yl-2-ylidene))bis(hydrazinecar-boxylate) (24b).

White powder (1.03 g, 85% yield); mp: 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.23–1.36 (m, 6H, 2OCH$_2$*CH$_3$*), 1.84 (s, 3H, *CH$_3$*), 2.08 (s, 3H, *CH$_3$*), 3.73 (brs, 3H, *CH$_3$*), 3.73 (s, 3H, OCH_3), 4.18 (s, 1H, CH), 4.19–4.30 (m, 4H, 2O*CH$_2$*CH$_3$), 4.67 (brs, 1H, CH), 7.43–7.55 (m, 5H, Ar), 7.98 (brs, 1H, NH), 8.17 (brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 14.4 (q), 14.6 (q), 17.5 (q), 52.7 (q), 52.8 (q), 53.0 (q), 53.1 (q), 58.1 (s), 58.9 (d), 59.0 (d), 62.1 (t), 128.2 (d), 128.3 (d), 129.3 (d), 136.0 (d), 145.4(s), 146.0 (s), 153.3 (s), 153.4 (s), 154.1 (s),168.4 (s), 169.4 (s), 171.1 (s), 204.0 (s); IR (nujol): ν_{max} = 3241, 3160, 1741, 1720, 1690 cm^{-1}; MS m/z (ESI): 608.72 (M - H$^+$); anal. calcd. for $C_{25}H_{31}N_5O_9S_2$ (609.6717): C 49.25, H 5.13, N 11.49; found: C 49.14, H 5.10, N 11.65.



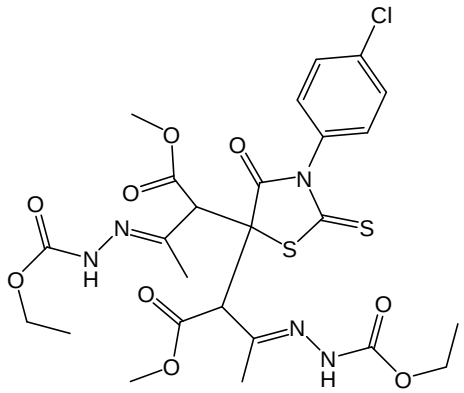
Diethyl 2,2'-((4-oxo-3-pheny-2-thioxothiazolidine-5,5-diyl)bis(4-methoxy-4-oxobutan-3-yl-2-ylidene))bis(hydrazinecarboxylate) (24c).

White powder (1.12 g, 92% yield); mp: 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.28–1.37 (m, 6H, 2OCH$_2$*CH$_3$*), 1.85 (s, 3H, *CH$_3$*), 2.08 (s, 3H, *CH$_3$*), 3.74 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.18 (s, 1H, CH), 4.19–4.31 (m, 4H, 2O*CH$_2$*CH$_3$), 4.69 (brs, 1H, CH), 7.44–7.56 (m, 5H, Ar), 7.80 (brs, 1H, NH), 7.95 (brs, H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 14.6 (q), 15.2 (q), 17.5 (q), 52.8 (q), 53.1 (q), 58.2 (s), 62.1 (t), 62.3 (d), 65.8 (d), 128.3 (d), 129.3 (d), 129.4 (d), 136.0 (s), 146.0(s), 153.1 (s), 153.9 (s), 168.4 (s), 169.4 (s), 171.2 (s), 204.0 (s); IR (nujol): ν_{max} = 3234, 3159, 3127, 1741, 1701 cm^{-1}; HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{25}H_{32}N_5O_9S_2$: 610.1641; found: 610.1641; MS m/z (ESI): 610.55 ($M + H^+$); anal. calcd. for $C_{25}H_{31}N_5O_9S_2$ (609.6717): C 49.25, H 5.13, N 11.49; found: C 49.12, H 5.11, N 11.62.



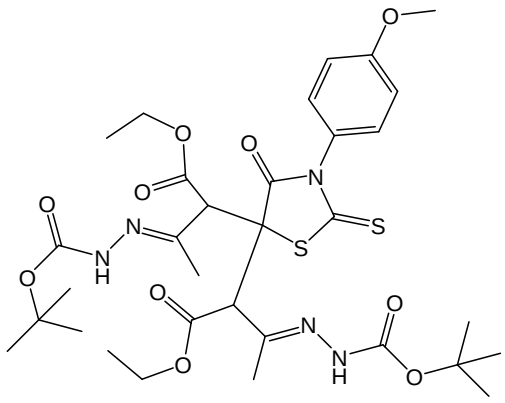
Di-*tert*-butyl 2,2'-((4-oxo-3-pheny-2-thioxothiazolidine-5,5-diyl)bis(4-ethoxy-4-oxobutan-3-yl-2-ylidene))bis(hydrazinecarboxylate) (24d).

White powder (1.29 g, 93% yield) mp: 138–141 °C ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.23–1.27 (m, 6H, 2OCH$_2$*CH$_3$*), 1.51 (s, 9H, (*CH$_3$*)$_3$), 1.56 (s, 9H, (*CH$_3$*)$_3$), 1.84 (s, 3H, *CH$_3$*), 2.09 (s, 3H, *CH$_3$*), 4.11 (s, 1H, CH), 4.17–4.23 (m, 4H, 2O*CH$_2$*CH$_3$), 4.67 (brs, 1H, CH), 7.45–7.56 (m, 5H, Ar), 7.65 (brs, 1H, NH), 7.77 (brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.9 (q), 14.0 (q), 14.5 (q), 17.6 (q), 28.2 (q), 28.4 (q), 58.2 (s), 59.1 (d), 59.2 (d), 61.9 (t), 62.1 (t), 62.2 (t), 81.5 (s), 81.7 (s), 128.4 (d), 129.3 (d), 136.2 (s), 145.3(s), 151.9 (s), 152.6 (s), 168.0 (s), 169.0 (s), 177.4 (s), 204.4 (s); IR (nujol): ν_{max} = 3328, 3225, 1724, 1746, 1700 cm^{-1}; MS m/z (ESI): 694.00 ($M + H^+$); anal. calcd. for $C_{31}H_{43}N_5O_9S_2$ (693.8312): C 53.66, H 6.25, N 10.09; found: C 53.78, H 6.29, N 10.02.



Diethyl 2,2'-((3-(4-chlorophenyl)-4-oxo-2-thioxothiazolidine-5,5-diyl)bis(4-methoxy-4-oxobutan-3-yl-2-ylidene))bis(hydra-zinecarboxlate) (24e).

White powder (1.13 g, 88% yield); mp: 182–184 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.29–1.37 (m, 6H, 2OCH$_2$*CH$_3$*), 1.83 (s, 3H, *CH$_3$*), 2.09 (s, 3H, *CH$_3$*), 3.73 (s, 3H, OCH$_3$), 3.74 (s, 3H, OCH$_3$), 4.17 (s, 1H, CH), 4.20–4.33 (m, 4H, 2O*CH$_2$*CH$_3$), 4.68 (s, 1H, CH), 7.40 (d, 2H, *J* = 8.4 Hz, Ar), 7.51 (d, 2H, *J* = 9.2 Hz, Ar), 7.84 (brs, 1H, NH), 7.98 (brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 14.4 (q), 14.5 (q), 15.2 (q), 17.6 (q), 52.9 (q), 53.2 (q), 58.2 (s), 59.0 (d), 62.1 (t), 65.8 (d), 129.7 (d), 129.8 (d), 134.5 (s), 135.4 (s), 145.8(s), 153.1 (s), 155.8 (s), 168.5 (s), 169.5 (s), 177.1 (s), 203.7 (s); IR (nujol): ν_{max} = 3332, 3209, 1718, 1752, 1698 cm^{-1}; MS m/z (ESI): 643.31 (M - H$^+$); anal. calcd. for $C_{25}H_{30}ClN_5O_9S_2$ (644.1168): C 46.62, H 4.69, N 10.87; found: C 46.75, H 4.71, N 10.73.



Di-*tert*-butyl 2,2'-((3-(4-methoxypheny)-4-oxo 2-thioxothiazolidine-5,5-diyl)bis(4-ethoxy-4-oxobutan-3-yl-2-ylidene))bis(hydrazinecar-boxylate) (24f).

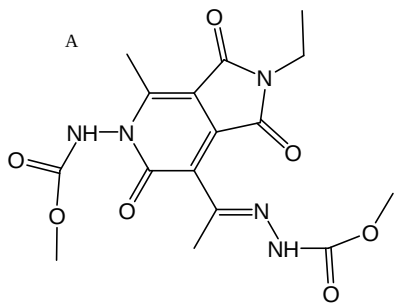
White powder (1.34 g, 93% yield) mp: 169–171 °C (dec.) ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.21–1.77 (m, 6H, 2OCH$_2$*CH$_3$*), 1.51 (s, 9H, (*CH$_3$*)$_3$), 1.55 (s, 9H, (*CH$_3$*)$_3$), 1.83 (s, 3H, *CH$_3$*), 2.08 (s, 3H, *CH$_3$*), 3.84 (s, 3H, O*CH$_3$*), 4.09 (s, 1H, CH), 4.16–4.23 (m, 4H, 2O*CH$_2$*CH$_3$), 4.66 (s, 1H, CH), 7.03 (d, 2H, *J* = 9.2 Hz, Ar), 7.37 (d, 2H, *J* = 8.4 Hz, Ar), 7.64 (brs, 1H, NH), 7.75 (brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.0 (q), 14.0 (q), 14.5 (q), 17.6 (q), 28.2 (q), 28.4 (q), 55.4 (q), 58.2 (s), 59.2 (d), 61.9 (t), 61.9 (t), 62.2 (t), 65.8 (d), 81.5 (s), 81.7 (s), 114.6 (d), 128.7 (s), 129.4 (d), 145.4 (s), 151.8 (s), 160.0 (s), 168.0 (s), 169.0 (s), 177.5 (s), 204.7 (s); IR (nujol): ν_{max} = 3246, 3150, 3109, 1754, 1693 cm^{-1}; MS m/z (ESI): 724.67 (M + H$^+$); anal. calcd. for $C_{32}H_{45}N_5O_{10}S_2$ (723.8572): C 53.10, H 6.27, N 9.68; found: C 53.01, H 6.26, N 9.78.

General procedure for synthesis of 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridine-1,3,6-triones (26a,c–e,j,l) starting from 24a–f (Path A).

To a magnetically stirred solution of bis-hydrazone-functionalized rhodanines **4a–f** (1.0 mmol) in THF (5 mL) heated at 55 °C in an oil bath, potassium carbonate (5.0 mmol) was added. The flask was kept open in order to remove CS_2 by evaporation (b.p. 49.3 °C). After the disappearance of the products **24** (2.0–5.0 h) (TLC monitoring), K_2CO_3 was filtered, the reaction solvent was evaporated under reduced pressure and the crude mixture was purified by column chromatography on silica gel (cyclohexane : ethyl acetate mixtures) to afford the corresponding 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-*c*]pyridine-1,3,6-triones **(26a,c–e,j,l)**, that were crystallized from diethyl ether-petroleum ether (b.p. 40–60 °C).

General procedure for synthesis of 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridine-1,3,6-triones (26a–l) in one-pot procedure (Path B). To a magnetically stirred solution of 1,2-diaza-1,3-dienes **1a–g** (2.1 mmol) and 3-ethyl or 3-phenyl or 3-(4-chlorophenyl) or 3-(4-methoxyphenyl)-2-thioxothiazolidin-4-ones **22a–d** (1.0 mmol) in THF (5 mL) heated at 55 °C in an oil bath, potassium carbonate (5.0 mmol) was added. The flask was kept open in order to remove CS_2 by evaporation. After the disappearance of the reagents (1.0–5.0 h) (TLC monitoring), K_2CO_3 was filtered, the reaction solvent was evaporated under reduced pressure and the crude mixture was purified by column chromatography on silica gel (cyclohexane : ethyl acetate mixtures) to afford the corresponding 2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridine-1,3,6-triones (**6a–l**), that were crystallized from diethyl ether-petroleum ether (b.p. 40–60 °C).

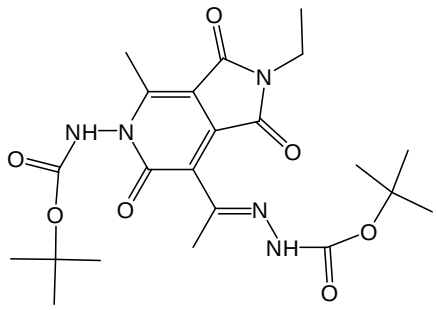
A



Methyl 2-(1-(2ethyl-5-((methoxycarbonyl)amino)-4-methyl-1,3,6-trioxo-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridin-7-yl)ethylidene)-hydrazinecarboxylate (26a).

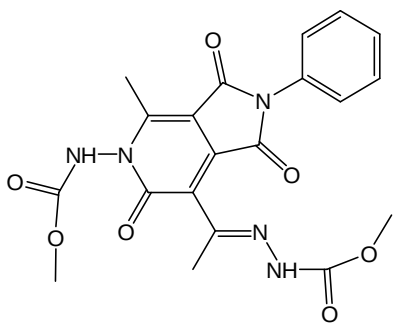
Pale yellow powder (149.7 mg, 38% yield, path A; 239.8, 61% yield, path B); mp: 235−237 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.22 (t, *J* = 7.2 Hz, 3H, $NCH_2\mathit{CH_3}$), 2.16 (s, 3H, $\mathit{CH_3}$), 2.79 (s, 3H, $\mathit{CH_3}$), 3.68 (q, *J* = 7.2 Hz, 2H, $N\mathit{CH_2}CH_3$), 3.83 (s, 6H, 2 $O\mathit{CH_3}$), 7.60 (brs, 1H, NH), 8.16 (brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.4 (q), 14.4 (q), 15.6 (q), 33.4 (t), 53.2 (q), 54.2 (q), 105.7 (s), 125.4 (s), 137.2 (s), 142.5 (s), 152.2 (s), 156.2 (s), 161.0 (s), 164.0 (s), 165.5 (s); IR (nujol): ν_{max} = 3331, 3239, 1753, 1721, 1672 cm^{-1}; HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{16}H_{20}N_5O_7$: 394.1363; found: 394.1363; MS *m/z* (ESI):

394.11 (M + H^+); anal. calcd. for $C_{16}H_{19}N_5O_7$ (393.1284): C 48.85, H 4.87, N 17.80; found: C 48.94, H 4.85, N 17.68.



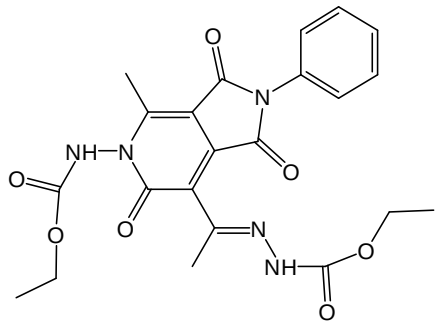
***Tert*-butyl 2-(1-(5-((*tert*-butoxycarbonyl)amino)-2ethyl-4-methyl-1,3,6-trioxo-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridin-7-yl)ethy-lidene)hydrazinecarboxylate (26b).**

Pale yellow powder (277.7, 58% yield, path B); mp: 218–220 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.22 (t, *J* = 7.2 Hz, 3H, NCH_2CH_3), 1.50 (s, 18H, 2 $C(CH_3)_3$), 2.13 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 3.67 (q, *J* = 7.2 Hz, 2H, NCH_2CH_3), 7.38 (brs, 1H, NH), 7.89 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.4 (q), 14.4 (q), 15.6 (q), 28.0 (q), 28.2 (q), 33.3 (t), 81.4 (s), 83.8 (s), 105.1 (s), 125.6 (s), 136.7 (s), 141.4 (s), 152.1 (s), 154.5 (s), 160.9 (s), 164.2 (s), 165.7 (s); IR (nujol): ν_{max} = 3358, 3190, 1735, 1718, 1678 cm^{-1}; MS *m/z* (ESI): 476.38 (M - H^+); anal. calcd. for $C_{22}H_{31}N_5O_7$ (477.5108): C 55.34, H 6.54, N 14.67; found: C 55.22, H 6.50, N 14.78.

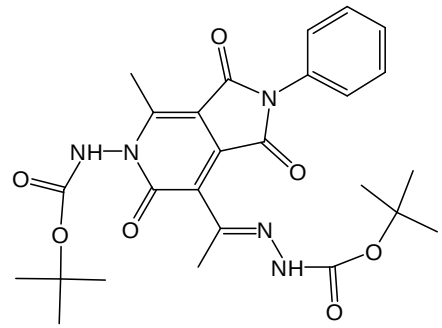


Methyl 2-(1-(5-((methoxycarbonyl)amino)-4-methyl-1,3,6-trioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridin-7-yl)ethyli-dene)hydrazinecarboxylate (26c)

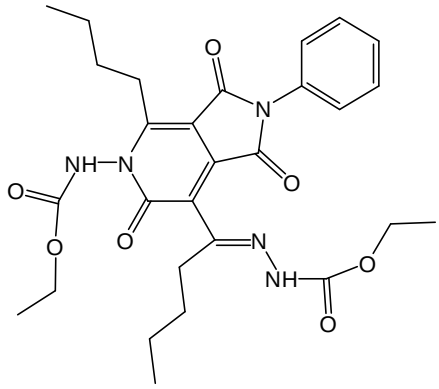
Pale yellow powder (209.3 mg, 37% yield, path A; 340.2, 60% yield, path B); mp: 169–172 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.16 (s, 3H, CH_3), 2.83 (s, 3H, CH_3), 3.82 and 3.85 (2s, 6H, 2 OCH_3), 7.34–7.37 (m, 2H, Ph), 7.39–7.43 (m, 1H, Ph), 7.47–7.51 (m, 2H, Ph), 7.80 (brs, 1H, NH), 8.20 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 14.6 (q), 15.5 (q), 15.6 (q), 53.3 (q), 54.1 (q), 54.2 (q), 105.1 (s), 125.8 (s), 126.5 (d), 126.6 (d), 129.1 (d), 131.2 (s), 136.7 (s), 142.5 (s), 153.5 (s), 156.2 (s), 161.0 (s), 163.3 (s), 164.7 (s); δ = IR (nujol): ν_{max} = 3352, 3195, 1738, 1722, 1681 cm^{-1}; MS *m/z* (ESI): 442.27 (M + H^+); anal. calcd. for $C_{20}H_{19}N_5O_7$ (441.3942): C 54.42, H 4.34, N 15.87; found: C 54.57, H 4.38, N 15.76.



Ethyl 2-(1-(5-((ethoxycarbonyl)amino)-4-methyl-1,3,6-trioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridin-7-yl)ethylide-ne)hydrazinecarboxylate (26d). Pale yellow powder (165.2 mg, 35% yield, path A; 258.6, 55% yield, path B); mp: 182–184 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.28–1.34 (m, 6H, 2 $OCH_2\mathit{CH_3}$), 2.16 (s, 3H, $\mathit{CH_3}$), 2.84 (s, 3H, $\mathit{CH_3}$), 4.25–4.33 (m, 4H, 2 $O\mathit{CH_2}CH_3$), 7.34–7.37 (m, 2H, Ph), 7.39–7.42 (m, 1H, Ph), 7.47–7.51 (m, 2H, Ph), 7.58 (brs, 1H, NH), 8.04 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.3 (q), 14.5 (q), 14.6 (q), 15.6 (q), 62.1 (t), 63.6 (t), 105.1 (s), 125.8 (s), 126.5 (d), 128.5 (d), 129.1 (d), 131.2 (s), 136.7 (s), 142.4 (s), 153.6 (s), 155.8 (s), 161.0 (s), 163.4 (s), 164.7 (s); IR (nujol): ν_{max} = 3343, 3239, 1722, 1675, 1631 cm^{-1}; MS *m/z* (ESI): 470.15 (M + H$^+$); anal. calcd. for $C_{22}H_{23}N_5O_7$ (469.4473): C 56.29, H 4.94, N 14.92; found: C 56.36, H 4.96, N 14.81.

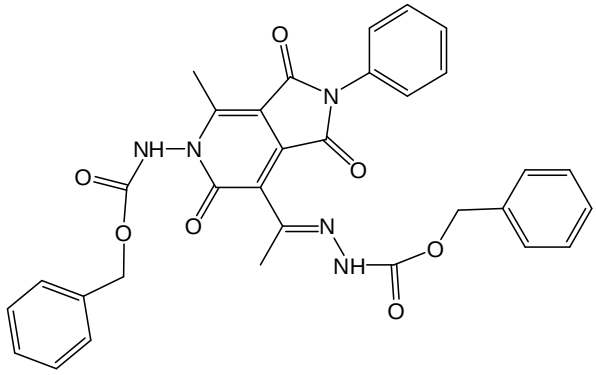


***Tert*-butyl 2-(1-(5-((*tert*-butoxycarbonyl)amino)-4-methyl-1,3,6-trioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridin-7-yl)ethylidene)hydrazinecarboxylate (26e).** Pale yellow powder (216.2 mg, 41% yield, path A; 346.1, 66% yield, path B); mp: 220–222 °C (dec.); ^{1}H NMR (400 MHz, $DMSO_{\mathit{d6}}$, 25 °C): δ = 1.45 and 1.48 (2s, 18H, 2 $C(\mathit{CH_3})_3$), 2.06 (s, 3H, $\mathit{CH_3}$), 2.66 (s, 3H, $\mathit{CH_3}$), 7.38–7.40 (m, 2H, Ph), 7.44–7.46 (m, 1H, Ph), 7.49–7.53 (m, 2H, Ph), 9.93 (brs, 1H, NH), 10.23 (s, 1H, NH); ^{13}C NMR (100 MHz, $DMSO_{\mathit{d6}}$, 25 °C): δ = 14.0 (q), 16.9 (q), 27.8 (q), 28.0 (q), 79.4 (s), 81.4 (s), 104.2 (s), 125.9 (s), 127.4 (d), 128.3 (d), 128.7 (d), 131.7 (s), 135.6 (s), 142.7 (s), 152.4 (s), 152.7 (s), 154.2 (s), 160.0 (s), 163.3 (s), 164.7; (s); IR (nujol): ν_{max} = 3314, 3253, 1725, 1712, 1677 cm^{-1}; HRMS (ESI) *m/z* [M+H]$^+$ calcd. for $C_{26}H_{32}N_5O_7$: 526.2302; found: 526.2302; MS *m/z* (ESI): 524.38 (M - H$^+$); anal. calcd. for $C_{26}H_{31}N_5O_7$ (525.5536): C 59.42, H 5.95, N 13.33; found: C 59.49, H 5.99, N 13.20.



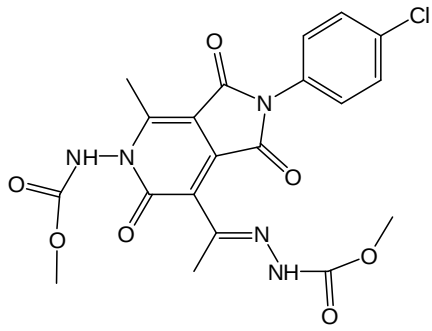
Ethyl 2-(1-(4-butyl-5-((etoxycarbonyl)amino)-1,3,6-trioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridin-7-yl)pentyli-dene)hydrazinecarboxylate (26f).

Pale yellow powder; (350.8, 63% yield, path B); mp: 194–196 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.85–0.98 (m, 8H, alkyl), 1.24–1.39 (m, 8H, alkyl), 1.56–1.74 (m, 4H, alkyl), 2.48–2.63 (m, 2H, alkyl), 2.71–3.09 (m, 2H, alkyl), 4.21–4.32 (m, 4H, 2 O*CH*$_2$CH$_3$), 7.08 (brs, 1H, NH), 7.37–7.41 (m, 3H, Ph), 7.47–7.50 (m, 2H, Ph), 8.06 and 8.27 (2brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (q), 13.7 (q), 14.3 (q), 14.5 (q), 22.7 (t), 23.0 (t), 27.0 (t), 27.9 (t), 29.7 (t), 30.7 (t), 63.6 (t), 104.7 (s), 126.4 (s), 126.5 (d), 128.5 (d), 129.1 (d), 131.2 (s), 137.5 (s), 139.3 (s), 156.0 (s), 157.6 (s), 161.5 (s), 163.4 (s), 164.5 (s), 165.3; IR (nujol): ν_{max} = 3348, 3192, 1729, 1721, 1680 cm^{-1}; MS *m/z* (ESI): 552.37 (M - H$^+$); anal. calcd. for $C_{28}H_{35}N_5O_7$ (553.6068): C 60.75, H 6.37, N 12.65; found: C 60.62, H 6.33, N 12.79.



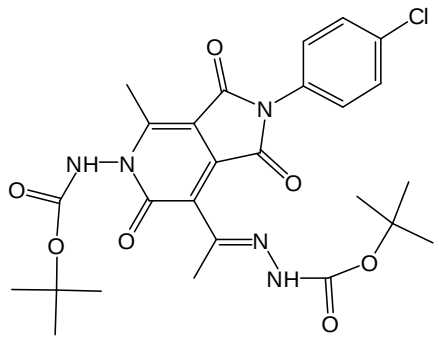
Benzyl 2-(1-(5-(((benzyloxy)carbonyl)amino)-4-methyl-1,3,6-trioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridin-7-yl) ethylidene)hydrazinecarboxylate (26g).

Pale yellow powder (253.8, 43% yield, path B); mp: 199–201 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.12 (s, 3H, *CH*$_3$), 2.80 (s, 3H, *CH*$_3$), 5.22 and 5.25 (2s, 4H, 2 O*CH*$_2$Ph), 7.34–7.51 (m, 15H, 3 Ph), 7.64 (s, 1H, NH), 8.12 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.6 (q), 14.8 (q), 27.8 (q), 67.8 (T), 69.1 (s), 105.1 (s), 126.4 (s), 126.5 (d), 128.3 (d), 128.5 (d), 128.6 (d), 128.6 (d), 128.7 (d), 128.8 (d), 129.1 (d), 129.2 (s), 131.2 (s), 134.7 (s), 136.8 (s), 142.5 (s), 153.5 (s), 153.5 (s), 155.5 (s), 160.9 (s), 163.3 (s), 164.6 (s); IR (nujol): ν_{max} = 3331, 3240, 1738, 1725, 1670; MS *m/z* (ESI): 592.12 (M + H$^+$); anal. calcd. for $C_{32}H_{27}N_5O_7$ (593.5861): C 64.75, H 4.58, N 11.80; found: C 64.66, H 4.54, N 11.88.



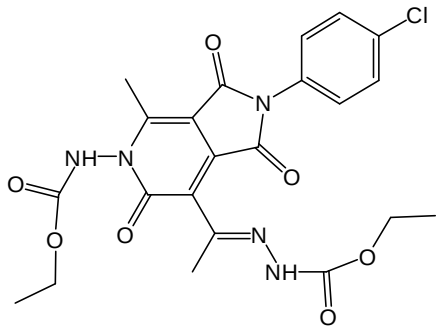
Methyl 2-(1-(2-(4-chlorophenyl)-5-((metoxycarbonyl)amino)-4-methyl-1,3,6-trioxo-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]py-ridin-7-yl)ethylidene)hydrazinecarboxylate (26h).

Pale yellow powder (220.2, 46% yield, path B); mp: 221–222 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.13 (s, 3H, *CH*$_3$), 2.80 (s, 3H, *CH*$_3$), 3.79 and 3.83 (2s, 6H, 2 O*CH*$_3$), 7.32 (d, *J* = 8.8 Hz, 2H, Ph), 7.45 (d, *J* = 8.8 Hz, 2H, Ph), 8.34 (s, 1H, NH), 8.61 (brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.6 (q), 14.7 (q), 15.5 (q), 15.6 (q), 53.1 (q), 53.3 (q), 54.1 (q), 54.3 (q), 104.9 (s), 126.0 (s), 127.6 (d), 129.3 (d), 129.6 (s), 134.4 (s), 136.5 (s), 142.2 (s), 153.8 (s), 156.2 (s), 160.9 (s), 163.0 (s), 164.4 (s); IR (nujol): ν_{max} = 3325, 3239, 1752, 1735, 1718, 1670 cm^{-1}; HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{20}H_{19}ClN_5O_7$: 476.0973; found: 476.0973; MS *m/z* (ESI): 476.10 ($M + H^+$); anal. calcd. for $C_{20}H_{18}ClN_5O_7$ (475.8392): C 50.48, H 3.81, N 14.72; found: C 50.59, H 3.82, N 14.65.



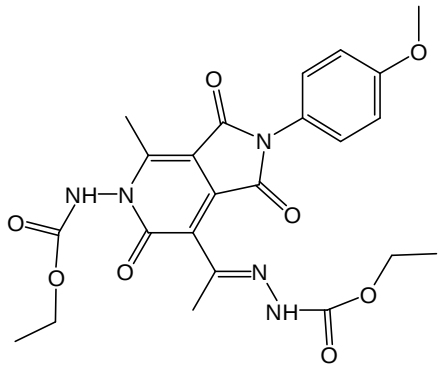
***Tert*-butyl 2-(1-(5-((*tert*-butoxycarbonyl)amino)-2-(4-chlorophenyl)-4-methyl-1,3,6-trioxo-2,3,5,6-tetrahydro1*H*-pyr-rolo[3,4-c]pyridin-7-yl)ethylidene)hydrazinecarboxylate (26i).**

Pale yellow solid; (284.8, 51% yield, path B); mp: 171–173 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.49 (s, 18H, 2 C(*CH*$_3$)$_3$), 2.13 (s, 3H, *CH*$_3$), 2.82 (s, 3H, *CH*$_3$), 7.33 (d, *J* = 8.8 Hz, 2H, Ph), 7.45 (d, *J* = 8.8 Hz, 2H, Ph), 7.48 (brs, 1H, NH), 7.86 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 14.7 (q), 15.5 (q), 15.7 (q), 27.9 (q), 28.0 (q), 28.2 (q), 28.3 (q), 81.5 (s), 83.9 (s), 104.4 (s), 126.1 (s), 127.8 (d), 127.8 (d), 129.2 (d), 129.3 (d), 129.8 (d), 134.2 (s), 136.1 (s), 141.7 (s), 152.1 (s), 153.7 (s), 154.4 (s), 160.9 (s), 163.2 (s), 164.6 (s); IR (nujol): ν_{max} = 3328, 3205, 1732, 1718, 1685 cm^{-1}; MS *m/z* (ESI): 558.30 ($M - H^+$); anal. calcd. for $C_{26}H_{30}ClN_5O_7$ (559.9987): C 55.76, H 5.40, N 12.51; found: C 55.84, H 5.44, N 12.53.



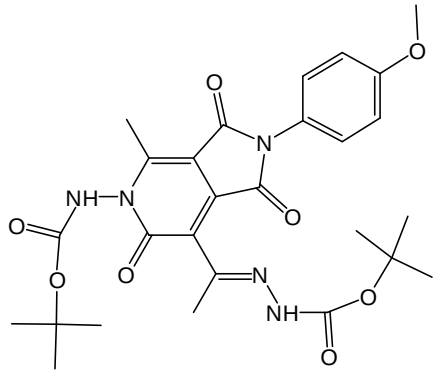
Ethyl 2-(1-(2-(4-chlorophenyl)-5-((ethoxycarbonyl)amino-4-methyl-1,3,6-trioxo-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridin-7-yl)ethylidene)hydrazinecarboxylate (26j).

Pale yellow powder (194.8 mg, 39% yield, path A; 293.7, 58% yield, path B); mp: 177–180 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.23–1.29 (m, 6H, 2 $OCH_2\mathit{CH_3}$), 2.13 (s, 3H, $\mathit{CH_3}$), 2.80 (s, 3H, $\mathit{CH_3}$), 4.12–4.33 (m, 4H, 2 $O\mathit{CH_2}CH_3$), 7.32 (d, *J* = 8.0 Hz, 2H, Ph), 7.44 (d, *J* = 8.0 Hz, 2H, Ph), 7.93, 8.03, 8.16 and 8.33 (4brs, 2H, 2NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14. 1(q), 14.3 (q), 14.5 (q), 14.7 (q), 60.4 (t), 63.5 (t), 104.8 (s), 126.0 (s), 127.8 (d), 129.3 (d), 129.8 (s), 134.3 (s), 136.4 (s), 142.6 (s), 153.9 (s), 155.8 (s), 158.8 (s), 161.0 (s), 163.2 (s), 164.5 (s); IR (nujol): ν_{max} = 3325, 3200, 1731, 1714, 1680 cm^{-1}; MS m/z (ESI): 502.76 (M - H^{+}); anal. calcd. for $C_{22}H_{22}ClN_5O_7$ (503.8924): C 52.44, H 4.40, N 13.90; found: C 52.51, H 4.38, N 14.01.



Ethyl 2-(1-(5-((ethoxycarbonyl)amino)-2-(4-methoxyphenyl)-4-methyl-1,3,6-trioxo-2,3,5,6-tetrahydro1*H*-pyrrolo[3,4-*c*]pyridin-7-yl)ethylidene)hydrazinecarboxylate (26k).

Pale yellow oil; (295.4, 59% yield, path B); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.26 (q, 6H, *J* = 7.2 Hz, 2 $OCH_2\mathit{CH_3}$), 2.12 (s, 3H, $\mathit{CH_3}$), 2.78 (s, 3H, $\mathit{CH_3}$), 3.81 (s, 3H, $O\mathit{CH_3}$), 4.22-4.28 (m, 4H, 2 $O\mathit{CH_2}CH_3$), 6.97 (d, *J* = 8.8 Hz, 2H, Ph), 7.25 (d, *J* = 8.8 Hz, 2H, Ph), 8.14 (brs, 1H, NH), 8.30 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14. 2(q), 14.4 (q), 14.4 (q), 15.6 (q), 60.4 (t), 55.4 (q), 60.3 (t), 63.3 (t), 105.0 (s), 114.4 (d), 123.8 (s), 125.8 (s), 127.8 (d), 136.6 (s), 142.7 (s), 153.4 (s), 155.7 (s), 159.4 (s), 161.0 (s), 163.6 (s), 165.0 (s); IR (nujol): ν_{max} = 3305, 3148, 1737, 1720, 1701 cm^{-1}; MS *m/z* (ESI): 498.44 (M - H^{+}); anal. calcd. for $C_{23}H_{25}N_5O_8$ (499.4733): C 55.31, H 5.05, N 14.02; found: C 55.22, H 5.03, N 14.11.

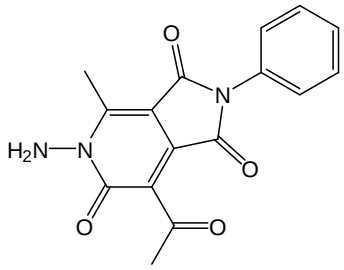


***Tert*-butyl 2-(1-(5-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)-4-methyl-1,3,6-trioxo-2,3,5,6-tetrahydro1*H*-pyrrolo[3,4-*c*]pyridin-7-yl)ethylidene)hydrazinecarboxylate (26l).**

Pale yellow solid; (338.9 mg, 61% yield, path A; 355.1, 64% yield, path B); mp: 171−173 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.47 (s, 18H, 2 C(*CH*$_3$)$_3$), 2.12 (s, 3H, *CH*$_3$), 2.78 (s, 3H, *CH*$_3$), 3.81 (s, 3H, O*CH*$_3$), 6.97 (d, *J* = 9.2 Hz, 2H, Ph), 7.26 (d, *J* = 9.2 Hz, 2H, Ph), 7.73 (brs, 1H, NH), 7.96 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 15.7 (q), 27.9 (q), 28.2 (q), 55.4 (q), 81.3 (s), 83.4 (s), 104.6 (s), 114.3(d), 123.9 (s), 125.7 (s), 127.8 (d), 136.4 (s), 141.8 (s), 152.2 (s), 153.3 (s), 154.5 (s), 159.4 (s), 161.0 (s), 163.8 (s), 165.2 (s); IR (nujol): ν_{max} = 3302, 3150, 2982, 1739, 1719, 1709 cm^{-1}; HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{27}H_{33}N_5O_8$: 556.2407; found: 556.2407; MS *m/z* (ESI): 554.44 (M - H^+); anal. calcd. for $C_{27}H_{33}N_5O_8$ (555.5796): C 58.37, H 5.99, N 12.61; found: C 58.50, H 6.01, N 12.52.

General procedure for synthesis of 7-acetyl-5-amino-4-methyl-2-phenyl-1H-pyrrolo[3,4-c]pyridine-1,3,6(2*H*,5*H*)-trione 27a starting from 26e.

To a magnetically stirred solution of *tert*-butyl 2-(1-(5-((*tert*-butoxycarbonyl)amino)-4-methyl-1,3,6-trioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*-pyrrolo[3,4-c]pyridin-7-yl)ethylidene)hydrazine-carboxylate **26e** (1.0 mmol) in acetone/water (4.5:0.5 mL) at room temperature, Amberlyst 15H (500 mg) was added. After the disappearance of the product **26e** (3.0 h) (TLC monitoring), Amberlyst 15H was filtered, the reaction solvent was evaporated under reduced pressure and the crude mixture was purified by column chromatography on silica gel (cyclohexane : ethyl acetate mixtures) to afford the corresponding 7-acetyl-5-amino-4-methyl-2-phenyl-1*H*-pyrrolo[3,4-c]pyridine-1,3,6(2*H*,5*H*)-trione **27a**, that was crystallized from diethyl ether-petroleum ether (b.p. 40-60 °C).



7-Acetyl-5-amino-4-methyl-2-phenyl-1*H*-pyrrolo[3,4-c]pyridine-1,3,6-(2*H*,5*H*)-trione (27a).

White powder (257.8, 83% yield); mp: 171−173 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.62 (s, 3H, *CH*$_3$), 2.96 (s, 3H, *CH*$_3$), 5.05 (brs, 2H, *NH*$_2$), 7.36-7.51 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 15. 1 (q), 31.0 (q), 104.2 (s), 126.4 (d), 128.6 (d), 129.1 (d), 131.7 (s), 131.1 (s), 134.0 (s), 151.4 (s), 159.2 (s), 163.5 (s), 164.9 (s), 197.5 (s); IR (nujol): ν_{max} = 3300, 3201, 1720, 1685, 1615 cm^{-1}; MS m/z (ESI): 312.14 (M + H$^+$); anal. calcd. for $C_{16}H_{13}N_3O_4$ (311.2921): C 61.73, H 4.21, N 13.50; found: C 61.85, H 4.25, N 13.39.

CHAPTER 4

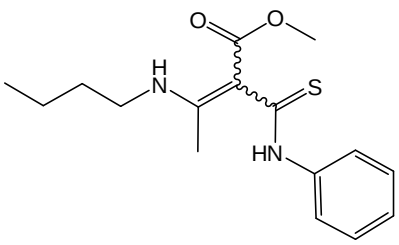
General experimental details.

All the commercially available reagents and solvents were used without further purification. 1,2-Diaza-1,3-dienes **1a–g** were synthesized as a mixture of *E/Z* isomers as previously reported.[99] Chromatographic purification of compounds was carried out on silica gel (60–200 μm). TLC analysis was performed on pre-loaded (0.25 mm) glass supported silica gel plates (Kieselgel 60); compounds were visualized by exposure to UV light and by dipping the plates in 1% $Ce(SO_4)\cdot 4H_2O$, 2.5% $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 10% sulphuric acid followed by heating on a hot plate. All ^1H NMR and ^{13}C NMR spectra were recorded at 400 and 100.56 MHz, respectively. Proton and carbon spectra were referenced internally to solvent signals, using values of δ = 2.49 ppm for proton (middle peak) and δ = 39.50 ppm for carbon (middle peak) in DMSO-d_6 and δ = 7.27 ppm for proton and δ = 77.00 ppm for carbon (middle peak) in $CDCl_3$. The following abbreviations are used to describe peak patterns where appropriate: s = singlet, d = doublet, t = triplet q = quartet, sep = septet, m = multiplet and brs = broad signal. All coupling constants (*J*) are given in Hz. FT-IR spectra were obtained as Nujol mulls. Mass spectra were recorded in the EI mode (70eV). Melting points were determined in open capillary tubes and are uncorrected.

Experimental procedures and spectral data.

General procedure for synthesis of 3-alkylamino-2-(carbamothioyl)but-2-enoates (ACTs) 28a-n.

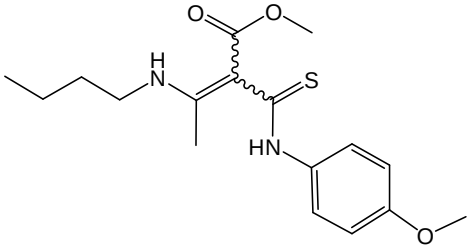
Alkyl amines **1a,b** (0.5 mmol) were added to β-ketoesters **30a-i** (0.55 mmol) or to ethyl phenylpropiolate **33a** (0.55mmol) under solvent-free conditions at room temperature and vigorously stirred. After 0.5 h aryl isothiocyanates **31a-c** (0.5mmol) in MeOH (1.5 mL) were added and the reactions were stirred until the disappearance of the enamino esters **32** (6.0-18.0 h monitored by TLC). The compounds **28a-c,e-i,l** crystallized directly from the reaction medium and were collected as pure products by filtration. In the other cases, the reaction solvent was evaporated under reduced pressure and the final ACTs **28d,j,k,m,n** were purified by chromatography on silica gel column (elution mixture: cyclohexane: ethyl acetate).



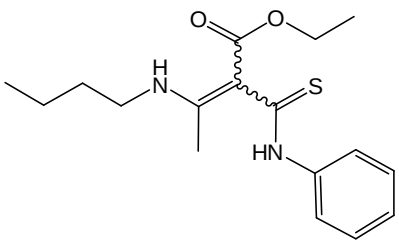
Methyl 3-(butylamino)-2-(phenylcarbamothioyl)but-2-enoate 28a.

28a was isolated by precipitation in methanol in 72% yield. Light yellow solid; mp: 109-110 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.93 (t, 3H, *J*=7,6 Hz, *n*-But), 1.33-1.64 (m, 4H, *n*-But), 2.18 and 2.21 (2s, 3H, *CH*$_3$), 3.20, 3.30 (2q, 2H, *J*=6,0 Hz, *J*=6,8 Hz, *n*-But), 3.40, 3.71 (2s, 3H, O*CH*$_3$), 7.02-7.60 (m, 5H, Ph), 9.54, 9.59, 10.40, 11.69 (4brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (q), 17.0 (t), 19.0 (t), 19.8 (t), 20.1 (t), 31.2 (q), 31.8 (q), 43.0 (t), 43.6 (t), 50.3 (q), 51.00 (q), 97.5 (s), 100.3 (s), 122.4 (d), 124.6 (d), 126.1 (d), 128.5 (d), 128.9 (d), 139.2 (s), 139.3 (s), 161.7 (s), 165.3 (s), 166.5 (s), 169.6 (s), 192.2 (s), 192.3 (s), 201.5 (s), 201.5 (s); IR (nujol): ν_{max} = 3277, 3246, 1629, 1594 cm^{-1}; MS *m/z* (ESI): 307.13 (M + H$^+$); anal. calcd. for $C_{16}H_{22}N_2O_2S$ (306.4231): C 62.71, H 7.24, N 9.14; found: C 62.62, H 7.21, N 9.21.

Methyl 3-(butylamino)-2-((4-methoxyphenyl)carbamothioyl)but-2-enoate 28b.

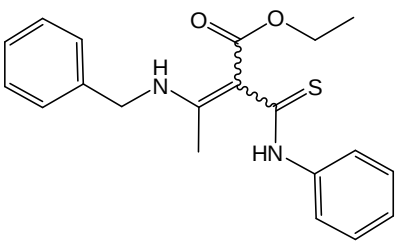


28b was isolated by precipitation in methanol in 49% yield. Light yellow solid; mp: 107-109 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.89-0.95 (m, 3H, *n*-But), 1.33-1.65 (m, 4H, *n*-But), 2.16, 2.21 (2s, 3H, *CH*$_3$), 3.16-3.22, 3.36 (m and q, 2H, *J*=5,2 Hz, *n*-But), 3.46, 3.60, 3.72 (3s, 3H, O*CH*$_3$), 3.76, 3.79, 3.80 (3s, 3H, O*CH*$_3$), 6.78-7.46 (m, 5H, Ph), 9.46, 9.70, 10.26, 11.67 (4brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (q), 16.9 (q), 18.9 (t), 19.8 (q), 20.1 (q), 31.3 (t), 31.8 (t), 32.3 (t), 42.6 (t), 43.0 (t), 43.5 (t), 49.7 (q), 50.4 (q), 51.0 (q), 55.3 (q), 55.4 (q), 81.2 (s), 97.2 (s), 100.1 (s), 113.8 (d), 114.0 (d), 114.7 (d), 124.3 (s), 126.4 (d), 126.8 (d), 132.4 (s), 157.7 (s), 161.3 (s), 165.1 (s), 166.5 (s), 169.6 (s), 192.6 (s), 201.6 (s); IR (nujol): ν_{max} = 3257, 3252, 1631, 1597 cm^{-1}; MS *m/z* (ESI): 337.21 (M + H$^+$); anal. calcd. for $C_{17}H_{24}N_2O_3S$ (336.4491): C 60.69, H 7.19, N 8.33; found: C 60.79, H 7.18, N 8.48.



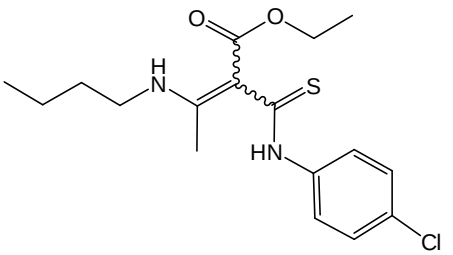
Ethyl 3-(butylamino)-2-(phenylcarbamothioyl)but-2-enoate 28c.

28c was isolated by precipitation in methanol in 58% yield. Light yellow solid; mp: 107-109 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.95 (t, 3H, *J*=7,6 Hz, *n*-But), 1.30 (t, 3H, *J*=7,2 Hz, $OCH_2\mathit{CH_3}$), 1.37-1.69 (m, 4H, *n*-But), 2.23 and 2.25 (2s, 3H, $\mathit{CH_3}$), 3.24, 3.33 (2q, 2H, *J*=6,0 Hz, *J*=6,8 Hz, *n*-But), 3.87, 4.21 (2q, 2H, *J*=6,8 Hz, *J*=7,2 Hz, $O\mathit{CH_2}CH_3$), 7.03-7.56 (m, 5H, Ph), 9.65, 9.70, 10.58, 12.07 (4brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (q), 14.2 (q), 17.2 (t), 19.4 (t), 19.9 (t), 20.1 (t), 31.2 (q), 31.9 (q), 43.1 (t), 43.7 (t), 59.1 (t), 60.00 (t), 97.8 (s), 100.3 (s), 122.0 (d), 125.0 (d), 125.7 (d), 126.1 (d), 128.6 (d), 129.0 (d), 139.4 (s), 161.9 (s), 165.8 (s), 166.1 (s), 169.5 (s), 191.7 (s), 191.7 (s), 191.8 (s), 201.4 (s); IR (nujol): ν_{max} = 3232, 3142, 1653, 1634 cm^{-1}; MS *m/z* (ESI): 321.18 (M + H$^+$); anal. calcd. for $C_{17}H_{24}N_2O_2S$ (320.4497): C 63.72, H 7.55, N 8.74; found: C 63.84, H 7.57, N 8.68.



Ethyl 3-(benzylamino)-2-(phenylcarbamothioyl)but-2-enoate 28d.

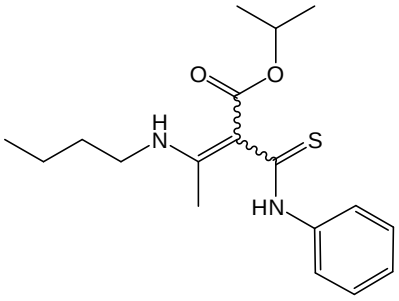
28d was isolated by precipitation in methanol in 35% yield. Light yellow solid; mp: 97–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.09 and 1.25-1.32 (t and m, 3H, *J*=7,2 Hz, $OCH_2\mathit{CH_3}$), 1.93, 2.19 and 2.26 (3s, 3H, $\mathit{CH_3}$), 3.94, 4.11and 4.22 (3q, 2H, *J*=7,2 Hz, *J*=7,2 Hz, *J*=7,2 Hz, $O\mathit{CH_2}CH_3$), 7.04-7.42 (m, 9H,2Ph), 7.59 (d, 1H, *J*=8,0 Hz, Ph), 8.97, 9.55, 9.95, 10.33, 12.14 (5brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.3 (q), 14.6 (q), 17.2 (q), 19.2 (q), 19.4 (q), 46.8 (t), 47.0 (t), 47.5 (t), 58.4 (t), 59.4 (t), 60.2 (t), 83.2 (s), 99.0 (s), 101.6 (s), 122.4 (d), 124.8 (d), 125.7 (d), 126.3 (d), 126.6 (d), 126.7 (d), 127.1 (d), 127.3 (d), 127.7 (d), 128.7 (d), 128.7 (d), 128.8 (d), 128.9 (d), 128.9 (d), 129.1 (d), 129.5 (d), 136.8 (s), 138.7 (s), 139.4 (s), 161.4 (s), 161.8 (s), 165.2 (s), 166.2 (s), 169.2 (s), 170.6 (s), 192.7 (s), 191.7 (s), 201.7 (s); IR (nujol): ν_{max} = 3130, 3088, 1648, 1589 cm^{-1}; MS *m/z* (ESI): 355.99 (M + H$^+$); anal. calcd. for $C_{20}H_{22}N_2O_2S$ (354.1402): C 67.77, H 6.26, N 7.90; found: C 67.63, H 6.22, N 7.96.



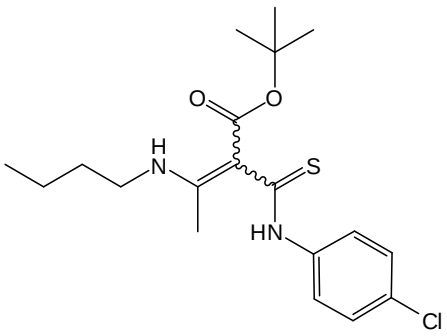
Ethyl 3-(butylamino)-2-((4-chlorophenyl)carbamothioyl)but-2-enoate 28e.

28e was isolated by precipitation in methanol in 51% yield. Light yellow solid; mp: 89–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.96 (t, 3H, *J*=7,2 Hz, *n*-But), 1.31 (t, 3H, *J*=7,2 Hz, $OCH_2\mathit{CH_3}$),1.43-1.70 (m, 4H, *n*-But), 2.26 (s, 3H, $\mathit{CH_3}$), 3.36 (q, 2H, *J*=5,6 Hz, *n*-But), 4.22 (q, 2H, $O\mathit{CH_2}CH_3$), 7.32 (d, 2H, *J*=8.4 Hz, Ph), 7.49 (d, 2H, *J*=8.4 Hz, Ph), 10.86, (brs, 1H, *NH*), 12.49 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (q), 13.7 (q), 14.2 (q), 14.6 (q), 19.3 (t), 20.0 (t), 20.2 (t), 31.2 (q), 32.4 (q), 42.7 (t), 43.8 (t), 58.2 (t), 60.2 (t), 81.6 (s), 99.9 (s), 126.5 (d), 126.9 (d), 128.7 (d), 130.0 (d), 131.2 (s), 132.9 (s), 138.0 (s), 161.9 (s), 166.7 (s), 169.9 (s), 170.6 (s), 195.9 (s), 206.9 (s); IR (nujol): ν_{max} = 3257, 3187, 1632, 1602 cm^{-1}; MS *m/z* (ESI): 355.03 ($M + H^+$); anal. calcd. for $C_{17}H_{23}ClN_2O_2S$ (354.8947): C 57.53, H 6.53, N 7.89; found: C 57.62, H 6.52, N 7.83.

Isopropyl 3-(butylamino)-2-(phenylcarbamothioyl)but-2-enoate 28f.

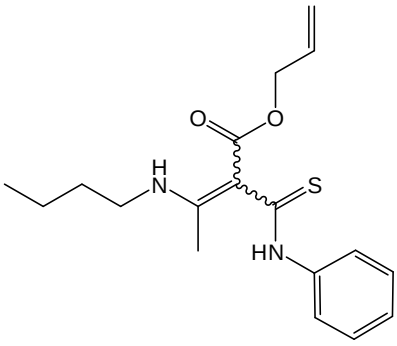


28f was isolated 1by precipitation in methanol in 55% yield. Light yellow solid; mp: 97–99 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.75, 1.12, 1.28 (3d, 6H, *J*=5,2 Hz, *J*=6,0 Hz, *J*=6,4 Hz, $O\mathit{CH(CH_3)_2}$), 0.94 (t, 3H, *J*=7,2 Hz, *n*-But), 1.37-1.69 (m, 4H, *n*-But), 2.22 and 2.25 (2s, 3H, $\mathit{CH_3}$), 3.23, 3.33 (2q, 2H, *J*=6,0 Hz, *J*=6,8 Hz, *n*-But), 4.75 and 5.09 (2ep, 2H, *J*=6,4 Hz, *J*=6,4 Hz, $O\mathit{CH(CH_3)_2}$), 7.03-7.54 (m, 5H, Ph), 9.61, 9.69, 10.70, 12.27 (4brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (q), 19.7 (t), 20.2 (t), 21.9 (q), 31.2 (q), 31.9 (q), 43.1 (t), 43.7 (t), 66.4 (d), 67.6 (d), 98.1 (s), 100.4 (s), 121.6 (d), 125.1 (d), 125.5 (d), 126.0 (d), 128.5 (d), 129.0 (d), 139.5 (s), 161.7 (s), 165.5 (s), 165.9 (s), 169.2 (s), 191.2 (s), 201.4 (s); IR (nujol): ν_{max} = 3255, 3167, 1622, 1612 cm^{-1}; MS *m/z* (ESI): 344.23 ($M + H^+$); anal. calcd. for $C_{18}H_{26}N_2O_2S$ (343.4762): C 64.64, H 7.84, N 8.38; found: C 64.73, H 7.86, N 8.32.



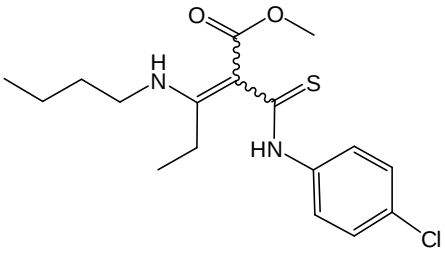
***Tert*-butyl 3-(butylamino)-2-((4-chlorophenyl)carbamothioyl)but-2-enoate 28g.**

28g was isolated by precipitation in methanol in 75% yield. Light yellow solid; mp: 92–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.94 (t, 3H, *J*=7,2 Hz, *n*-But), 1.42-1.70 (m, 4H, *n*-But), 1.51 (s, 9H, C(*CH*$_3$)$_3$), 2.24 (s, 3H, *CH*$_3$), 3.33 (q, 2H, *J*=6,8 Hz, *n*-But), 7.30 (d, 2H, *J*=8.4 Hz, Ph), 7.45 (d, 2H, *J*=8.4 Hz, Ph), 9.47, 9.61, 10.97, (3brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (q), 20.2 (t), 28.4 (q), 31.2 (t), 43.8 (t), 81.0 (s), 101.2 (s), 124.4 (s), 124.5 (s), 126.6 (d), 128.6 (d), 131.0 (s), 132.9 (s), 138.1 (s), 166.3 (s), 169.6 (s), 190.0 (s), (s); IR (nujol): ν_{max} = 3257, 3191, 1624, 1615 cm^{-1}; MS *m/z* (ESI): 383.59 (M + H$^+$); anal. calcd. for $C_{19}H_{27}ClN_2O_2S$ (382.9479): C 59.59, H 7.11, N 7.32; found: C 59.67, H 7.08, N 7.37.



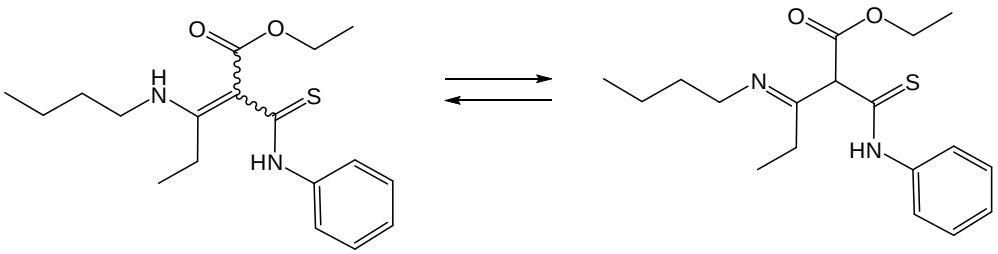
Allyl 3-(butylamino)-2-(phenylcarbamothioyl)but-2-enoate 28h.

28h was isolated by precipitation in methanol in 40% yield. Light yellow solid; mp: 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.95 (t, 3H, *J*=7,6 Hz, *n*-But), 1.37-1.68 (m, 4H, *n*-But), 2.22 and 2.26 (2s, 3H, *CH*$_3$), 3.18-3.26, 3.33 (m, q, 2H, *J*=6,0 Hz, *n*-But), 4.28-4.67 (m, 2H, O*CH*$_2$CH=CH$_2$), 5.06-5.37 (m, 2H, OCH$_2$CH=*CH*$_2$), 5.62-6.00 (m, 1H, OCH$_2$*CH*=CH$_2$), 7.02-7.58 (m, 5H, Ph), 8.54, 9.58, 10.42, 11.88 (4brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (t), 13.7 (q), 19.3 (t), 19.4 (t), 19.9 (t), 20.2 (t), 31.3 (q), 32.4 (q), 42.7 (t), 43.7 (t), 63.1 (t), 64.7 (d), 81.3 (s), 97.4 (s), 100.2 (s), 116.8 (t), 118.1 (t), 122.3 (d), 124.9 (d), 125.7 (d), 126.2 (d), 127.2 (d), 128.6 (d), 129.0 (d), 129.5 (d), 132.4 (s), 133.6 (s), 139.4 (s), 162.0 (s), 162.3 (s), 165.7 (s), 165.7 (s), 168.8 (s), 170.1 (s), 192.2 (s), 192.3 (s),201.5 (s); IR (nujol): ν_{max} = 3240, 3141, 1654, 1626 cm^{-1}; MS *m/z* (ESI): 333.12 (M + H$^+$); anal. calcd. for $C_{18}H_{24}N_2O_2S$ (332.4604): C 65, H 7.28, N 8.43; found: C 64.86, H 7.24, N 8.52.



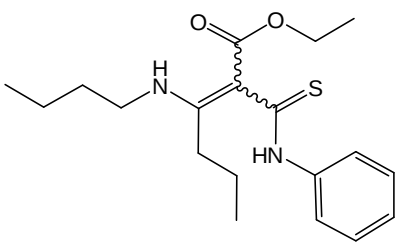
Methyl 3-(butylamino)-2-((4-chlorophenyl)carbamothioyl)pent-2-enoate 28i.

28i was isolated by precipitation in methanol in 50% yield. Light yellow solid; mp: 102–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.95 (t, 3H, *J*=7,2 Hz, *n*-But), 1.27 (t, 3H, *J*=7,2 Hz, $CH_2\mathit{CH_3}$),1.47 (sex, 2H, *J*=7,6 Hz, *n*-But),1.63-1.70 (m, 2H, *n*-But), 2.55 (q, 2H, *J*=7,2 Hz, $\mathit{CH_2}CH_3$), 3.37 (q, 2H, *J*=6,4 Hz, *n*-But), 3.74 (s, 3H, $O\mathit{CH_3}$), 7.33 (d, 2H, *J*=8.4 Hz, Ph), 7.53 (d, 2H, *J*=8.4 Hz, Ph), 9.58, 9.63, 10.48, 11.90 (4brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.6 (q), 13.6 (q), 20.0 (t), 20.1 (t), 25.2 (t), 30.8 (q), 31.6 (q), 43.2 (t), 51.1 (q), 51.2 (q), 99.8 (s), 123.4 (d), 126.1 (d), 126.3 (d), 128.6 (d), 130.0 (s), 131.2 (s), 137.9 (s), 166.6 (s), 169.7 (s), 170.0 (s), 180.5 (s), 199.2 (s), 199.2 (s), 201.5 (s); IR (nujol): ν_{max} = 3289, 3267, 1654, 1632 cm^{-1}; MS *m/z* (ESI): 355.58 (M + H$^+$); anal. calcd. for $C_{17}H_{23}ClN_2O_2S$ (354.8947): C 57.53, H 6.53, N 7.89; found: C 57.39, H 6.49, N 8.01.



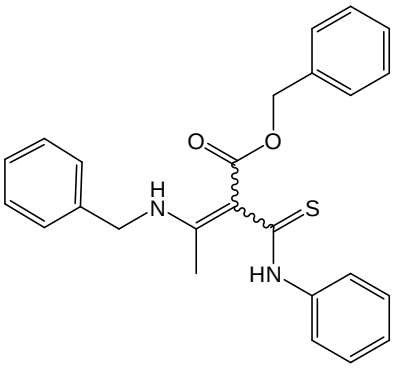
Ethyl 3-(butylamino)-2-(phenylcarbamothioyl)pent-2-enoate 28j (Ethyl 3-(butylimino)-2-(phenylcarbamothioyl)pentanoate 28j').

28j is in tautomeric equilibrium between the enamino **j** (51%) and the imino **j'** forms. **g** was isolated by precipitation in methanol in 76% yield. Light yellow solid; mp: 94–95 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.92-0.98 (m, 3.3 H), 1.14 (t, 1.4 H, *J*=7,6 Hz), 1.24-1.33 (m, 4.3 H), 1.39-1.68 (m, 4.4 H), 2.22 (q, 1.0 H, *J*=7,6 Hz), 2.59 (q, 1.0 H, *J*=7,6 Hz), 3.20 (q, 0.9 H, *J*=6,4 Hz), 3.37 (q, 1.1 H, *J*=6,4 Hz), 4.09 (q, 0.9 H, *J*=7,2 Hz), 4.21 (q, 0.9 H, *J*=7,2 Hz), 4.45 (s, 0.4 H), 7.06 (brs, 0.34 H), 7.21-7.57 (m, 4.9 H), 8.57 (brs, 0.34 H), 9.51, 9.67 (brs, 0.18 H), 10.36 (brs, 0.37 H), 11.79 (brs, 0.37 H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.2, 13.7, 13.7, 14.6, 20.0, 20.2, 25.3, 32.4, 42.3, 43.2, 58.2, 60.1, 79.8, 100.2, 124.9, 125.6, 125.7, 125.7, 126.2, 127.2, 128.6, 129.5, 129.5, 129.5, 139.5, 167.1, 171.0, 192.5, 201.7; IR (nujol): ν_{max} = 3248, 3135, 1625, 1598 cm^{-1}; MS *m/z* (ESI): 335.20 (M + H$^+$); anal. calcd. for $C_{18}H_{26}N_2O_2S$ (334.4762): C 64.64, H 7.84, N 8.38; found: C 64.76, H 7.82, N 8.45.



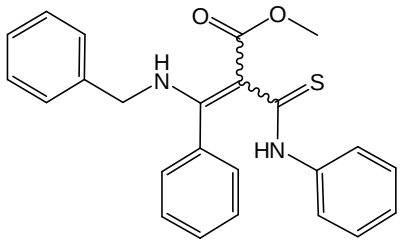
Ethyl 3-(butylamino)-2-(phenylcarbamothioyl)hex-2-enoate 28k.

28k was isolated by precipitation in methanol in 45% yield. Light yellow solid; mp: 88–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.95 (t, 3H, *J*=7,2 Hz, *n*-But), 1.01 (t, 3H, *J*=7,2 Hz, prop), 1.31 (t, 3H, *J*=7,2 Hz, $OCH_2\mathit{CH_3}$), 1.42-1.52 (m, 2H, prop), 1.56-1.74 (m, 4H, *n*-But), 2.51-2.55 (m, 2H, prop), 3.34 (q, 2H, *J*= 5.6 Hz, *n*-But), 3.81-3.84, 4.20 (m, and q, 2H, *J*=6,8 Hz, $O\mathit{CH_2}CH_3$), 7.04-7.57 (m, 5H, Ph), 9.57, 9.71, 10.27, 11.71 (4brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.7 (q), 14.3 (q), 20.2 (q), 21.9 (t), 31.8 (t), 33.7 (t), 43.2 (t), 60.1 (t), 97.1 (s), 100.4 (s), 121.6 (s), 124.9 (d), 126.2 (s), 128.6 (d), 129.0 (s), 139.4 (s), 165.7 (s), 166.3 (s), 168.0 (s), 169.5 (s), 192.6 (s), 192.7 (s), 192.8 (s), 201.4 (s); IR (nujol): ν_{max} = 3282, 3214, 1649, 1616 cm^{-1}; MS *m/z* (ESI): 349.09 (M + H$^+$); anal. calcd. for $C_{19}H_{28}N_2O_2S$ (348.5028): C 65.48, H 8.10, N 8.04; found: C 65.39, H 8.06, N 8.11.



Benzyl 3-(butylamino)-2-(phenylcarbamothioyl)but-2-enoate 28l.

28lwas isolated by precipitation in methanol in 34% yield. Light yellow solid; mp: 120–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.23 and 2.24 (2s, 3H, $\mathit{CH_3}$), 4.42, 4.48 (2d, 2H, *J*=6,4 Hz, *J*=6,0 Hz, $N\mathit{CH_2}Ph$), 5.20 (d, 2H, *J*=4,4 Hz, $O\mathit{CH_2}Ph$), 7.32-7.40 (m, 14H, Ph), 7.58 (d, 1H, *J*=7,6 Hz, Ph), 9.87, 9.94, 10.10, 11.50 (4brs, 2H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 16.8 (q), 18.5 (t), 47.3 (t), 49.8 (t), 65.6 (t), 66.9 (t), 82.7 (s), 98.5 (s), 101.8 (s), 126.6 (d), 124.3 (d), 126.2 (d), 126.3 (d), 126.9 (d), 127.4 (d), 127.5 (d), 127.8(d), 128.2(d), 128.2(d), 128.3(d), 128.3(d), 128.4(d), 128.5(d), 128.7(d), 128.8(d), 135.1 (s), 136.1 (s), 136.5 (s), 136.7 (s), 137.5 (s), 138.9 (s), 139.1 (s), 161.0 (s), 164.4 (s), 165.9 (s), 166.8 (s), 168.1 (s), 193.7 (s), 200.3 (s), 201.2(s); IR (nujol): ν_{max} = 3319, 3193, 1634, 1611 cm^{-1}; MS *m/z* (ESI): 417.27 (M + H$^+$); anal. calcd. for $C_{25}H_{24}N_2O_2S$ (416.5353): C 72.09, H 5.81, N 6.73; found: C 72.21, H 5.79, N 6.78.

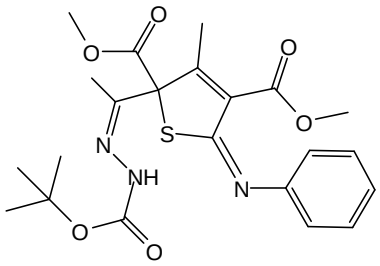


Methyl 3-(benzylamino)-3-phenyl-2-(phenylcarbamothioyl)acrylate 28m.

28m was isolated by precipitation in methanol in 27% yield. Light yellow solid; mp: 110–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.58-0.68, 1.17-1.36 (2m, 3H, $OCH_2\mathit{CH_3}$), 3.55-3.68, 4.14-4.20 (2m, 2H, $O\mathit{CH_2}CH_3$), 4.22-4.29 (m, 2H, $\mathit{CH_2}Ph$), 7.17-7.99, (m, 15H, *Ph*, NH*Ph*, Bn), 11.73 (brs, H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.0 (q), 14.0 (q), 14.5 (q), 45.8 (t), 48.2 (t), 48.7 (t), 60.0 (t), 60.1 (t), 61.3 (t), 100.8 (s), 100.9 (s), 125.4 (d), 125.9 (d), 126.7 (d), 127.0 (d), 127.0 (d), 127.0 (d), 127.3 (d), 127.3 (d), 127.7 (d), 128.2 (d), 128.3 (d), 128.5 (d), 128.9 (d), 129.2 (d), 133.6 (s), 136.3 (s), 137.2 (s), 139.1 (s), 167.3 (s), 168.3 (s), 170.1 (s), 170.8 (s), 188.4 (s), 192.3 (s), 199.7 (s); IR (nujol): ν_{max} = 3317, 3298, 1679, 1645 cm^{-1}; MS *m/z* (ESI): 403.28 ($M + H^+$); anal. calcd. for $C_{24}H_{22}N_2O_2S$ (402.5087): C 71.62, H 5.51, N 6.96; found: C 71.51, H 5.48, N 7.04.

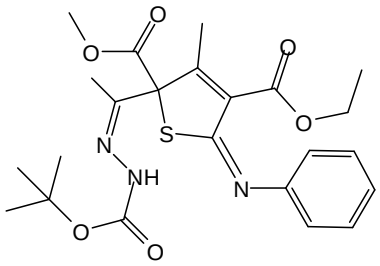
One-pot procedure for synthesis of 2,5-dihydrothiophenes 29a-x.

Alkyl amines **31a,b** (0.5 mmol) were added to β-ketoesters **30a-i** (0.55 mmol) or to ethyl phenylpropiolate **33a** (0.55mmol) under solvent-free conditions at room temperature and vigorously stirred at room temperature. After 0.5 h aryl isothiocyanates **34a-c** (0.5mmol) in MeOH (1.5 mL) were added and the reactions were stirred until the disappearance of the enamino esters **32** (6-18h monitored by TLC). DDs **1a-j** (1.0 mmol) in MeOH (1.5mL) were added to the reaction medium and magnetically stirred until the complete disappearance of the ACTs **28a-m** (3.0-5.0 h monitored by TLC). Then, the reaction solvent was evaporated under reduced pressure and the desired 2,5-dihydrothiophenes **29a-x** were purified by chromatography on silica gel column (elution mixture: cyclohexane: ethyl acetate).



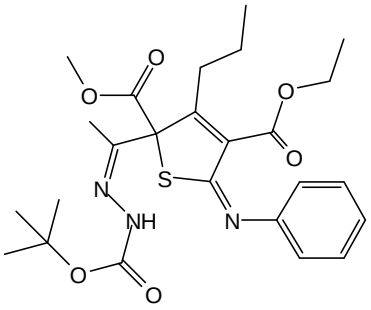
Dimethyl 2-(1-(2-*tert*butoxycarbonyl)hydrazono)ethyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29a.

29a was isolated by column chromatography on silica gel (acetate/cyclohexane) in 78% yield. Pale yellow solid; mp: 151–153 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.50 (s, 9H, C(*CH*$_3$)$_3$), 1.83 (s, 3H, *CH*$_3$), 2.24 (s, 3H, *CH*$_3$), 3.80 (s, 3H, O*CH*$_3$), 3.93 (s, 3H, O*CH*$_3$), 7.04(d, 2H, *J* = 8.0 Hz, Ph), 7.15 (t, 1H, *J* = 7.2 Hz, Ph), 7.35 (t, 2H, *J* = 7.6 Hz, Ph), 7.85 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.3 (q), 16.2 (q), 28.2 (q), 52.7 (q), 64.4 (q), 75.1 (s), 81.7 (s), 120.1 (d), 125.2 (d), 129.1 (d), 136.0 (s), 144.6 (s), 150.7 (s), 152.2 (s), 157.7 (s), 163.8 (s), 164.2 (s), 168.1 (s); IR (nujol): ν_{max} = 3231, 1740, 1698, 1629 cm^{-1}; MS *m/z* (ESI): 462.30 (M + H$^+$); anal. calcd. for $C_{22}H_{27}N_3O_6S$ (461.5313): C 57.25, H 5.90, N 9.10; found: C 57.16, H 5.87, N 9.16.



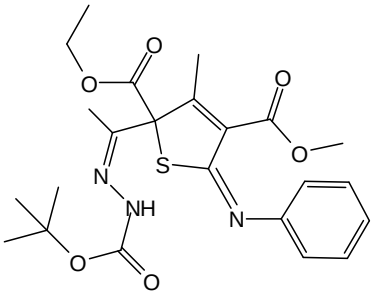
4-Ethyl-2-methyl 2-(1-(2-*tert*butoxycarbonyl)hydrazono)ethyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29b.

29b was isolated by column chromatography on silica gel (acetate/cyclohexane) in 83% yield. Pale yellow solid; mp: 136–139 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.30 (t, 3H, *J* = 7.2 Hz OCH$_2$*CH*$_3$), 1.45 (s, 9H, C(*CH*$_3$)$_3$), 1.84 (s, 3H, *CH*$_3$), 2.13 (s, 3H, *CH*$_3$), 3.73 (s, 3H, O*CH*$_3$), 4.33 (q, 2H, *J* = 7.2 Hz, O*CH*$_2$CH$_3$), 6.97 (d, 2H, *J* = 7.2 Hz, Ph), 7.18 (t, 1H, *J* = 7.6 Hz, Ph), 7.40 (t, 2H, *J* = 7.6 Hz, Ph), 9.88 (s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 13.4 (q), 13.9 (q), 15.4 (q), 27.9 (q), 53.4 (q), 61.4 (t), 75.4 (s), 79.7 (s), 119.6 (d), 125.2 (d), 129.3 (d), 135.7 (s), 145.9 (s), 150.2 (s), 152.6 (s), 156.5 (s), 163.1 (s), 163.2 (s), 167.8 (s); IR (nujol): ν_{max} = 3227, 1739, 1695, 1636 cm^{-1}; MS *m/z* (ESI): 476.29 (M + H$^+$); anal. calcd. for $C_{23}H_{29}N_3O_6S$ (475.5579): C 58.09, H 6.15, N 8.84; found: C 58.21, H 6.17, N 8.70.



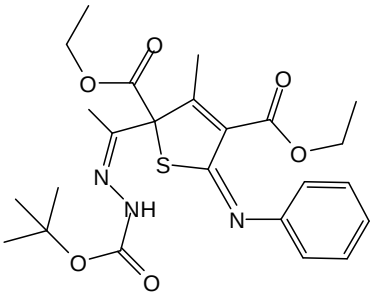
4-Ethyl-2-methyl 2-(1-(2-*tert*butoxycarbonyl)hydrazono)ethyl)-5-(phenylimino)-3-propyl--2,5-dihydrothiophene-2,4-dicarboxylate 29c.

29c was isolated by column chromatography on silica gel (acetate/cyclohexane) in 58% yield. Pale yellow solid; mp: 142−144 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 0.95 (t, 3H, *J* = 7.2 Hz $CH_2CH_2CH_3$), 1.39 (t, 3H, *J* = 7.2 Hz OCH_2CH_3), 1.51 (s, 9H, $C(CH_3)_3$), 1.46-1.60 (m, 2H, $CH_2CH_2CH_3$), 1.83 (s, 3H, CH_3), 2.54-2.68 (m, 2H, $CH_2CH_2CH_3$), 3.80 (s, 3H, OCH_3), 4.41 (q, 2H, *J* = 7.2 Hz, OCH_2CH_3), 7.04 (dd, 2H, *J* = 8.8 Hz, *J* = 1.2 Hz, Ph), 7.14 (t, 1H, *J* = 7.2 Hz, Ph), 7.35 (t, 2H, *J* = 7.6 Hz, Ph), 7.72 (s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 12.7 (q), 14.1 (q), 14.7 (q), 22.2 (t), 28.2 (q), 32.1 (t), 53.3 (q), 53.4 (q), 61.8 (t), 75.4 (s), 81.7 (s), 120.2 (d), 125.1 (d), 129.1 (d), 136.7 (s), 145.0 (s), 150.9 (s), 152.3 (s), 160.1 (s), 164.0 (s), 164.2 (s), 168.4 (s); IR (nujol): ν_{max} = 3237, 1745, 1695, 1636 cm^{-1}; MS *m/z* (ESI): 504.45 (M + H$^+$); anal. calcd. for $C_{25}H_{33}N_3O_6S$ (503.6110): C 59.62, H 6.60, N 8.34; found: C 59.49, H 6.56, N 8.48.



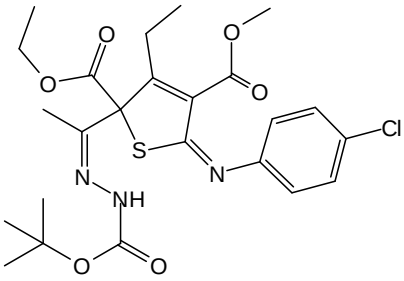
2-Ethyl-4-methyl 2-(1-(2(*tert*-butoxycarbonyl)hydrazono)ethyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29d.

29d was isolated by column chromatography on silica gel (acetate/cyclohexane) in 88 % yield. Pale yellow solid; mp: 152−156 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.27 (t, 3H, *J* = 7.2 Hz OCH_2CH_3), 1.47 (s, 9H, $C(CH_3)_3$), 1.82 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 4.23 (q, 2H, *J* = 7.2 Hz, OCH_2CH_3), 7.01 (dd, 2H, *J* = 8.4 Hz, *J* = 1.2 Hz, Ph), 7.11 (t, 1H, *J* = 7.6 Hz Ph), 7.32 (t, 2H, *J* = 8.4 Hz, Ph), 8.35 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.5 (q), 12.6 (q), 13.8 (q), 16.1 (q), 16.1 (q), 28.0 (q), 52.4 (q), .52.5 (q), 62.6 (t), 75.1 (s), 81.3 (s), 119.9 (d), 125.0 (d), 129.0 (d), 135.7 (s), 135.7 (s), 144.8 (s), 150.8 (s), 152.8 (s), 157.8 (s), 157.9 (s), 163.8 (s), 163.9 (s), 164.1 (s), 167.5 (s); IR (nujol): ν_{max} = 3254, 1713, 1676, 1628 cm^{-1}; MS *m/z* (ESI): 476.38 (M + H$^+$); anal. calcd. for $C_{23}H_{29}N_3O_6S$ (475.5579): C 58.09, H 6.15, N 8.84; found: C 57.95, H 6.11, N 8.98.



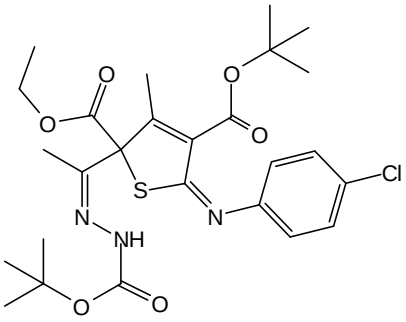
Diethyl 2-(1-(2(*tert*-butoxycarbonyl)hydrazono)ethyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29e.

29e was isolated by column chromatography on silica gel (acetate/cyclohexane) in 79 % yield. Pale yellow solid; mp: 136–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.31 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.39 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.51 (s, 9H, $C(\mathit{CH_3})_3$), 1.83 (s, 3H, $\mathit{CH_3}$), 2.25 (s, 3H, $\mathit{CH_3}$), 4.27 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 4.42 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 7.06 (d, 2H, *J* = 8.4 Hz, Ph), 7.15 (t, 1H, *J* = 7.6 Hz Ph), 7.36 (t, 2H, *J* = 8.0 Hz, Ph), 7.64 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.4 (q), 14.0 (q), 14.2 (q), 16.0 (q), 28.2 (q), 61.7 (t), .62.7 (t), 75.2 (s), 81.6 (s), 120.19 (d), 125.1 (d), 129.1 (d), 136.3 (s), 144.9 (s), 150.9 (s), 152.5 (s), 156.9 (s), 163.7 (s), 163.9 (s), 167.6 (s); IR (nujol): ν_{max} = 3230,1731,1693,1622 cm^{-1}; MS *m/z* (ESI): 490.29 ($M + H^+$); anal. calcd. for $C_{24}H_{31}N_3O_6S$ (489.5844): C 58.88, H 6.38, N 8.58; found: C 58.76, H 6.35, N 8.61.



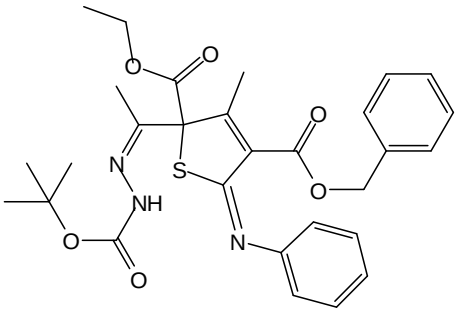
2-Ethyl-4-methyl 2-(1-(2(*tert*-butoxycarbonyl)hydrazono)ethyl)-5-((4-chlorophenyl)imino)-3-ethyl-2,5-dihydrothiophene-2,4-dicarboxylate 29f.

29f was isolated by column chromatography on silica gel (acetate/cyclohexane) in 63 % yield. Pale yellow solid; mp: 107–109 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.10 (t, 3H, *J* = 7.6 Hz $CH_2\mathit{CH_3}$), 1.30 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.49 (s, 9H, $C(\mathit{CH_3})_3$), 1.84 (s, 3H, $\mathit{CH_3}$), 2.63-2.70 (m, 2H, $\mathit{CH_2}CH_3$), 3.92 (s, 3H, $O\mathit{CH_3}$), 4.22-4.29 (m, 2H, $O\mathit{CH_2}CH_3$), 6.97 (d, 2H, *J* = 8.8 Hz, Ph), 7.30 (d, 2H, *J* = 8.8 Hz Ph), 8.01 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.9 (q), 13.0 (q), 13.9 (q), 22.4 (t), 28.2 (q), 52.7 (q), 62.8 (t), 75.8 (s), 81.6 (s), 121.6 (d), 129.2 (d), 130.3 (s), 135.8 (s), 144.8 (s), 149.3 (s), 152.6 (s), 162.9 (s), 164.5 (s), 165.2 (s), 167.6 (s); IR (nujol): ν_{max} = 3223,1717,1684,1621 cm^{-1}; MS *m/z* (ESI): 524.81 ($M + H^+$); anal. calcd. for $C_{24}H_{30}ClN_3O_6S$ (524.0295): C 55.01, H 6.77, N 8.02; found: C 54.95, H 6.79, N 8.15.



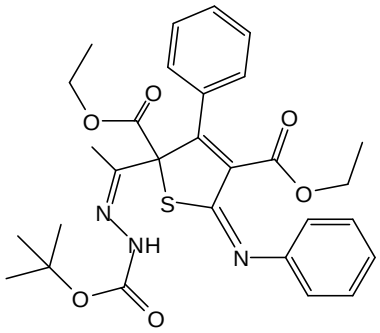
4-*Tert*-butyl 2-ethyl 2-((1-(2(*tert*-butoxycarbonyl)hydrazono)ethyl)-5-((4-chlorophenyl)imino)--3-methyl-2,5-dihydrothiophene-2,4-dicarboxylate 29g.

29g was isolated by column chromatography on silica gel (acetate/cyclohexane) in 81 % yield. Pale yellow solid; mp: 120–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.28 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.47 (s, 9H, $C(\mathit{CH_3})_3$), 1.56 (s, 9H, $C(\mathit{CH_3})_3$), 1.81 (s, 3H, $\mathit{CH_3}$), 2.20 (s, 3H, $\mathit{CH_3}$), 4.23 (q, 2H, *J* = 7.2 Hz $O\mathit{CH_2}CH_3$), 6.96 (d, 2H, *J* = 8.8 Hz, Ph), 7.27 (d, 2H, *J* = 8.8 Hz Ph), 8.46 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.4 (q), 13.9 (q), 15.8 (q), 28.1 (q), 62.6 (t), 75.4 (s), 81.4 (s), 83.0 (s), 121.4 (d), 129.9 (d), 130.1 (d), 137.1 (s), 144.8 (s), 149.3 (s), 152.7 (s), 155.9 (s), 162.8 (s), 164.4 (s), 167.5 (s); IR (nujol): ν_{max} = 3255,1736,1685,1624 cm^{-1}; MS *m/z* (ESI): 553.04 (M + H$^+$); anal. calcd. for $C_{26}H_{34}ClN_3O_6S$ (552.1890): C 56.56, H 6.21, N 7.61; found: C 56.68, H 6.24, N 7.51.



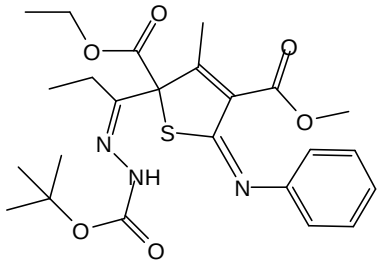
4-Benzyl-2-ethyl 2-(1-(2-*tert*butoxycarbonyl)hydrazono)ethyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29h.

29h was isolated by column chromatography on silica gel (acetate/cyclohexane) in 65% yield. Pale yellow solid; mp: 109–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.30 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.49 (s, 9H, $C(\mathit{CH_3})_3$), 1.82 (s, 3H, $\mathit{CH_3}$), 2.21 (s, 3H, $\mathit{CH_3}$), 4.26 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 5.39 (s, 2H, $O\mathit{CH_2}Ph$), 7.06 (dd, 2H, *J* = 8.4 Hz, *J* = 1.2 Hz, Ph), 7.16 (t, 1H, *J* = 7.6 Hz, Ph), 7.34-7.39 (m, 5H, Ph), 7.48 (dd, 2H, *J* = 8.0 Hz, *J* = 1.6 Hz, Ph), 7.88 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.4 (q), 14.0 (q), 16.1 (q), 28.2 (q), 62.7 (t), 67.4 (t), 75.3 (s), 81.6 (s), 120.1 (d), 125.1 (d), 128.3 (d), 128.3 (d), 128.5 (d), 129.1 (d), 135.3 (s), 135.9 (s), 144.7 (s), 150.7 (s), 152.3 (s), 157.6 (s), 163.5 (s), 163.7 (s), 167.55 (s); IR (nujol): ν_{max} = 3255,1751,1681,1643 cm^{-1}; MS *m/z* (ESI): 552.37 (M + H$^+$); anal. calcd. for $C_{29}H_{33}N_3O_6S$ (551.6538): C 63.14, H 6.03, N 7.62; found: C 63.02, H 6.00, N 7.74.



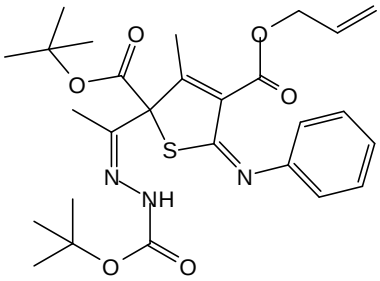
Diethyl-2-methyl 2-(1-(2(*tert*-butoxycarbonyl)hydrazono)ethyl)-3-phenyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29i.

29i was isolated by column chromatography on silica gel (acetate/cyclohexane) in 58 % yield. Pale yellow solid; mp: 105–110 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.00 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.14 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.49 (s, 9H, $C(\mathit{CH_3})_3$), 1.88 (s, 3H, $\mathit{CH_3}$), 4.10-4.18 (m, 4H, 2 $O\mathit{CH_2}CH_3$), 7.01-7.18 (m, 3H, Ph), 7.30-7.39 (m, 5H, Ph), 7.42-7.44 (m, 2H, Ph), 7.92 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (q), 13.7 (q), 13.8 (q), 28.1 (q), 28.2 (q), 61.6 (t), 62.7 (t), 76.1 (s), 81.6 (s), 120.2 (d), 120.3 (d), 125.3 (d), 125.4 (d), 127.9 (d), 129.1 (d), 129.1 (d), 133.4 (s), 139.0 (s), 144.7 (s), 150.7 (s), 152.5 (s), 156.0 (s), 163.3 (s), 163.7 (s), 167.5 (s); IR (nujol): ν_{max} = 3236,1747,1698,1623 cm^{-1}; MS *m/z* (ESI): 552.42 ($M + H^+$); anal. calcd. for $C_{29}H_{33}N_3O_6S$ (551.6538): C 63.14, H 6.03, N 7.62; found: C 63.01, H 5.99, N 7.78.



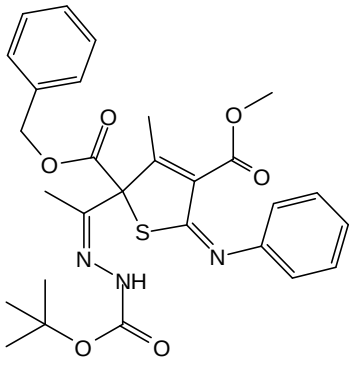
Dimethyl 2-(1-(2-*tert*butoxycarbonyl)hydrazono)propyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29j.

29j was isolated by column chromatography on silica gel (acetate/cyclohexane) in 78% yield. Pale yellow solid; mp: 120–124 °C; ^{1}H NMR (400 MHz, $DMSO_{d6}$, 25 °C): δ = 0.96 (t, 3H, *J* = 7.6 Hz $OCH_2\mathit{CH_3}$), 1.45 (s, 9H, $C(\mathit{CH_3})_3$), 2.11 (s, 3H, $\mathit{CH_3}$), 2.44 (q, 2H, *J* = 7.6 Hz $O\mathit{CH_2}CH_3$), 3.71 (s, 3H, $O\mathit{CH_3}$), 3.85 (s, 3H, $O\mathit{CH_3}$), 6.97(dd, 2H, *J* = 8.4 Hz, *J* = 0.8 Hz, Ph), 7.19 (t, 1H, *J* = 7.2 Hz, Ph), 7.41 (t, 2H, *J* = 7.6 Hz, Ph), 10.09 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $DMSO_{d6}$, 25 °C): δ = 10.5 (q), 15.8 (q), 19.8 (t), 28.0 (q), 52.7 (q), 53.4 (q), 75.3 (s), 79.9 (s), 119.6 (d), 125.3 (d), 129.5 (d), 135.6 (s), 140.8 (s), 150.4 (s), 152.6 (s), 157.2 (s), 163.4 (s), 163.7 (s), 167.9 (s); IR (nujol): ν_{max} = 3220,1749,1715,1654 cm^{-1}; MS *m/z* (ESI): 476.29 ($M + H^+$); anal. calcd. for $C_{22}H_{27}N_3O_6S$ (475.5579): C 58.09, H 6.15, N 8.84; found: C 57.96, H 6.12, N 8.96.



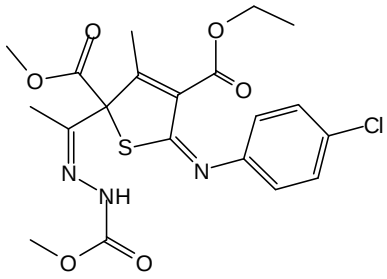
4-Allyl 2-tert-butyl-2-(1-(2(*tert*-butoxycarbonyl)hydrazono)ethyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29k.

29k was isolated by column chromatography on silica gel (acetate/cyclohexane) in 86 % yield. Pale yellow solid; mp: 121–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.46 (s, 18H, 2 OC(*CH*$_3$)$_3$), 1.81 (brs, 3H, *CH*$_3$), 2.22 (s, 3H, *CH*$_3$), 4.80 (d, 2H, *J* = 5.6 Hz, O*CH*$_2$CH=CH$_2$), 5.24 (dd, 1H, *J* = 10.4 Hz, *J* = 1.2 Hz, OCH$_2$CH=*CH*$_2$), 5.43 (dd, 1H, *J* = 17.2 Hz, *J* = 1.6 Hz, OCH$_2$CH=*CH*$_2$), 5.75 (brs, 2H, NH_2), 5.92-6.01 (m, 1H, OCH$_2$*CH*=CH$_2$), 7.01 (dd, 2H, *J* = 8.4 Hz, *J* = 1.2 Hz, Ph), 7.10 (t, 1H, *J* = 7.6 Hz, Ph), 7.31 (t, 2H, *J* = 8.0 Hz, Ph), 8.44 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 16.1 (q), 26.7 (q), 27.6 (q), 65.9 (t), 75.9 (s), 118.7 (t), 120.0 (d), 124.8 (d), 128.9 (d), 131.4 (d), 135.4 (s), 145.2 (s), 150.8 (s), 152.7 (s), 158.1 (s), 163.4 (s), 163.9 (s), 166.2 (s); IR (nujol): ν_{max} = 3235,1752,1727,1676 cm^{-1}; MS *m/z* (ESI): 530.36 (M + H$^+$); anal. calcd. for $C_{27}H_{35}N_3O_6S$ (529.6483): C 61.23, H 6.66, N 7.93; found: C 61.10, H 6.64, N 8.02.



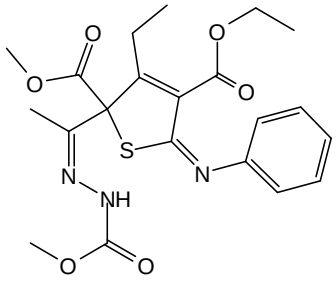
2-Benzyl-4-methyl 2-(1-(2-*tert*butoxycarbonyl)hydrazono)ethyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29l.

29l was isolated by column chromatography on silica gel (acet39ate/cyclohexane) in 86% yield. Pale yellow solid; mp: 135–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.49 (s, 9H, C(*CH*$_3$)$_3$), 1.82 (s, 3H, *CH*$_3$), 2.22 (s, 3H, *CH*$_3$), 3.92 (s, 3H, O*CH*$_3$), 5.39 (s, 2H, O*CH*$_2$Ph), 7.03 (dd, 2H, *J* = 8.4 Hz, *J* = 1.2 Hz, Ph), 7.14 (t, 1H, *J* = 7.6 Hz, Ph), 7.32-7.37 (m, 7H, Ph), 7.97 and 8.38 (2 brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.4 (q), 16.2 (q), 28.1 (q), 52.6 (q), 68.2 (t), 75.3 (s), 81.6 (s), 120.0 (d), 125.1 (d), 128.2 (d), 128.3 (d), 128.4 (d), 129.1 (d), 135.0 (s), 136.0 (s), 144.6 (s), 150.8 (s), 152.6 (s), 157.8 (s), 163.9 (s), 164.2 (s), 167.5 (s); IR (nujol): ν_{max} = 3216,1735,1722,1634 cm^{-1}; MS *m/z* (ESI): 538.19 (M + H$^+$); anal. calcd. for $C_{28}H_{31}N_3O_6S$ (537.6272): C 62.55, H 5.81, N 7.82; found: C 62.62, H 5.83, N 7.71.



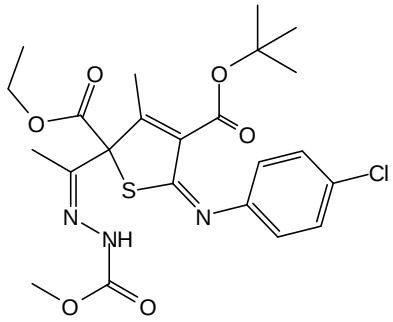
4-Ethyl-2-methyl 5-((4-chlorophenyl)imino)-2-(1-(2(methoxycarbonyl)hydrazono)ethyl)-3-methyl-2,5-dihydrothiophene-2,4-dicarboxylate 29m.

29m was isolated by column chromatography on silica gel (acetate/cyclohexane) in 84 % yield. Pale yellow solid; mp: 106–109 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.35 (t, 3H, *J* = 7.6 Hz, $OCH_2\mathit{CH_3}$), 1.84 (s, 3H, $\mathit{CH_3}$), 2.20 (s, 3H, $\mathit{CH_3}$), 3.76 (s, 3H, $O\mathit{CH_3}$), 3.78 (s, 3H, $O\mathit{CH_3}$), 4.37 (q, 2H, *J* = 7.6 Hz, $O\mathit{CH_2}CH_3$), 6.95 (d, 2H, *J* = 8.8 Hz, Ph), 7.28 (d, 2H, *J* = 8.8 Hz Ph), 8.08 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.1 (q), 14.1 (q), 15.8 (q), 52.9 (q), 53.3 (q), 75.3 (s), 121.5 (d), 129.1 (d), 130.4 (s), 136.3 (s), 146.0 (s), 149.1 (s), 154.4 (s), 156.9 (s), 163.5 (s), 164.2 (s), 167.8 (s); IR (nujol): ν_{max} = 3243,1747,1736,1684 cm^{-1}; MS *m/z* (ESI): 468.64 ($M + H^+$); anal. calcd. for $C_{20}H_{22}ClN_3O_6S$ (467.9232): C 51.34, H 4.74, N 8.98; found: C 54.15, H 4.69, N 9.13.



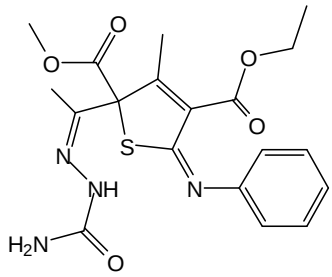
4-Ethyl-2-methyl 3-ethyl-2-(1-(2(metoxycarbonyl)hydrazono)ethyl)-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29n.

29n was isolated by column chromatography on silica gel (acetate/cyclohexane) in 63 % yield. Pale yellow solid; mp: 136–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.10 (t, 3H, *J* = 7.6 Hz $CH_2\mathit{CH_3}$), 1.37 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.85 (s, 3H, $\mathit{CH_3}$), 2.64 (q, 2H, *J* = 7.6 Hz $\mathit{CH_2}CH_3$), 3.76 (s, 3H, $O\mathit{CH_3}$), 3.78 (s, 3H, $O\mathit{CH_3}$), 4.39 (q, 2H, *J* = 7.6 Hz $O\mathit{CH_2}CH_3$), 7.01 (dd, 2H, *J* = 8.8 Hz, *J* = 0.8 Hz, Ph), 7.12 (t, 1H, *J* = 7.2 Hz Ph), 7.33 (t, 2H, *J* = 7.6 Hz Ph), 8.38 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.8 (q), 12.9 (q), 14.1 (q), 23.1 (t), 26.9 (q), 53.0 (q), 53.3 (q), 61.9 (t), 75.4 (s), 120.1 (d), 125.2 (d), 129.1 (d), 136.6 (s), 146.5 (s), 150.8 (s), 154.4 (s), 161.2 (s), 164.0 (s), 164.1 (s), 168.4 (s); IR (nujol): ν_{max} = 3262, 1732, 1624, 1598 cm^{-1}; MS *m/z* (ESI): 448.24 ($M + H^+$); anal. calcd. for $C_{21}H_{25}N_3O_6S$ (447.5047): C 56.36, H 5.63, N 9.39; found: C 56.45, H 5.66, N 9.32.



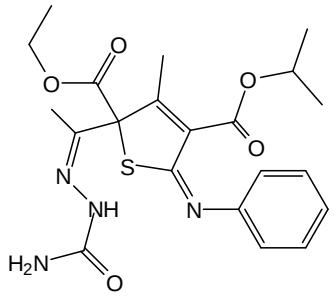
4-*Tert*-butyl 2-ethyl 5-((4-chlorophenyl)imino)-2-((1-(2(methoxycarbonyl)hydrazono)ethyl)-3-methyl-2,5-dihydrothiophene-2,4-dicarboxylate 29o.

29o was isolated by column chromatography on silica gel (acetate/cyclohexane) in 86 % yield. Pale yellow solid; mp: 132–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.28 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.56 (s, 9H, $C(\mathit{CH_3})_3$), 1.81 (s, 3H, $\mathit{CH_3}$), 2.18 (s, 3H, $\mathit{CH_3}$), 3.75 (s, 3H, $O\mathit{CH_3}$), 4.16-4.34 (m, 2H, $O\mathit{CH_2}CH_3$), 6.95 (d, 2H, *J* = 8.4 Hz, Ph), 7.28 (d, 2H, *J* = 8.8 Hz Ph), 8.43 (brs, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.0 (q), 13.9 (q), 15.7 (q), 28.2 (q), 53.0 (q), 62.8 (t), 75.4 (s), 83.2 (s), 121.7 (d), 129.2 (s), 130.3 (d), 137.8 (s), 146.2 (s), 149.3 (s), 154.3 (s), 155.4 (s), 162.9 (s), 164.2 (s), 167.4 (s); IR (nujol): ν_{max} = 3243, 1741, 1703, 1612 cm^{-1}; MS *m/z* (ESI): 510.54 (M + H$^+$); anal. calcd. for $C_{23}H_{28}ClN_3O_6S$ (510.0029): C 54.17, H 5.53, N 8.24; found: C 54.30, H 5.57, N 8.11.



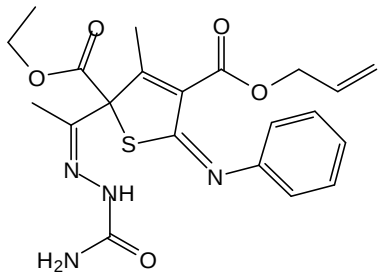
4-Ethyl-2-methyl 2-(1-(2-carbomoylhydrazono)ethyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29p.

29p was isolated by column chromatography on silica gel (acetate/cyclohexane) in 78 % yield. Pale yellow solid; mp: 165–169 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.38 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.90 (s, 3H, $\mathit{CH_3}$), 2.18 (s, 3H, $\mathit{CH_3}$), 3.78 (s, 3H, $O\mathit{CH_3}$), 4.41 (q, 2H, *J* = 7.2 Hz , $O\mathit{CH_2}CH_3$), 5.17 and 5.80 (2brs, 2H, $\mathit{NH_2}$), 7.03 (dd, 2H, *J* = 8.4 Hz, *J* = 1.2 Hz, Ph), 7.15 (t, 1H, *J* = 7.6 Hz, Ph), 7.35 (t, 2H, *J* = 7.6 Hz, Ph), 9.06 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.7 (q), 14.2 (q), 15.6 (q), 53.3 (q), 61.9 (t), 75.1 (s), 120.1 (d), 125.3 (d), 129.2 (d), 136.8 (s), 144.4 (s), 150.7 (s), 155.6 (s), 157.3 (s), 163.2 (s), 163.7 (s), 168.1 (s); IR (nujol): ν_{max} = 3412, 3278, 3243, 1753, 1644, 1623 cm^{-1}; MS *m/z* (ESI): 419.02 (M + H$^+$); anal. calcd. for $C_{19}H_{22}N_4O_5S$ (418.4668): C 54.53, H 5.30, N 13.39; found: C 54.63, H 5.33, N 13.27.



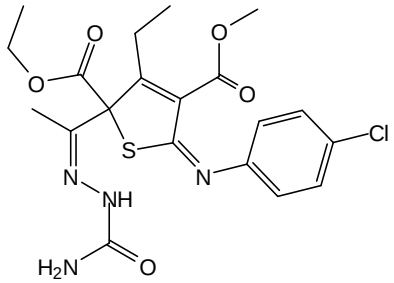
2-Ethyl 4-*iso*-propyl 2-((1-(2-carbamoylhydrazono)ethyl)-3-methyl-5-((phenyl)imino)-2,5-dihydrothiophene-2,4-dicarboxylate 29q.

29q was isolated by column chromatography on silica gel (acetate/cyclohexane) in 75 % yield. Pale yellow solid; mp: 170–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.27 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.35 (d, 6H, *J* = 6.4 Hz, $CH(\mathit{CH_3})_2$), 1.90 (s, 3H, $\mathit{CH_3}$), 2.16 (s, 3H, $\mathit{CH_3}$), 4.23 (q, 2H, *J* = 7.2 Hz $O\mathit{CH_2}CH_3$), 5.29 (hept, 1H, *J* = 6.4 Hz, $C\mathit{H}(CH_3)_2$),5.60 and 5.82 (2 brs, 2H, $\mathit{NH_2}$), 7.04 (dd, 2H, *J* = 8.8 Hz, *J* = 1.2 Hz, Ph), 7.13 (t, 1H, *J* = 7.2 Hz, Ph), 7.34 (d, 2H, *J* = 8.4 Hz Ph), 9.40 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.8 (q), 14.0 (q), 15.4 (q), 27.8 (q), 62.6 (t), 69.7 (d), 75.3 (s), 120.1 (d), 125.1 (d), 129.1 (d), 136.9 (s), 144.7 (s), 150.6 (s), 155.0 (s), 157.9 (s), 163.1 (s), 163.3 (s), 167.5 (s); IR (nujol): ν_{max} = 3421, 3264, 3257, 1742, 1613, 1606cm^{-1}; MS *m/z* (ESI): 446.87 (M + H^+); anal. calcd. for $C_{21}H_{26}N_4O_5S$ (446.1624): C 56.49, H 5.87, N 12.55; found: C 56.37, H 5.89, N 12.68.



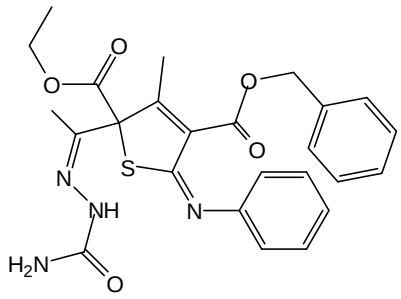
4-Allyl 2-tert-butyl-2-(1-(2(carbamoylhydrazono)ethyl)-3-methyl-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29r.

29r was isolated by column chromatography on silica gel (acetate/cyclohexane) in 63 % yield. Pale yellow solid; mp: 155–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.26 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.89 (s, 3H, $\mathit{CH_3}$), 2.18 (s, 3H, $\mathit{CH_3}$), 4.22 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 4.83 (d, 2H, *J* = 5.2 Hz, $O\mathit{CH_2}CH{=}CH_2$), 5.29 (dd, 1H, *J* = 10.4 Hz, *J* = 1.2 Hz, $OCH_2CH{=}\mathit{CH_2}$), 5.46 (dd, 1H, *J* = 17.2 Hz, *J* = 1.6 Hz, $OCH_2CH{=}\mathit{CH_2}$), 5.75 (brs, 2H, NH_2), 5.94-6.02 (m, 1H, $OCH_2\mathit{CH}{=}CH_2$), 7.03 (d, 2H, *J* = 7.6 Hz, Ph), 7.13 (t, 1H, *J* = 7.6 Hz Ph), 7.34 (t, 2H, *J* = 7.6 Hz, Ph), 9.48 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.8 (q), 13.9 (q), 15.7 (q), 62.6 (t), .66.2 (t), 75.3 (s), 119.0 (t), 120.0 (d), 125.2 (d), 129.0 (d), 131.3 (d), 136.2 (s), 144.5 (s), 150.6 (s), 156.4 (s), 157.9 (s), 163.2 (s), 163.3 (s), 167.4 (s); IR (nujol): ν_{max} = 3476, 3316, 3197, 1739, 1732, 1695 cm^{-1}; MS *m/z* (ESI): 445.13 (M + H^+); anal. calcd. for $C_{21}H_{24}N_4O_5S$ (444.5041): C 56.74, H 5.44, N 12.60; found: C 56.63, H 5.40, N 12.71.



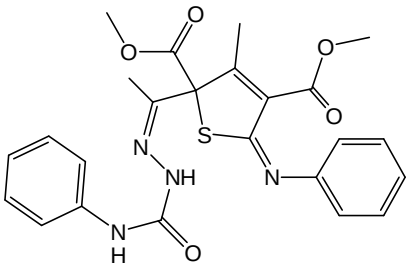
2-Ethyl-4-methyl 2-(1-(2(carbamoylhydrazono)ethyl)-5-((4-chlorophenyl)imino)-3-ethyl-2,5-dihydrothiophene-2,4-dicarboxylate 29s.

29s was isolated by column chromatography on silica gel (acetate/cyclohexane) in 78 % yield. Pale yellow solid; mp: 158–162 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.00 (t, 3H, *J* = 7.6 Hz $CH_2\mathit{CH_3}$), 1.19 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.84 (s, 3H, $\mathit{CH_3}$), 2.48-2.58 (m, 2H, $\mathit{CH_2}CH_3$), 3.85 (s, 3H, $O\mathit{CH_3}$), 4.17-4.26 (m, 2H, $O\mathit{CH_2}CH_3$), 6.23 (brs, 2H, $\mathit{NH_2}$), 7.02 (d, 2H, *J* = 8.8 Hz, Ph), 7.45 (d, 2H, *J* = 8.8 Hz Ph), 9.62 (s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 12.6 (q), 13.5 (q), 13.7 (q), 22.7 (t), 52.7 (q), 62.6 (t), 75.8 (s), 81.6 (s), 121.6 (d), 129.4 (s), 129.4 (d), 135.4 (s), 142.2 (s), 149.0 (s), 156.3 (s), 162.2 (s), 163.8 (s), 164.8 (s), 167.2 (s); IR (nujol): ν_{max} = 3446, 3321, 3223, 1747, 1730, 1684 cm^{-1}; MS *m/z* (ESI): 467.63 (M + H$^+$); anal. calcd. for $C_{20}H_{23}ClN_4O_5S$ (466.9384): C 51.44, H 4.96, N 12.00; found: C 51.36, H 4.97, N 12.11.



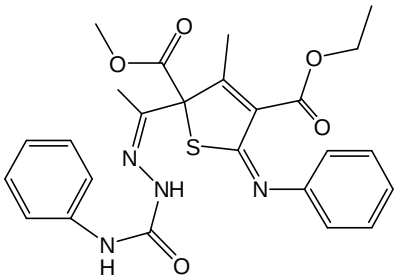
4-benzyl 2-ethyl 2-((1-(2-carbamoylhydrazono)ethyl)-3-methyl-5-((phenyl)imino)-2,5-dihydrothiophene-2,4-dicarboxylate 29t.

29t was isolated by column chromatography on silica gel (acetate/cyclohexane) in 63 % yield. Pale yellow solid; mp: 148–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.27 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.88 (s, 3H, $\mathit{CH_3}$), 2.14 (s, 3H, $\mathit{CH_3}$), 4.22 (dq, 2H, *J* = 7.2 Hz, *J* = 1.6 Hz,$O\mathit{CH_2}CH_3$), 5.38 (AB system, 2H, *J* = 12.4 Hz, $O\mathit{CH_2}Ph$), 5.78 (brs, 2H, $\mathit{NH_2}$), 7.05 (dd, 2H, *J* = 8.4 Hz, *J* = 1.2 Hz, Ph), 7.16 (t, 1H, *J* = 7.2 Hz, Ph), 7.34-7.40 (m, 5H, Ph), 7.46 (dd, 2H, *J* = 8.0 Hz, *J* = 1.6 Hz, Ph), 9.32 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 12.9 (q), 14.0 (q), 15.7 (q), 62.6 (t), 67.5 (t), 75.3 (s), 120.1 (d), 125.2 (d), 128.3 (d), 128.4 (d), 128.5 (d), 129.1 (d), 135, 2 (s), 136.2 (s), 144.5 (s), 150.6 (s), 156.5 (s), 157.7 (s), 163.1 (s), 163.6 (s), 167.4 (s); IR (nujol): ν_{max} = 3470, 3361, 3196, 1732, 1697, 1682 cm^{-1}; MS *m/z* (ESI): 494.96 (M + H$^+$); anal. calcd. for $C_{25}H_{26}N_4O_5S$ (494.1624): C 60.71, H 5.30, N 11.33; found: C 60.86, H 5.34, N 11.21.



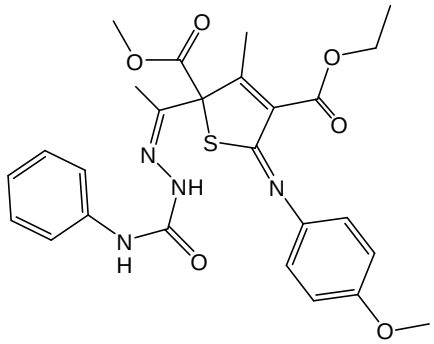
Dimethyl 3-methyl 2-(1-(2(phenylcarbamoyl)hydrazono)ethyl)-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29u.

29u was isolated by column chromatography on silica gel (acetate/cyclohexane) in 65% yield. Pale yellow solid; mp: 126–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.01 (s, 3H, *CH₃*), 2.24 (s, 3H, *CH₃*), 3.86 (s, 3H, O*CH₃*), 3.97 (s, 3H, O*CH₃*), 7.05-7.11 (m, 3H, Ph), 7.16 (t, 1H, *J* = 7.6 Hz, Ph), 7.31-7.39 (m, 4H, Ph), 7.45 (d, 2H, *J* = 8.4 Hz, Ph), 7.97 (s, 1H, *NH*), 9.46 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.2 (q), 15.9 (q), 52.8 (q), .53.5 (q), 75.2 (s), 119.0 (d), 120.1 (d), 123.6 (d), 125.4 (d), 129.1 (d), 129.2 (d), 136.6 (s), 137.7 (s), 144.3 (s), 150.6 (s), 153.4 (s), 156.5 (s), 163.2 (s), 164.1 (s), 168.1 (s); IR (nujol): ν_{max} = 3335, 3215, 1712, 1684, 1677 cm^{-1}; MS *m/z* (ESI): 481.22 (M + H$^+$); anal. calcd. for $C_{24}H_{24}N_4O_5S$ (480.5362): C 59.99, H 5.03, N 11.66; found: C 60.10, H 5.06, N 11.55.



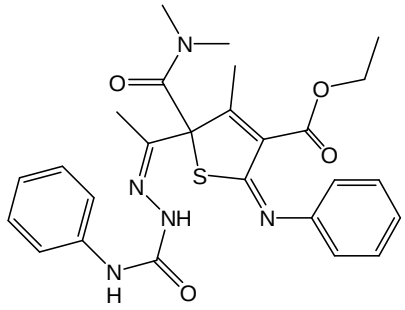
4-Ethyl-2-methyl 3-methyl-2-(1-(2-phenylcarbomoyl)hydrazono)ethyl)-5-(phenylimino)-2,5-dihydrothiophene-2,4-dicarboxylate 29v.

29v was isolated by column chromatography on silica gel (acetate/cyclohexane) in 60 % yield. Pale yellow solid; mp: 137–141 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.42 (t, 3H, *J* = 7.2 Hz OCH_2CH_3), 2.07 (s, 3H, *CH₃*), 2.53 (s, 3H, *CH₃*), 3.87 (s, 3H, O*CH₃*), 4.46 (q, 2H, *J* = 7.2 Hz, OCH_2CH_3), 7.07-7.12 (m, 3H, Ph), 7.17 (t, 1H, *J* = 7.6 Hz, Ph), 7.32-7.40 (m, 4H, Ph), 7.46 (dd, 2H, *J* = 8.4 Hz, *J* = 1.2 Hz, Ph), 8.03 (s, 1H, *NH*), 10.15 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.2 (q), 14.2 (q), 15.6 (q), 53.4 (q), 61.9 (t), 75.2 (s), 118.8 (d), 120.2 (d), 123.4 (d), 125.2 (d), 129.0 (d), 129.1 (d), 136.8 (s), 137.6 (s), 144.6 (s), 150.6 (s), 153.8 (s), 155.5 (s), 163.1 (s), 163.7 (s), 168.2 (s); IR (nujol): ν_{max} = 3357, 3241, 1707, 1698, 1653 cm^{-1}; MS *m/z* (ESI): 494.76 (M + H$^+$); anal. calcd. for $C_{25}H_{26}N_4O_5S$ (494.1624): C 60.71, H 5.30, N 11.33; found: C 60.57, H 5.26, N 11.45.



4-Ethyl 2-methyl 5-((4-methoxyphenyl)imino)-3methyl-1-(2-(phenylcarbamoyl)hydrazono)ethyl)-2,5-dihydrothiophene-2,4-dicarboxylate 29w.

29w was isolated by column chromatography on silica gel (acetate/cyclohexane) in 60 % yield. Pale yellow solid; mp: 133–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.34 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 2.01 (s, 3H, $\mathit{CH_3}$), 2.23 (s, 3H, $\mathit{CH_3}$), 3.81 (s, 3H, $O\mathit{CH_3}$), 3.97 (s, 3H, $O\mathit{CH_3}$), 4.26-4.39 (q, 2H, *J* = 7.2 Hz $O\mathit{CH_2}CH_3$), 6.91 (d, 2H, *J* = 8.8 Hz, Ph), 7.06-7.11 (m, 3H, Ph), 7.33 (t, 2H, *J* = 7.6 Hz, Ph), 7.45 (dd, 2H, *J* = 8.8 Hz, *J* = 1.2 Hz, Ph), 7.98 (s, 1H, *NH*), 9.29 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.2 (q), 14.1 (q), 15.8 (q), 52.8 (q), 55.4 (q), 62.8 (t), 75.4 (s), 114.3 (d), 119.1 (d), 121.9 (d), 123.6 (d), 129.1 (d), 136.8 (s), 137.7 (d), 143.5 (s), 144.6 (s), 153.4 (s), 155.4 (s), 157.4 (s), 162.0 (s), 164.3 (s), 167.8 (s); IR (nujol): ν_{max} = 3349, 3208, 1733, 1675, 1649 cm^{-1}; MS *m/z* (ESI): 524.79 ($M + H^+$); anal. calcd. for $C_{26}H_{28}N_4O_6S$ (524.1730): C 59.53, H 5.38, N 10.68; found: C 59.38, H 5.34, N 10.79.

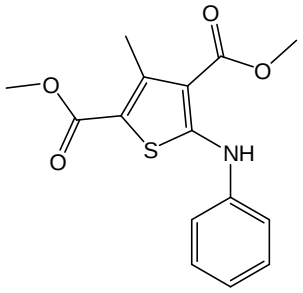


Ethyl 5-(dimethylcarbamoyl)-4-methyl-5-(-1-(2(phenylcarbamoyl)hydrazono)ethyl)-2-(phenylimino)-2,5-dihydrothiophene-3-carboxylate 29x

29x was isolated by column chromatography on silica gel (acetate/cyclohexane) in 77 % yield. Pale yellow solid; mp: 125–131 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.39 (t, 3H, *J* = 7.2 Hz, $OCH_2\mathit{CH_3}$), 2.04 (s, 3H, $\mathit{CH_3}$), 2.23 (s, 3H, $\mathit{CH_3}$), 2.81 (s, 3H, $N\mathit{CH_3}$), 3.05 (s, 3H, $N\mathit{CH_3}$), 4.42 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$),7.05 (d, 2H, *J* = 7.6 Hz, Ph), 7.10 (t, 1H, *J* = 7.2 Hz, Ph), 7.17 (t, 1H, *J* = 7.2 Hz, Ph), 7.32-7.40 (m, 4H, Ph), 7.43 (d, 2H, *J* = 7.6 Hz, Ph), 8.01 (s, 1H, *NH*), 9.48 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.3 (q), 14.2 (q), 16.6 (q), 36.8 (q), .39.0 (q), 61.9 (t), 73.8 (s), 119.0 (d), 120.1 (d), 123.7 (d), 125.3 (d), 129.2 (d), 129.2 (d), 135.6 (s), 137.5 (s), 144.7 (s), 150.8 (s), 153.4 (s), 159.2 (s), 163.0 (s), 163.8 (s), 167.2 (s); IR (nujol): ν_{max} = 3374, 3239, 1754, 1636, 1625 cm^{-1}; MS *m/z* (ESI): 507.72 ($M + H^+$); anal. calcd. for $C_{26}H_{29}N_5O_4S$ (507.1940): C 61.52, H 5.76, N 13.80; found: C 61.64, H 5.79, N 13.72.

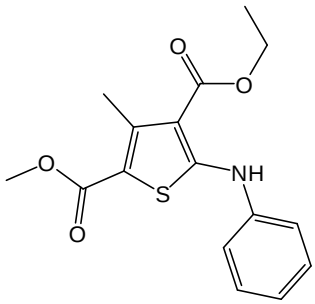
General procedure for synthesis of 5-amino thiophene-2,4-dicarboxylates 9a-i.

To a solution of 2,5-dihydrothiophenes **29a-c,e-g,l,m,r,t** (0.5 mmol) in acetone/water (90/10, 5.0mL) were added Amberlyst 15 H (4 equiv) and the reaction mixture was softly stirred at room temperature. At the disappearance of the starting 2,5-dihydrothiophenes **29** (2.0-4.0 h monitored by TLC) the reaction solvent was evaporated under reduced pressure and the desired 2,5-5-amino thiophene-2,4-dicarboxylates **35a-j** were purified by chromatography on silica gel column (elution mixture: cyclohexane: ethyl acetate).



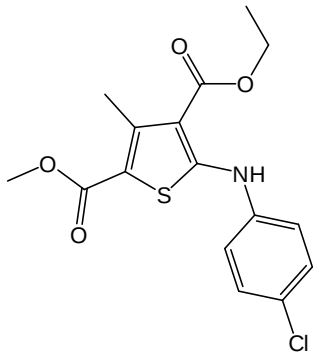
Dimethyl 3-methyl-5-(phenylamino)thiophene-2,4-dicarboxylate 35a.

35a was isolated by column chromatography on silica gel (acetate/cyclohexane) in 82% yield. White solid; mp: 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.75 (s, 3H, *CH*$_3$), 3.81 (s, 3H, O*CH*$_3$), 3.90 (s, 3H, O*CH*$_3$), 7.15 (t, 1H, *J* = 7.2 Hz, Ph), 7.34 (d, 2H, *J* = 7.6 Hz, Ph), 7.40 (t, 2H, *J* = 7.2 Hz, Ph), 10.58 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 15.9 (q), 51.3 (q), 51.5 (q), 108.2 (s), 109.0 (s), 120.0 (d), 124.4 (d), 129.6 (d), 139.8 (s), 147.7 (s), 162.6 (s), 163.1 (s), 167.3 (s); IR (nujol): ν_{max} = 3225, 1700, 1662 cm^{-1}; MS *m/z* (ESI): 306.09 (M + H$^+$); anal. calcd. for $C_{15}H_{15}NO_4S$ (305.3489): C 59.00, H 4.95, N 4.95; found: C 58.88, H 4.92, N 4.99.



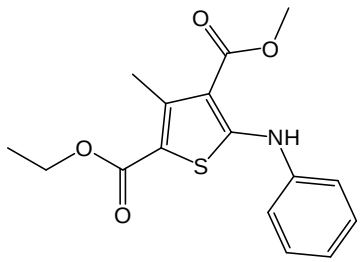
4-Ethyl-2-methyl 3-methyl-5-(phenylamino)thiophene-2,4-dicarboxylate 35b.

35b was isolated by column chromatography on silica gel (acetate/cyclohexane) in 80% yield. White solid; mp: 136–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.42 (t, 3H, *J* = 7.2 Hz OCH$_2$*CH*$_3$), 2.77 (s, 3H, *CH*$_3$), 3.81 (s, 3H, O*CH*$_3$), 4.36 (q, 2H, *J* = 7.2 Hz, O*CH*$_2$CH$_3$), 7.15 (t, 1H, *J* = 7.2 Hz, Ph), 7.33-7.42 (m, 4H, Ph), 10.62 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.3 (q), 16.0 (q), 51.5 (q), 60.4 (t), 108.1 (s), 109.2 (s), 119.9 (d), 124.3 (d), 129.6 (d), 139.8 (s), 147.8 (s), 162.4 (s), 163.2 (s), 166.9 (s); IR (nujol): ν_{max} = 3217, 1714, 1650 cm^{-1}; MS *m/z* (ESI): 320.04 (M + H$^+$); anal. calcd. for $C_{16}H_{17}NO_4S$ (319.3755): C 60.17, H 5.37, N 4.39; found: C 60.26, H 5.39, N 4.32.



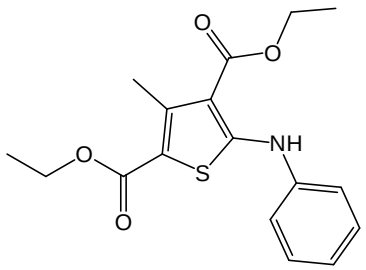
4-Ethyl 2-methyl 5-((4-chlorophenyl)amino) 3-methylthiophene-2,4-dicarboxylate 35c.

35c was isolated by column chromatography on silica gel (acetate/cyclohexane) in 82% yield. White solid; mp: 140–141 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.42 (t, 3H, *J* = 7.6 Hz $OCH_2\mathit{CH_3}$), 2.77 (s, 3H, $\mathit{CH_3}$), 3.82 (s, 3H, O $\mathit{CH_3}$), 4.37 (q, 2H, *J* = 7.6 Hz, OCH_2CH_3), 7.28 (d, 2H, *J* = 9.6 Hz, Ph), 7.36 (d, 2H, *J* = 9.6 Hz, Ph), 10.62 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.3 (q), 16.0 (q), 51.6 (q), 60.6 (t), 108.6 (s), 109.6 (s), 121.2 (d), 129.4 (s), 129.6 (d), 138.4 (s), 147.8 (s), 162.0 (s), 163.1 (s), 166.9 (s); IR (nujol): ν_{max} = 3160, 1704, 1652 cm^{-1}; MS *m/z* (ESI): 354.55 (M + H$^+$); anal. calcd. for $C_{16}H_{16}ClNO_4S$ (353.8205): C 54.31, H 4.56, N 3.96; found: C 54.42, H 4.58, N 3.88.



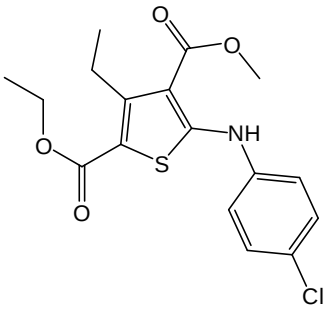
2-Ethyl-4-methyl 3-methyl-5-(phenylamino)thiophene-2,4-dicarboxylate 35d.

35d was isolated by column chromatography on silica gel (acetate/cyclohexane) in 78% yield. White solid; mp: 128–131 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.35 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 2.76 (s, 3H, $\mathit{CH_3}$), 3.91 (s, 3H, O$\mathit{CH_3}$), 4.29 (q, 2H, *J* = 7.2 Hz, OCH_2CH_3), 7.16 (t, 1H, *J* = 7.2 Hz, Ph), 7.35-7.44 (m, 4H, Ph), 10.55 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 15.9 (q), 51.3 (q), 60.5 (t), 108.8 (s), 109.1 (s), 120.1 (d), 124.5 (d), 129.6 (d), 139.9 (s), 147.4 (s), 162.6 (s), 162.8 (s), 167.4 (s); IR (nujol): ν_{max} = 3163, 1698, 1667 cm^{-1}; MS *m/z* (ESI): 320.05 (M + H$^+$); anal. calcd. for $C_{16}H_{17}NO_4S$ (319.3755): C 60.17, H 5.37, N 4.39; found: C 60.05, H 5.34, N 4.43.



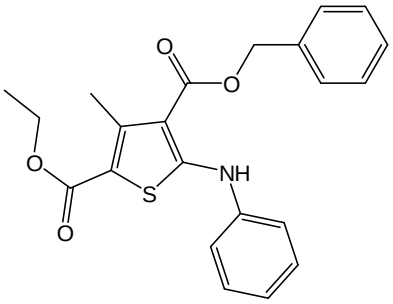
Diethyl 3-methyl-5-(phenylamino)thiophene-2,4-dicarboxylate 35e.

35e was isolated by column chromatography on silica gel (acetate/cyclohexane) in 78% yield. White solid; mp: 106–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.34 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 1.41 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 2.76 (s, 3H, $\mathit{CH_3}$), 4.28 (q, 2H, *J* = 7.2 Hz, OCH_2CH_3), 4.35 (q, 2H, *J* = 7.2 Hz, OCH_2CH_3), 7.12-7.41 (m, 5H, Ph), 10.62 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.0 (q), 14.2 (q), 15.7 (q), 60.2 (t), 108.4 (s), 108.9 (s), 119.6 (d), 124.0 (d), 129.3 (d), 139.6 (s), 147.2 (s), 162.0 (s), 162.6 (s), 166.1 (s); IR (nujol): ν_{max} = 3224, 1693, 1655 cm^{-1}; MS *m/z* (ESI): 334.07 (M + H$^+$); anal. calcd. for $C_{17}H_{19}NO_4S$ (333.4021): C 61.24, H 5.74, N 4.20; found: C 61.39, H 5.79, N 4.12.



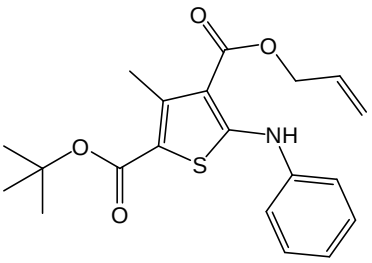
2-Ethyl 4-methyl 5-((4-chlorophenyl)amino) 3-ethylthiophene-2,4-dicarboxylate 35f.

35f was isolated by column chromatography on silica gel (acetate/cyclohexane) in 78% yield. White solid; mp: 149–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.19 (t, 3H, *J* = 7.6 Hz $CH_2\mathit{CH_3}$), 1.35 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 3.31 (q, 2H, *J* = 7.2 Hz, $\mathit{CH_2}CH_3$), 3.91 (s, 3H, O $\mathit{CH_3}$), 4.30 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 7.30 (d, 2H, *J* = 8.8 Hz, Ph), 7.37 (d, 2H, *J* = 8.8 Hz, Ph), 10.58 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 14.8 (q), 22.3 (t), 51.5 (q), 60.6 (t), 108.5 (s), 108.8 (s), 121.4 (d), 129.5 (s), 129.7 (d), 138.5 (s), 153.7 (s), 162.3 (s), 162.6 (s), 167.1 (s); IR (nujol): ν_{max} = 3223, 1696, 1664 cm^{-1}; MS *m/z* (ESI): 368.42 (M + H^+); anal. calcd. for $C_{17}H_{18}ClNO_4S$ (367.8471): C 55.51, H 4.93, N 3.81; found: C 55.40, H 4.95, N 3.86.



4-Benzyl 2-Ethyl 3-methyl-5-(phenylamino)thiophene-2,4-dicarboxylate 35g.

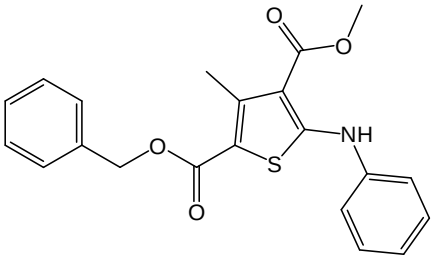
35g was isolated by column chromatography on silica gel (acetate/cyclohexane) in 81% yield. White solid; mp: 117–119 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.34 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 2.77 (s, 3H, $\mathit{CH_3}$), 4.28 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 5.38 (s, 2H, $O\mathit{CH_2}Ph$), 7.16 (t, 1H, *J* = 7.2 Hz, Ph), 7.33-7.46 (m, 9H, Ph), 10.54 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 16.2 (q), 60.5 (t), 66.2 (t), 108.8 (s), 108.9 (s), 120.2 (d), 124.5 (d), 128.1 (d), 128.3 (d), 128.7 (d), 129.6 (d), 135.9 (s), 139.8 (s), 147.4 (s), 162.8 (s), 166.6 (s); IR (nujol): ν_{max} = 3242, 1701, 1648 cm^{-1}; MS *m/z* (ESI): 395.98 (M + H^+); anal. calcd. for $C_{22}H_{21}NO_4S$ (395.4714): C 66.82, H 5.35, N 3.54; found: C 66.95, H 5.38, N 3.59.



4-Allyl 2-*tert-but*yl 3-methyl-5-(phenylamino)thiophene-2,4-dicarboxylate 35h.

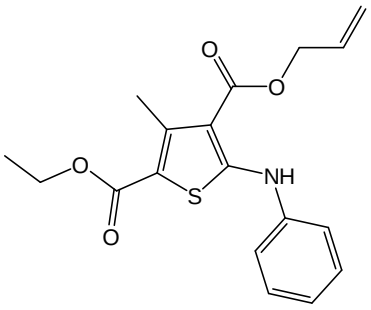
35h was isolated by column chromatography on silica gel (acetate/cyclohexane) in 70% yield. White solid; mp: 77–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.56 (s, 9H, $OC(\mathit{CH_3})_3$), 2.75 (s, 3H, $\mathit{CH_3}$), 4.82 (d, 2H, *J* = 5.2 Hz, $O\mathit{CH_2}CH{=}CH_2$), 5.31 (dd, 1H, *J* = 10.4 Hz, *J* = 0.8 Hz, $OCH_2CH{=}\mathit{CH_2}$), 5.42 (dd, 1H, *J* = 17.2 Hz, *J* = 1.2 Hz, $OCH_2CH{=}\mathit{CH_2}$), 6.00-6.10 (m, 1H, $OCH_2\mathit{CH}{=}CH_2$), 7.15 (t, 1H, *J* = 7.2 Hz, Ph), 7.34-7.42 (m, 4H, Ph), 10.54 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 15.9 (q), 28.4 (q), 65.0 (s), 81.3 (s), 108.9 (s), 110.6 (s), 118.2 (t), 120.0 (d), 124.3 (d), 129.6 (d), 132. 2 (s), 139.9 (s), 146.2 (s), 162.3 (s), 162.3 (s),

166.6 (s); IR (nujol): ν_{max} = 3188, 1704, 1664 cm^{-1}; MS *m/z* (ESI): 374.08 ($M + H^+$); anal. calcd. for $C_{20}H_{23}NO_4S$ (373.4659): C 64.32, H 6.21, N 3.75; found: C 64.39, H 6.22, N 3.75.



2-Benzyl 4-methyl 3-methyl-5-(phenylamino)thiophene-2,4-dicarboxylate 35i.

35i was isolated by column chromatography on silica gel (acetate/cyclohexane) in 81% yield. White solid; mp: 116–118 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.78 (s, 3H, *CH*$_3$), 3.91 (s, 3H, O*CH*$_3$), 5.29 (s, 2H, O*CH*$_2$Ph), 7.16 (t, 1H, *J* = 7.2 Hz, Ph), 7.34-7.43 (m, 9H, Ph), 10.59 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 16.0 (q), 51.4 (q), 66.0 (t), 108.2 (s), 109.2 (s), 120.1 (d), 124.5 (d), 128.0 (d), 128.1 (d), 128.5 (d), 129.6 (d), 136.2 (s), 139.8 (s), 148.1 (s), 162.6 (s), 162.8 (s), 167.3 (s); IR (nujol): ν_{max} = 3221, 1695, 1666 cm^{-1}; MS *m/z* (ESI): 382.04 ($M + H^+$); anal. calcd. for $C_{21}H_{19}NO_4S$ (381.4449): C 66.12, H 5.02, N 3.67; found: C 65.98, H 4.98, N 3.72.

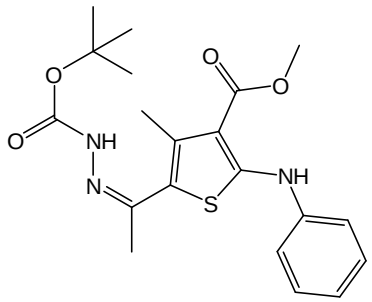


4-Allyl 2-ethyl 3-methyl-5-(phenylamino)thiophene-2,4-dicarboxylate 35j.

35j was isolated by column chromatography on silica gel (acetate/cyclohexane) in 67% yield. White solid; mp: 70–73 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.35 (t, 3H, *J* = 7.2 Hz OCH$_2$*CH*$_3$), 2.79 (s, 3H, *CH*$_3$), 4.29 (q, 2H, *J* = 7.2 Hz, O*CH*$_2$CH$_3$), 4.83 (dt, 2H, *J* = 5.6 Hz, *J* = 1.2 Hz, O*CH*$_2$CH=CH$_2$), 5.31 (dd, 1H, *J* = 10.4 Hz, *J* = 1.2 Hz, OCH$_2$CH=*CH*$_2$), 5.46 (dd, 1H, *J* = 17.2 Hz, *J* = 1.2 Hz, OCH$_2$CH=*CH*$_2$), 6.00-6.10 (m, 1H, OCH$_2$*CH*=CH$_2$), 7.16 (t, 1H, *J* = 7.2 Hz, Ph), 7.34-7.43 (m, 4H, Ph), 10.56(s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 16.4 (q), 16.1 (q), 60.5 (t), 65.1 (t), 108.8 (s), 108.9 (s), 118.4 (t), 120.1 (d), 124.5 (d), 129.6 (d), 132.2 (d), 139.8 (s), 147.4 (s), 162.7 (s), 162.8 (s), 166.5 (s); IR (nujol): ν_{max} = 3283, 1708, 1663 cm^{-1}; MS *m/z* (ESI): 346.11 ($M + H^+$); anal. calcd. for $C_{18}H_{19}NO_4S$ (345.4128): C 62.59, H 5.54, N 4.06; found: C 62.46, H 5.51, N 4.12.

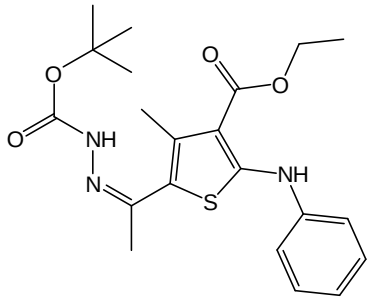
General procedure for the synthesis of 2-arylamino 5-hydrazono thiophene-3 carboxylates 41a-g by basic treatment of DHT 29.

To a solution of 2,5-dihydrothiophenes **29a,b,d-f,h,j,m,n** (0.5 mmol) in THF (5.0mL) NaH (2.0 equiv.) were added and the reaction mixture was softly stirred at room temperature. At the disappearance of the starting 2,5-dihydrothiophenes **29** (3.0-5.0 h monitored by TLC) the reaction solvent was evaporated under reduced pressure and the desired 2-arylamino 5-hydrazono thiophene-3 carboxylates **41a-g** were purified by chromatography on silica gel column (elution mixture: cyclohexane: ethyl acetate).



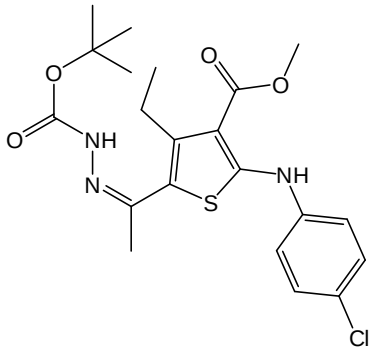
***Tert*-butyl 2-(1-(4-(methoxycarbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)ethylidene)hydrazinecarboxylate 41a.**

41a was isolated by column chromatography on silica gel (acetate/cyclohexane) in 71% yield. Pale yellow solid; mp: 123–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.49 and 1.52 (brs and s, 9H, OC(CH_3)$_3$), 2.16 and 2.48 (2s, 3H, CH_3), 2.19 and 2.23 (2s, 3H, CH_3), 3.86 and 3.88 (2s, 3H, OCH_3), 7.04-7.14 (m, 1H, Ph), 7.27-7.39 (m, 4H, Ph), 7.73 and 7.83 (2brs, 1H, *NH*), 10.28 and 10.35 (2s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 16.6 (q), 16.8 (q), 25.4 (q), 28.2 (s), 28.3 (s), 51.1 (q), 51.3 (q), 81.2 (s), 107.0 (s), 108.6 (s), 109.7 (s), 119.4 (s), 119.5 (d), 119.6 (d), 123.5 (d), 124.1 (d), 129.5 (d), 129.6 (d), 135.2 (s), 135.2 (s), 140.2 (s), 140.3 (s), 142.9 (s), 152.5 (s), 159.9 (s), 161.5 (s), 167.0(s), 167.2 (s); IR (nujol): ν_{max} = 3288, 3167, 1743, 1653 cm^{-1}; MS *m/z* (ESI): 403.68 (M + H^{+}); anal. calcd. for $C_{20}H_{25}N_3O_4S$ (403.1566): C 59.53, H 6.25, N 10.41; found: C 59.68, H 6.28, N 10.31.



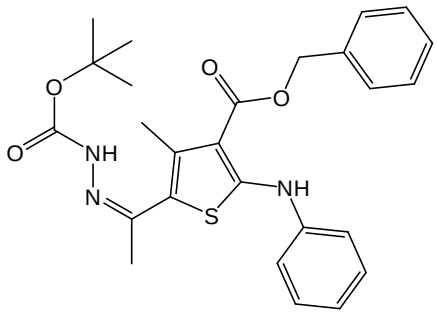
***Tert*-butyl 2-(1-(4-(ethoxycarbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)ethylidene)hydrazinecarboxylate 41b.**

41b was isolated by column chromatography on silica gel (acetate/cyclohexane) in 78% yield. Pale yellow solid; mp: 170–172 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.31 (t, 3H, *J* = 7.2 Hz OCH$_2$*CH$_3$*), 1.41 and 1.45 (2s, 9H, OC(*CH$_3$*)$_3$), 2.05 and 2.43 (2s, 3H, *CH$_3$*), 2.10 and 2.18 (2s, 3H, *CH$_3$*), 4.29 (q, 2H, *J* = 7.2 Hz, O*CH$_2$*CH$_3$), 7.12 (t, 1H, *J* = 7.2 Hz, Ph), 7.35-7.43 (m, 4H, Ph), 9.16 and 9.77 (2s, 1H, *NH*), 10.00 (s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 14.2 (q), 14.2 (q), 16.4 (q), 16.4 (q), 17.5 (q), 25.2 (q), 28.0 (s), 28.1 (s), 59.9 (t), 79.3 (s), 79.4 (s), 107.6 (s), 108.9 (s), 111.6 (s), 119.5 (d), 119.7 (d), 120.4 (s), 123.7 (d), 123.8 (d), 129.6 (d), 129.6 (d), 134.1 (s), 134.3(s), 140.4 (s), 140.7 (s), 147.2 (s), 152.6 (s), 152.8 (s), 158.4 (s), 159.9 (s), 165.4(s), 165.4 (s); IR (nujol): ν_{max} = 3281, 3221, 1740, 1653 cm^{-1}; MS *m/z* (ESI): 418.13 (M + H$^+$); anal. calcd. for $C_{21}H_{27}N_3O_4S$ (417.5288): C 60.41, H 6.52, N 10.06; found: C 60.28, H 6.47, N 10.22.



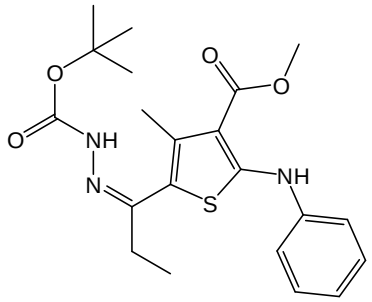
***Tert*-butyl 2-(1-(5-((4-chlorophenyl)amino)-3-ethyl--4-(methoxycarbonyl)thiophen-2-yl)ethylidene)hydrazinecarboxylate 41c.**

41c was isolated by column chromatography on silica gel (acetate/cyclohexane) in 77% yield. Pale yellow solid; mp: 150–152 °C; ^{1}H NMR (400 MHz, CDCl$_3$,, 25 °C): δ = 1.09 and 1.21 (2t, 3H, *J* = 7.6 Hz, *J* = 7.2 Hz, CH$_2$*CH$_3$*), 1,48 and 1.53 (2s, 9H, OC(*CH$_3$*)$_3$), 2.20 and 2.24 (2s, 3H, *CH$_3$*), 2.58 and 2.96 (2q, 2H, *J* = 7.2 Hz, *J* = 7.2 Hz, *CH$_2$*CH$_3$), 3.88 and 3.90 (2s, 3H, O*CH$_3$*), 4.29-4.37 (m, 2H, O*CH$_2$*CH$_3$), 7.22-7.34 (m, 4H, Ph), 7.68 and 7.82 (2brs, 1H, *NH*), 10.31 and 10.41 (2s, 1H, *NH*); ^{13}C NMR (100 MHz, CDCl$_3$,, 25 °C): δ = 15.0 (q), 15.3 (q), 16.2 (q), 22.7 (t), 23.1 (t), 25.8 (q), 28.2 (s), 28.2 (s), 51.1 (q), 51.4 (q), 81.3 (s), 106.5 (s), 108.0 (s), 109.1 (s), 119.4 (s), 120.7 (d), 120.8 (d), 128.4 (s), 129.0 (s), 129.4 (d), 129.6 (d), 138.8 (s), 139.0 (s), 141.4 (s), 141.7 (s), 142.8 (s), 152.2 (s), 152.3 (s), 159.7 (s), 161.4 (s), 166.6(s), 167.0 (s); IR (nujol): ν_{max} = 3291, 3210, 1746, 1702 cm^{-1}; MS *m/z* (ESI): 452.57 (M + H$^+$); anal. calcd. for $C_{21}H_{26}ClN_3O_4S$ (451.9668): C 55.81, H 5.80, N 9.30; found: C 55.73, H 5.78, N 9.37.



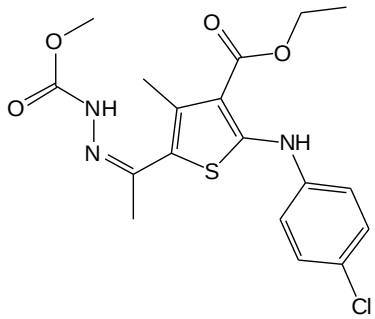
***Tert*-butyl 2-(1-(4-((benzyloxy)carbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)ethylidene)hydrazinecarboxylate 41d.**

41d was isolated by column chromatography on silica gel (acetate/cyclohexane) in 73% yield. Pale yellow solid; mp: 126–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.50 and 1.53 (2s, 9H, OC(*CH*$_3$)$_3$), 2.18 and 2.52 (2s, 3H, *CH*$_3$), 2.20 and 2.23 (2s, 3H, *CH*$_3$), 5.35and 5.37 (2s, 2H, *J* = 7.2 Hz, O*CH*$_2$Ph), 7.08-7.14 (m, 1H, *J* = 7.2 Hz, Ph), 7.30-7.45 (m, 9H, Ph), 7.63 and 7.81 (2brs, 1H, *NH*), 10.28 and 10.36 (2s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$,, 25 °C): δ = 16.6 (q), 16.8 (q), 17.2 (q), 25.5 (q), 28.2 (s), 28.3 (s), 66.0 (t), 66.2 (t), 81.3 (s), 106.8 (s), 108.5 (s), 109.6 (s), 119.6 (d), 119.7 (d), 120.4 (s), 123.7 (d), 124.2 (d), 128.1 (d), 128.2 (d), 128.2 (d), 128.3 (d), 128.6 (d), 128.7 (d), 129.5 (d), 129.6 (d), 135.2 (s), 135.9(s), 136.1 (s), 140.2 (s), 140.2 (s), 152.5 (s), 152.5 (s), 160.3 (s), 161.8 (s), 166.2(s), 166.4 (s); IR (nujol): ν_{max} = 3296, 3145, 1745, 1624 cm^{-1}; MS *m/z* (ESI): 480.13 (M + H$^+$); anal. calcd. for $C_{26}H_{29}N_3O_4S$ (479.5912): C 65.11, H 6.09, N 8.76; found: C 65.24, H 6.11, N 8.71.



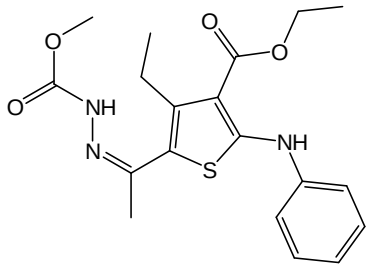
***Tert*-butyl 2-(1-(4-(methoxycarbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)propylidene)hydrazinecarboxylate 41e.**

41e was isolated by column chromatography on silica gel (acetate/cyclohexane) in 78% yield. Pale yellow solid; mp: 136–138 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.08 (t, 3H, *J* = 7.6 Hz, CH_2*CH*$_3$), 2.16 (s, 3H, *CH*$_3$), 2.54 (q, 2H, *J* = 7.6 Hz, *CH*$_2$$CH_3$), 3.90 (s, 3H, O*CH*$_3$), 7.13 (t, 1H, *J* = 7.2 Hz, *J* = 8.4 Hz, *J* = 0.8 Hz, Ph), 7.39 (t, 2H, *J* = 7.6 Hz, Ph), 7.82 (brs, 1H, *NH*), 10.30 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 11.0 (q), 16.6 (q), 28.2 (q), 32.3 (t), 25.7 (q), 51.3 (q), 81.2 (s), 107.0 (s), 108.5 (s), 119.6 (d), 124.1 (d), 129.6 (d), 135.8 (s), 140.2 (s), 144.5 (s), 147.6 (s), 152.6 (s), 161.5 (s), 167.0 (s); IR (nujol): ν_{max} = 3296, 3220, 1745, 1653 cm^{-1}; MS *m/z* (ESI): 417.85 (M + H$^+$); anal. calcd. for $C_{21}H_{27}N_3O_4S$ (417.1722): C 60.41, H 6.52, N 10.06; found: C 60.33, H 6.50, N 10.12.



Methyl 2-(1-(5-((4-chlorophenyl)amino)-4-(ethoxycarbonyl)-3-methylthiophen-2-yl)ethylidene)hydrazinecarboxylate 41f.

41f was isolated by column chromatography on silica gel (acetate/cyclohexane) in 69% yield. Pale yellow solid; mp: 136–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$,, 25 °C): δ = 1.36-1.40 (m, 3H, $OCH_2\mathit{CH_3}$), 2.17 and 2.46 (2s, 3H, $\mathit{CH_3}$), 2.22 and 2.24 (2s, 3H, $\mathit{CH_3}$), 3.80 and 3.83 (brs and s, 3H, $O\mathit{CH_3}$), 4.29-4.37 (m, 2H, $O\mathit{CH_2}CH_3$), 7.20-7.33 (m, 4H, Ph), 7.97 (brs, 1H, *NH*), 10.32 and 10.38 (2s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$,, 25 °C): δ = 14.3 (q), 16.7 (q), 16.7 (q), 16.9 (q), 25.3 (q), 53.0 (q), 60.2 (t), 60.4 (t), 107.5 (s), 109.1 (s), 109.5 (s), 119.1 (s), 120.5 (d), 120.7 (d), 128.3 (s), 129.0 (s), 129.4 (d), 129.6 (d), 135.5 (s), 135.6 (s), 138.7 (s), 138.9 (s), 143.9 (s), 153.9 (s), 154.2 (s), 159.1 (s), 160.8 (s), 166.4(s), 166.7 (s); IR (nujol): ν_{max} = 3293, 3191, 1736, 1653 cm^{-1}; MS *m/z* (ESI): 410.47 ($M + H^+$); anal. calcd. for $C_{18}H_{20}ClN_3O_4S$ (409.8871): C 52.74, H 4.92, N 10.25; found: C 52.58, H 4.89, N 10.34.

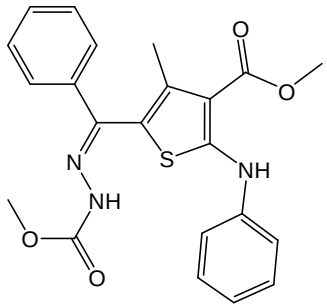


***(*Methyl 2-(1-(4-(ethoxycarbonyl)-3-ethyl-5-(phenylamino)thiophen-2-yl)ethylidene)hydrazinecarboxylate 41g.**

41g was isolated by column chromatography on silica gel (acetate/cyclohexane) in 68% yield. Pale yellow solid; mp: 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.03 and 1.16 (2t, 3H, *J* = 7.2 Hz, *J* = 7.2 Hz, $CH_2\mathit{CH_3}$), 1.33 (t, 3H, *J* = 7.2 Hz $OCH_2\mathit{CH_3}$), 2.14 and 2.18 (2s, 3H, $\mathit{CH_3}$), 2.51 and 2.90 (2q, 2H, *J* = 7.2 Hz, *J* = 7.2 Hz, $\mathit{CH_2}CH_3$), 3.73 and 3.76 (brs and s, 3H, $O\mathit{CH_3}$), 4.29 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 6.99-7.07 (m, 1H, Ph), 7.19-7.33 (m, 4H, Ph), 7.89 and 7.96 (brs and s, 1H, *NH*), 10.27 and 10.38 (2s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.2 (q), 15.2 (q), 15.4 (q), 16.4 (q), 17.5 (q), 22.8 (t), 23.3 (q), 25.7 (q), 53.3 (q), 60.2 (t), 60.4 (t), 79.3 (s), 106.1 (s), 107.8 (s), 108.2 (s), 118.4 (s), 119.6 (d), 119.8 (d), 123.6 (d), 124.2 (d), 129.5 (d), 129.6 (d), 140.3 (s), 140.4 (s), 141.7(s), 142.3 (s), 144.5 (s), 144.6 (s), 153.8 (s), 153.9 (s), 160.4 (s), 162.1 (s), 166.2 (s), 166.6 (s); IR (nujol): ν_{max} = 3288, 3120, 1756, 1654 cm^{-1}; MS *m/z* (ESI): 390.03 ($M + H^+$); anal. calcd. for $C_{19}H_{23}N_3O_4S$ (389.4686): C 58.59, H 5.95, N 10.79; found: C 58.71, H 5.99, N 10.67.

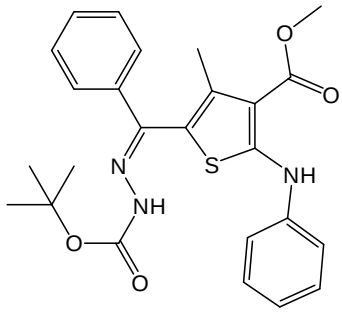
One pot sequential synthesis of 2-arylamino 5-hydrazono thiophene-3-carboxylates (AHTs) 41h-r starting from in situ generated 4-unsubstituted DDs.

Alkyl amines **31a,b** (0.5 mmol) were added to β-ketoesters **30a,b,i** (0.55 mmol) under solvent-free conditions at room temperature and vigorously stirred at room temperature. After 0.5 h aryl isothiocyanates **34a** (0.5mmol) in CH_2Cl_2 (1.5 mL) were added and the reactions were stirred until the disappearance of the enamino esters **32** (5-20h monitored by TLC). α-Halo hydrazones **15a-g** (1.0 mmol) in CH_2Cl_2 (2.0 mL) and potassium carbonate (4.0 mmol) were added to the reaction medium and magnetically stirred until the complete disappearance of the ACTs **28a,c,i** (5.0-7.0 h monitored by TLC). Then, the reaction solvent was evaporated under reduced pressure and the desired 2-arylamino 5-hydrazono thiophene-3-carboxylates (AHTs) **41h-r** were purified by chromatography on silica gel column (elution mixture: cyclohexane: ethyl acetate).



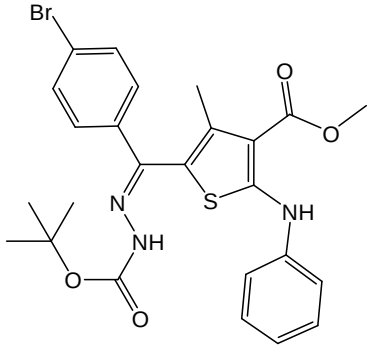
Methyl 2-((4-(methoxycarbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)(phenyl)methylene)hydrazinecarboxylate (41h).

41h was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 80% yield. White solid; mp: 169–172 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.17 (s, 3H, CH_3), 3.87 (brs, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 7.11 (t, 1H, *J* = 7.2 Hz, Ph), 7.29-7.38 (m, 7H, Ph), 7.63-7.68 (m, 2H, Ph), 8.22 (brs, 1H, NH), 10.39 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 16.5 (q), 51.3 (q), 53.1 (q), 106.0 (s), 106.9 (s), 119.6 (d), 124.1 (d), 127.3 (d), 128.4 (d), 129.6 (d), 129.7 (s), 136.6 (s), 137.8 (s), 140.1 (s), 144.1 (s), 153.6 (s), 162.2 (s), 166.9 (s); IR (nujol): νmax = 3218, 3113, 1699, 1660 cm-1; MS m/z (ESI): 424.05 ($M + H^+$); anal. calcd. for $C_{22}H_{21}N_3O_4S$ (423.4848): C 62.40, H 5.00, N 9.92; found: C 62.34, H 4.98, N 9.96.



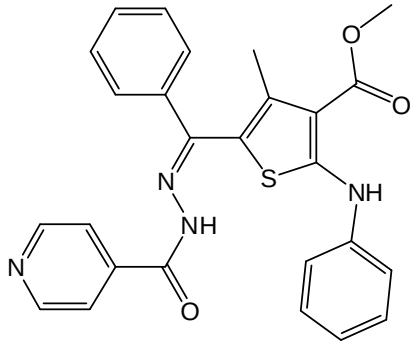
Tert-butyl 2-((4-(methoxycarbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)(phenyl)methylene)hydrazinecarboxylate (41i).

41i was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 81% yield. White solid; mp: 162–166 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.55 (s, 9H, $OC(CH_3)_3$), 2.18 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 7.11 (t, 1H, *J* = 7.2 Hz, Ar), 730-7.38 (m, 7H, Ph), 7.67-7.69 (m, 2H, Ph), 8.07 (brs, 1H, NH), 10.42 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 16.4 (q), 28.2 (q), 51.3 (q), 81.7 (s), 106.3 (s), 107.0 (s), 119.5 (d), 124.0 (d), 127.1 (d), 128.3 (d), 129.4 (d), 129.6 (d), 136.9 (s), 137.5 (s), 140.2 (s), 142.8 (s), 152.3 (s), 162.0 (s), 167.0 (s); IR (nujol): νmax = 3287, 3178, 1751, 1663 cm-1; MS m/z (ESI): 466.18 ($M + H^+$); anal. calcd. for $C_{25}H_{27}N_3O_4S$ (465.5646): C 64.50, H 5.85, N 9.03; found: C 64.44, H 5.86, N 9.09.



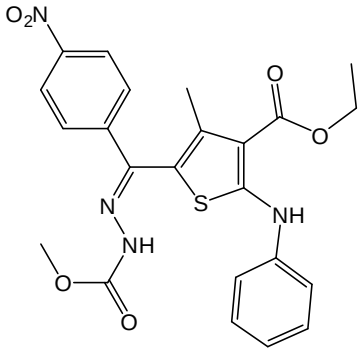
Tert-butyl 2-((4-bromophenyl)(4-(methoxycarbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)methylene)hydrazinecarboxylate (41j).

41j was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 87% yield. White solid; mp: 168–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.54 (s, 9H, OC(*CH*$_3$)$_3$), 2.15 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 7.12 (t, 1H, *J* = 7.2 Hz, Ph), 7.31 (dd, *J* = 8.8 Hz, *J* = 1.2 Hz, 2H, Ph), 7.37 (t, 2H, *J* = 7.2 Hz, Ph), 7.46 (d, 2H, *J* = 8.8 Hz, Ph), 7.54 (d, 2H, *J* = 8.8 Hz, Ph), 8.05 (brs, 1H, NH), 10.40 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 16.4 (q), 28.2 (q), 51.3 (q), 81.9 (s), 105.6 (s), 107.0 (s), 119.6 (d), 123.9 (s), 124.2 (d), 128.6 (d), 129.6 (d), 131.4 (d), 135.9 (s), 137.8 (s), 140.1 (s), 141.8 (s), 152.2 (s), 162.2 (s), 167.0 (s); IR (nujol): νmax = 3343, 3173, 1754, 1659 cm-1; MS m/z (ESI): 545.08 (M + H^+); anal. calcd. for $C_{25}H_{26}BrN_3O_4S$ (544.4606): C 55.15, H 4.81, N 7.72; found: C 55.02, H 4.78, N 7.74.



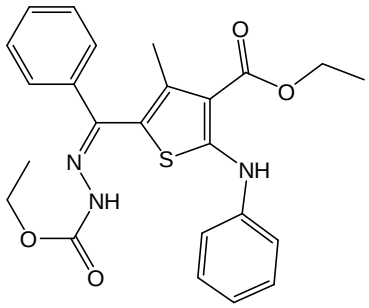
Methyl 5-((2-isonicotinoylhydrazono)(phenyl)methyl)-4-methyl-2-(phenylamino)thiophene-3-carboxylate (41k).

41k was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 53% yield. White solid; mp: 160–163 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.22 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 7.13 (t, 1H, *J* = 7.2 Hz, Ar), 7.30-7.42 (m, 7H, Ar), 7.51-7.62 (m, 2H, Ar), 7.76-7.83 (m, 2H, Ar), 7.67-8.79 (m, 2H, Ar), 9.17 and 9.48 (2brs, 1H, NH), 10.39 and 10.45 (2s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.1 (q), 16.6 (q), 51.1 (q), 51.3 (q), 51.4 (q), 105.0 (s), 105.7 (s), 107.0 (s), 119.6 (d), 119.8 (d), 119.9 (s), 120.0 (s), 123.7 (s), 124.4 (s), 127.3 (d), 128.0 (d), 128.1 (d), 129.5 (d), 129.6 (d), 130.2 (d), 130.2 (d), 130.6 (s), 130.6 (s), 136.2 (s), 138.5 (s), 139.8 (s), 140.0 (s), 145.2 (s), 149.6 (s), 150.7 (s), 162.4 (s), 162.5 (s), 166.8 (s), 167.8 (s); IR (nujol): νmax = 3355, 3241, 1661, 1593 cm-1; MS m/z (ESI): 471.13 (M + H^+); anal. calcd. for $C_{26}H_{22}N_4O_3S$ (470.5429): C 66.37, H 4.71, N 11.91; found: C 66.48, H 4.73, N 11.82.



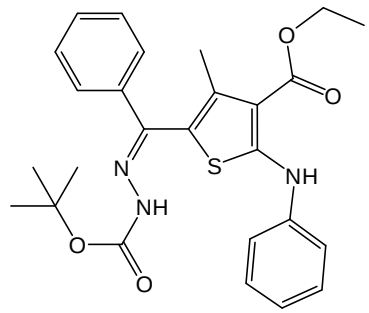
Methyl 2-((4-(ethoxycarbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)(4-nitrophenyl)methylene)hydrazinecarboxylate (41l).

41l was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 57% yield. White solid; mp: 164–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.43 (q, 3H, *J* = 7.2 Hz, $OCH_2\mathit{CH_3}$), 2.17 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 4.41 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 7.14 (t, 1H, *J* = 7.2 Hz, Ph), 7.30 (d, 2H, *J* = 7.6 Hz, Ph),7.38 (t, 2H, *J* = 7.6 Hz, Ph), 7.84 (d, 2H, *J* = 8.8 Hz, Ph), 8.20 (d, 2H, *J* = 8.4 Hz, Ph), 8.34 (brs, 1H, NH), 10.36 (brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 16.7 (q), 55.4 (q), 60.6 (t), 104.3 (s), 107.2 (s), 119.8 (d), 123.7 (d), 124.5 (d), 128.0 (d), 129.7 (d), 138.8 (s), 140.0 (s), 141.9 (s), 142.7 (s), 148.3 (s), 153.7 (s), 162.5 (s), 166.4 (s); IR (nujol): νmax = 3327, 3222, 1763, 1652 cm-1; MS m/z (ESI): 483.10 ($M + H^+$); anal. calcd. for $C_{23}H_{22}N_4O_6S$ (482.5090): C 57.25, H 4.60, N 11.61; found: C 57.16, H 4.63, N 11.74.



Ethyl 2-((4-(ethoxycarbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)(phenyl)methylene)hydrazinecarboxylate (41m).

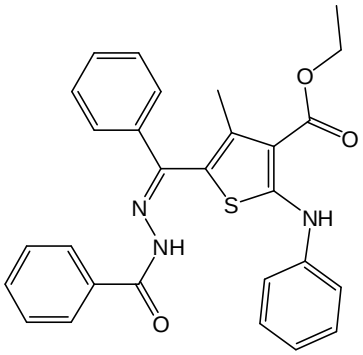
41m was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 79% yield. White solid; mp: 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.31-1.39 (m, 3H, $OCH_2\mathit{CH_3}$), 1.42 (t, 3H, *J* = 7.2 Hz, $OCH_2\mathit{CH_3}$), 2.19 (s, 3H, CH_3), 4.27-4.32 (m, 2H, $O\mathit{CH_2}CH_3$), 4.40 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 7.10 (t, 1H, *J* = 7.2 Hz, Ph), 7.29-7.38 (m, 7H, Ph), 7.67-7.69 (m, 2H, Ph), 8.20 (brs, 1H, NH), 10.44 (s, 1H, 2NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 14.5 (q), 16.6 (q), 60.4 (t), 62.1 (t), 106.1 (s), 107.1 (s), 119.4 (d), 124.0 (s), 127.3 (d), 128.3 (d), 129.6 (d), 129.7 (d), 136.8 (s), 137.8 (s), 140.2 (s) 153.6 (s), 162.0 (s), 166.6 (s); IR (nujol): νmax = 3209, 3131, 1697, 1650 cm-1; MS m/z (ESI): 452.12 ($M + H^+$); anal. calcd. for $C_{24}H_{25}N_3O_4S$ (451.5380): C 63.84, H 5.58, N 9.31; found: C 63.96, H 5.61, N 9.20.



Tert-butyl 2-((4-(ethoxycarbonyl)-3-methyl-5-(phenylamino)thiophen-2-yl)(phenyl)methylene)hydrazinecarboxylate (41n).

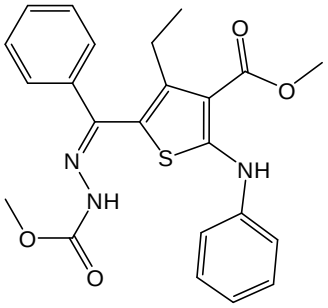
41n was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 85% yield. White solid; mp: 149–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.42 (t, 3H, *J* = 7.2 Hz, $OCH_2\mathit{CH_3}$), 1.55 (s, 9H, $OC(\mathit{CH_3})_3$), 2.19 (s, 3H, CH_3), 4.39 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 7.10 (t, 1H, *J* = 7.2 Hz, Ph), 730-7.38

(m, 7H, Ph), 7.67-7.70 (m, 2H, Ph), 8.09 (brs, 1H, NH), 10.46 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.3 (q), 16.5 (q), 28.2 (q), 60.3 (t), 81.7 (s), 106.2 (s), 107.1 (s), 119.3 (d), 123.9 (d), 127.1 (d), 128.2 (d), 129.4 (d), 129.5 (d), 136.8 (s), 137.6 (s), 140.2 (s), 143.0 (s), 152.2 (s), 161.7 (s), 166.6 (s); IR (nujol): νmax = 3277, 3226, 1750, 1652 cm-1; MS m/z (ESI): 480.16 (M + H^+); anal. calcd. for $C_{26}H_{29}N_3O_4S$ (479.5912): C 65.11, H 6.09, N 8.76; found: C 65.24, H 6.12, N 8.69.



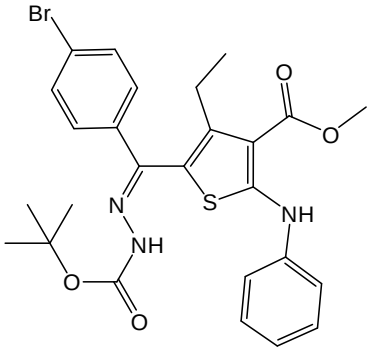
Ethyl 5-((2-(benzoylhydrazono)(phenyl)methyl)-4-methyl-2-(phenylamino)thiophene-3-carboxylate (41o).

41o was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 62% yield. White solid; mp: 142–144 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.43 (q, 3H, *J* = 7.2 Hz, $OCH_2\mathit{CH_3}$), 2.25 (s, 3H, CH_3), 4.41 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 7.11 (t, 1H, *J* = 7.2 Hz, Ph), 7.31-7.77 (m, 14H, Ph), 8.03, 9,02 (2brs, 1H, NH), 10.48 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.3 (q), 16.8 (q), 60.4 (t), 60.1 (t), 106.1 (s), 107.2 (s), 119.5 (d), 124.2 (d), 127.2 (d), 127.8 (d), 128.2 (d), 128.4 (d), 128.8 (d), 129.6 (d), 130.0 (d), 133.1 (s), 136.6 (s), 138.3 (s), 140.1 (s), 147.7 (s), 162.1 (s), 163.2 (s), 166.5 (s); IR (nujol): νmax = 3326, 3310, 1693, 1651 cm-1; MS m/z (ESI): 483.99 (M + H^+); anal. calcd. for $C_{28}H_{25}N_3O_3S$ (483.5814): C 69.54, H 5.21, N 8.69; found: C 69.66, H 5.24, N 8.60.



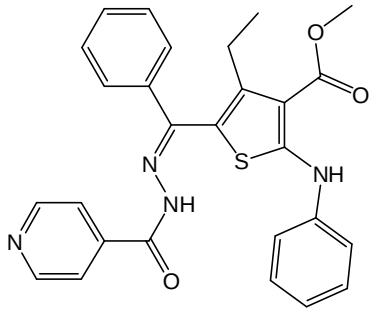
Methyl 2-((3-ethyl-4-(methoxycarbonyl)--5-(phenylamino)thiophen-2-yl)(phenyl)methylene)hydrazinecarboxylate (41p).

41p was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 87% yield. White solid; mp: 129–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.85 and 1.04 (2t, 3H, *J* = 7.2 Hz, *J* = 7.2 Hz, $CH_2\mathit{CH_3}$), 2.46 and 2.57 (q and brs, 2H, *J* = 7.2 Hz, $CH_2\mathit{CH_3}$), 3.79, 3.84, and 3.92 (brs and 2s, 6H, $2OCH_3$), 7.08-7.70 (m, 10H, 2Ph), 7.76 and 8.25 (2brs, 1H, NH), 10.40 and 10.48 (2brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.8 (q), 15.0 (q), 51.0 (q), 51.2 (q), 52.8 (q), 52.9 (q), 105.3 (s), 106.0 (s), 107.7 (s), 119.7 (s), 119.8 (d), 123.8 (d), 124.1 (d), 127.2 (d), 128.0 (d), 128.3 (d), 129.4 (d), 129.5 (d), 129.7 (s), 129.7 (d), 130.1 (s), 132.4 (s), 136.6 (s), 140.1 (s), 140.1 (s), 143.9 (s), 144.3 (s), 153.5 (s), 153.9 (s), 161.5 (s), 162.7 (s), 166.6 (s), 166.9 (s); IR (nujol): νmax = 3321, 3217, 1774, 1668 cm-1; MS m/z (ESI): 438.01 (M + H^+); anal. calcd. for $C_{23}H_{23}N_3O_4S$ (437.5114): C 63.14, H 5.30, N 9.60; found: C 63.23, H 5.34, N 9.55.



Tert-butyl 2-((4-bromophenyl)(-3-ethyl-4-(methoxycarbonyl)-5-(phenylamino)thiophen-2-yl)methylene)hydrazinecarboxylate (41q).

41q was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 91% yield. White solid; mp: 163–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.03 (t, 2H, *J* = 7.2 Hz, $CH_2\mathit{CH_3}$), 1.53 (s, 9H, $OC(\mathit{CH_3})_3$), 2.55 (q, 2H, $\mathit{CH_2}CH_3$), 3.94 (s, 3H, OCH_3), 7.12 (t, 1H, *J* = 7.2 Hz, Ph), 7.31 (dd, *J* = 8.8 Hz, *J* = 1.2 Hz, 2H, Ph), 7.37 (t, 2H, *J* = 7.2 Hz, Ph), 7.45 (d, 2H, *J* = 8.8 Hz, Ph), 7.56 (d, 2H, *J* = 8.4 Hz, Ph), 8.07 (brs, 1H, NH), 10.42 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.8 (q), 23.3 (t), 28.2 (q), 51.4 (q), 81.9 (s), 104.9 (s), 106.1 (s), 119.7 (d), 123.9 (s), 124.2 (d), 128.6 (d), 129.6 (d), 131.4 (d), 136.0 (s), 140.1 (s), 142.1 (s), 144.0 (s), 152.1 (s), 162.7 (s), 166.7 (s); IR (nujol): νmax = 3336, 3233, 1754, 1665 cm-1; MS m/z (ESI): 559.14 ($M + H^+$); anal. calcd. for $C_{26}H_{28}BrN_3O_4S$ (558.4872): C 55.92, H 5.05, N 7.52; found: C 55.82, H 5.03, N 7.53.



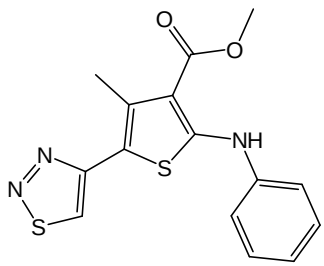
Methyl 4-methyl-5-((2-isonicotinoylhydrazono)(phenyl)methyl)-2-(phenylamino)thiophene-3-carboxylate (41r).

41r was isolated by column chromatography on silica gel (acetate/cyclohexane 20:80) in 73% yield. White solid; mp: 162–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.06-1.09 (m, 3H, $CH_2\mathit{CH_3}$), 2.51-2.71 (m, 2H, $\mathit{CH_2}CH_3$), 3.94 (s, 3H, OCH_3), 7.13 (t, 1H, *J* = 7.2 Hz, Ar), 7.33-7.43 (m, 7H, Ar), 7.53-7.59 (m, 2H, Ar), 7.79-7.82 (m, 2H, Ar), 7.66-8.90 (m, 2H, Ar), 9.22 and 9.51 (2brs, 1H, NH), 10.40 and 10.47 (2brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.9 (q), 15.1 (q), 23.4 (t), 23.4 (t), 51.1 (q), 51.3 (q), 51.4 (q), 104.2 (s), 104.9 (s), 106.0 (s), 119.7 (d), 120.0 (d), 120.1 (s), 120.8 (s), 123.7 (s), 124.4 (d), 127.2 (d), 127.9 (d), 128.4 (d), 128.5 (d), 129.6 (d), 130.0 (d), 130.2 (d), 130.6 (s), 136.2 (s), 139.9 (s), 140.1 (s), 144.6 (s), 145.4 (s), 149.3 (s), 149.6 (s), 150.7 (s), 161.2 (s), 162.9 (s), 163.1 (s), 166.5 (s), 167.6 (s); IR (nujol): νmax = 3226, 3161, 1692, 1650 cm-1; MS m/z (ESI): 485.04 ($M + H^+$); anal. calcd. for $C_{27}H_{24}N_4O_3S$ (484.5695): C 66.92, H 4.99, N 11.56; found: C 67.05, H 5.03, N 11.43.

General Procedure for the synthesis of 2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylates (ATTs) 43a-d starting from 2-arylamino 5-hydrazono thiophene-3-carboxylates (AHTs) 41a-c,g.

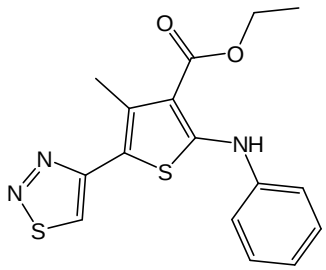
To a solution of 2-arylamino 5-hydrazono thiophene-3-carboxylates (AHTs) **41a-c,g**. (0.5 mmol) in CH_2Cl_2 (4.0 mL) thionyl chloride (5.0 mmol) were added and the reaction mixture was softly stirred at room temperature. At the disappearance of the starting 2-arylamino 5-hydrazono thiophene-3-carboxylates (AHTs) **41** (1.0-1.5 h monitored by TLC)

the crude was neutralized by adding saturated aqueous solution of sodium hydrogen carbonate until pH ~ 7 and then extracted with fresh CH_2Cl_2 (5.0 mL x 3). The organic layer was washed with water and dried on sodium sulphate. The reaction solvent was evaporated under reduced pressure and the desired 2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylates (ATTs) **43a-d** were purified by chromatography on silica gel column (elution mixture: cyclohexane: ethyl acetate).



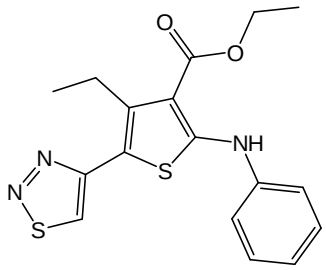
Methyl 4-methyl-2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylate 43a.

43a was isolated by column chromatography on silica gel (acetate/cyclohexane) in 63% yield. Pale brown solid; mp: 120–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.60 (s, 3H, *CH*$_3$), 3.94 (s, 3H, O*CH*$_3$), 7.12-7.15 (m, 1H, Ph), 7.37-7.42 (m, 4H, Ph), 8.38 (s, 1H, SCH), 10.42 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 16.5 (q), 51.3 (q), 108.2 (s), 109.5 (s), 119.6 (d), 123.9 (d), 129.3 (d), 129.6 (d), 133.6 (s), 140.3 (s), 156.7 (s), 160.6 (s), 167.2 (s); IR (nujol): ν_{max} = 3109, 1668 cm^{-1}; MS *m/z* (ESI): 33.97 (M + H$^+$); anal. calcd. for $C_{15}H_{13}N_3O_2S_2$ (331.4126): C 54.36, H 3.95, N 12.68; found: C 54.47, H 3.97, N 12.82.



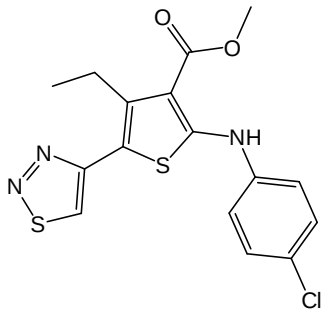
Ethyl 4-methyl-2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylate 43b.

43b was isolated by column chromatography on silica gel (acetate/cyclohexane) in 57% yield. Pale brown solid; mp: 99–102 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.44 (t, 3H, *J* = 7.2 Hz, $OCH_2\mathit{CH_3}$), 2.61 (s, 3H, $\mathit{CH_3}$), 4.40 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 7.09-7.14 (m, 1H, Ph), 7.36-7.42 (m, 4H, Ph), 8.37 (s, 1H, SCH), 10.45 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 16.5 (q), 60.3 (q), 108.3 (s), 109.5 (s), 119.5 (d), 123.8 (d), 129.2 (d), 129.6 (d), 135.6 (s), 140.3 (s), 156.7 (s), 160.4 (s), 166.8 (s); IR (nujol): ν_{max} = 3115, 1654 cm^{-1}; MS *m/z* (ESI): 346.01 (M + H$^+$); anal. calcd. for $C_{16}H_{15}N_3O_2S_2$ (345.4392): C 55.63, H 4.38, N 12.16; found: C 55.51, H 4.36, N 12.27.



Ethyl 4-ethyl-2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylate 43c.

43c was isolated by column chromatography on silica gel (acetate/cyclohexane) in 60% yield. Pale brown solid; mp: 105–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.32 (t, 3H, *J* = 7.6 Hz, $CH_2\mathit{CH_3}$), 1.45 (t, 3H, *J* = 7.2 Hz, $OCH_2\mathit{CH_3}$), 3.08 (q, 2H, *J* = 7.6 Hz, $\mathit{CH_2}CH_3$), 4.42 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 7.10-7.17 (m, 1H, Ph), 7.34-7.42 (m, 4H, Ph), 8.36 (s, 1H, SCH), 10.50 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.2 (q), 14.5 (q), 22.8 (t), 60.3 (t), 107.4 (s), 109.3 (s), 119.6 (d), 123.9 (d), 128.5 (d), 129.6 (d), 140.4 (s), 142.0 (s), 156.5 (s), 161.0 (s), 166.6 (s); IR (nujol): ν_{max} = 3114, 1647 cm^{-1}; MS *m/z* (ESI): 360.16 (M + H$^+$); anal. calcd. for $C_{17}H_{17}N_3O_2S_2$ (359.4658): C 56.80, H 4.77, N 11.69; found: C 56.96, H 4.79, N 11.59.

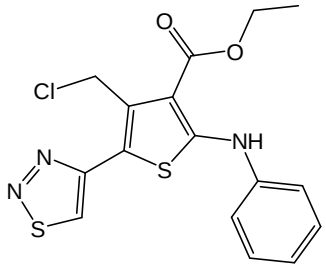


Methyl -2-((4-chlorophenyl)amino)-4-ethyl-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylate 43d.

43d was isolated by column chromatography on silica gel (acetate/cyclohexane) in 63% yield. Pale brown solid; mp: 120–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.30 (t, 3H, *J* = 7.6 Hz, $CH_2\mathit{CH_3}$), 3.06 (q, 2H, *J* = 7.6 Hz, $\mathit{CH_2}CH_3$), 3.95 (s, 3H, OCH_3), 7.30-7.37 (m, 4H, Ph), 8.37 (s, 1H, SCH), 10.45 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.4 (q), 22.8 (t), 51.4 (q), 107.7 (s), 109.7 (s), 120.9 (d), 128.7 (d), 128.9 (s), 129.6 (d), 138.9 (s), 142.0 (s), 156.3 (s), 160.6 (s), 167.0 (s); IR (nujol): ν_{max} = 3180, 1686 cm^{-1}; MS *m/z* (ESI): 380.43 (M + H$^+$); anal. calcd. for $C_{16}H_{14}ClN_3O_2S_2$ (379.8843): C 50.59, H 3.71, N 11.06; found: C 50.68, H 3.70, N 11.00.

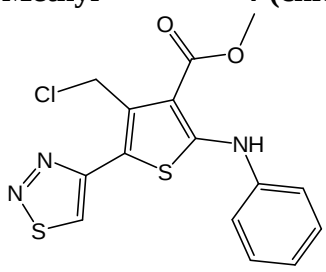
General Procedure for the synthesis of 2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylates (ATTs) 43e-h starting from 2-arylamino 5-hydrazono thiophene-3-carboxylates (AHTs) 41a,b,d,f.

To a solution of 2-arylamino 5-hydrazono thiophene-3-carboxylates (AHTs) **41a,b,d,f**. (0.5 mmol) in CH_2Cl_2 (4.0 mL) thionyl chloride (5.0 mmol) were added and the reaction mixture was softly stirred at room temperature. At the disappearance of the starting 2-arylamino 5-hydrazono thiophene-3-carboxylates (AHTs) **41** (15 h – 18h monitored by TLC). Then, the crude was neutralized by adding saturated aqueous solution of sodium hydrogen carbonate until pH ~ 7 and then extracted with fresh CH_2Cl_2 (5.0 mL x 3). The organic layer was washed with water and dried on sodium sulphate. The reaction solvent was evaporated under reduced pressure and the desired 2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylates (ATTs) **43e-h** were purified by chromatography on silica gel column (elution mixture: cyclohexane: ethyl acetate).



Ethyl 4-(chloromethyl)-2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylate 43e.

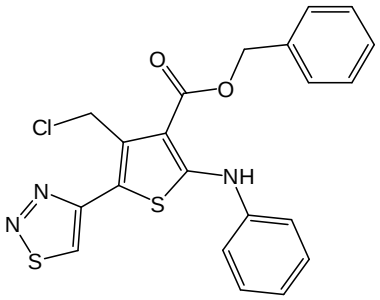
43e was isolated by column chromatography on silica gel (acetate/cyclohexane) in 56% yield. Dark green solid; mp: 98–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.49 (t, 3H, *J* = 7.2 Hz, $OCH_2\mathit{CH_3}$), 4.46 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 5.06 (s, 2H, $\mathit{CH_2}Cl$), 7.16 (t, 1H, *J* = 7.2 Hz, Ph), 7.36-7.44 (m, 4H, Ph), 8.79 (s, 1H, SCH), 10.40 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.2 (q), 39.6 (t), 60.8 (t), 106.0 (s), 114.5 (s), 120.0 (d), 124.5 (d), 129.7 (d), 130.9 (d), 133.5 (s), 140.0 (s), 155.0 (s), 161.2 (s), 165.9 (s); IR (nujol): ν_{max} = 3193, 1661 cm^{-1}; MS *m/z* (ESI): 380.49 ($M + H^+$); anal. calcd. for $C_{16}H_{14}ClN_3O_2S_2$ (379.8843): C 50.59, H 3.71, N 11.06; found: C 50.72, H 3.76, N 10.92.



Methyl 4-(chloromethyl)-2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylate 43f.

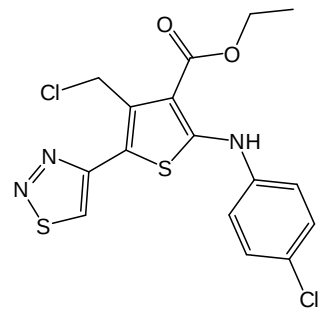
43f was isolated by column chromatography on silica gel (acetate/cyclohexane) in 58% yield. Dark green solid; mp: 97–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.00 (s, 3H, OCH_3), 5.00 (s, 2H, $\mathit{CH_2}Cl$), 7.18 (t, 1H, *J* = 6.8 Hz, Ph), 7.37-7.44 (m, 4H, Ph), 8.79 (s, 1H, SCH), 10.34 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.2 (q), 14.4 (q), 38.9 (t), 39.4 (t), 60.9 (t), 61.0 (t), 106.5 (s), 106.6 (s), 113.9 (s), 114.9 (s), 121.0 (d), 121.0 (d), 129.3 (s),

129.4 (s), 129.7 (d), 129.7 (d), 131.0 (d), 131.6 (d), 132.0 (s), 133.5 (s), 138.6 (s), 154.8 (s), 154.9(s), 160.6 (s), 160.7 (s), 165.8 (s), 165.8 (s) IR (nujol): ν_{max} = 3154, 1675 cm^{-1}; MS *m/z* (ESI): 366.26 ($M + H^+$); anal. calcd. for $C_{15}H_{12}ClN_3O_2S_2$ (365.8577): C 49.24, H 3.31, N 11.49; found: C 49.11, H 3.27, N 11.57.



Benzyl 4-(chloromethyl)-2-(phenylamino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylate 43g.

43g was isolated by column chromatography on silica gel (acetate/cyclohexane) in 62% yield. Dark green solid; mp: 116–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.97 (s, 2H, O*CH*$_2$Ph), 5.44 (s, 2H, *CH*$_2$Cl), 7.17 (t, 1H, *J* = 7.2 Hz, Ph), 7.34-7.45 (m, 7H, Ph),7.55 (d, 2H, *J* = 6.8 Hz, Ph), 8.78 (s, 1H, SCH), 10.35 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 39.5 (t), 66.7 (t), 105.6 (s), 114.6 (s), 120.1 (d), 124.6 (d), 128.4 (d), 128.4 (d), 128.7 (d), 129.7 (d), 130.9 (d), 133.5 (s), 135.7 (s), 139.9 (s), 154.9 (s), 161.5 (s), 165.5 (s); IR (nujol): ν_{max} = 3128, 1654 cm^{-1}; MS *m/z* (ESI): 442.58 ($M + H^+$); anal. calcd. for $C_{21}H_{16}ClN_3O_2S_2$ (441.9536): C 57.07, H 3.65, N 9.51; found: C 57.21, H 3.68, N 9.42.



Ethyl 4-(chloromethyl)-2-((4-chlorophenyl)amino)-5-(1,2,3-thiadiazol-4-yl)thiophene-3-carboxylate 43h.

43h was isolated by column chromatography on silica gel (acetate/cyclohexane) in 64% yield. Dark green solid; mp: 101–103 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.46-1.50 (m, 3H, OCH$_2$*CH*$_3$), 4.42-4.47 (m, 2H, O*CH*$_2$CH$_3$), 4.72 and 4.98 (2s, 2H, *CH*$_2$Cl), 7.28-7.31 (m, 2H, Ph), 7.34-7.37 (m, 2H, Ph), 8.79 and 8.84 (2s, 1H, SCH), 10.42 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.2 (q), 14.4 (q), 38.9 (t), 39.4 (t), 60.9 (t), 61.0 (t), 106.5 (s), 106.6 (s), 113.9 (s), 114.9 (s), 121.0 (d), 121.0 (d), 129.3 (s), 129.4 (s), 129.7 (d), 129.7 (d), 131.0 (d), 131.6 (d), 132.0 (s), 133.5 (s), 138.6 (s), 154.8 (s), 154.9(s), 160.6 (s), 160.7 (s), 165.8 (s), 165.8 (s) IR (nujol): ν_{max} = 3119, 1657 cm^{-1}; MS *m/z* (ESI): 415.88 ($M + H^+$); anal. calcd. for $C_{16}H_{13}Cl_2N_3O_2S_2$ (414.3293): C 46.38, H 3.16, N 10.14; found: C 46.25, H 3.19, N 10.05.

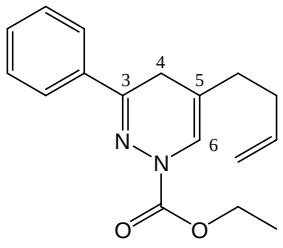
CHAPTER 5

Experimental Section

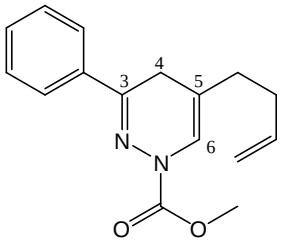
All chemicals and solvents were purchased from commercial suppliers and used as received. 1,2-Diaza-1,3-dienes were prepared as reported[99] and used as *EE/EZ* isomer mixtures. Melting points were determined in open capillary tubes and are uncorrected. FTIR spectra were obtained as Nujol mulls. All ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Proton and carbon spectra were referenced internally to solvent signals, using values of δ = 7.26 ppm for proton and δ = 77.00 ppm for carbon (middle peak) in $CDCl_3$. Precoated silica gel plates 0.25 mm were employed for analytical thin layer chromatography. All new compounds showed satisfactory elemental analysis. Mass spectra were recorded in the EI mode (70eV). The nomenclature was generated using ACD/IUPAC Name (version 3.50, 5 Apr. 1998), Advanced Chemistry Development Inc., Toronto, ON (Canada).

General Procedure for the Synthesis of the γ-adduct 3a–s.

To a magnetically stirred solution of tetrahydropyridazine **18** (0.1 mmol 1.0 eq) in DCM (2 mL) at room temperature, a nucleophile **44a-i** (0.1 eq), and $BF_3(OEt)_2$ (1.2 eq) as promoter, was added consequentially. After the disappearance of the reagents (0.1–0.5 h) (TLC monitoring), the reaction solvent was evaporated under reduced pressure and the crude mixture was purified by column chromatography on silica gel (ethyl acetate/cyclohexane mixtures) to afford the corresponding adduct.

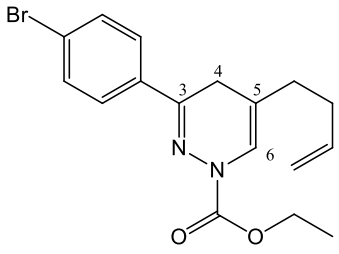


hyl 5-(but-3-enyl)-3-phenylpyridazine-1(4*H*)-carboxylate (45a):
Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 80% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.40 (t, 3H, $OCH_2\mathit{CH_3}$), 2.17–2.21 (m, 2H $\mathit{CH_2}CH_2CH{=}CH_2$), 2.25–2.31 (m, 2H $CH_2\mathit{CH_2}CH{=}CH_2$), 3.17 (s, 2H, $C\mathit{CH_2}C$), 4.34–4.40 (q, 2H, $O\mathit{CH_2}CH_3$), 4.99–5.10 (m, 2H, $CH_2CH_2CH{=}\mathit{CH_2}$), 5.78–5.88 (m, 1H, $CH_2CH_2\mathit{CH}{=}CH_2$), 6.99 (s, 1H, N*CH*), 7.40–7.42 (m, 3H, Ph), 7.82–7.84 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 26.3 (t), 30.7 (t), 33.3 (t), 62.9 (t), 113.7 (s), 116.0 (t), 117.6 (s), 125.9 (d), 128.3 (d), 129.8 (d), 136.4 (s), 137.4 (d), 146.5 (s), 152.6 (s); IR (nujol): ν_{max} = 1697 cm^{-1}; MS *m/z* (ESI): 285.03 ($M + H^+$); anal. calcd. for $C_{17}H_{20}N_2O_2$ (284,35): C 71.81, H 7.09, N 9.85; found: C 71.68, H 5.05, N 9.96.



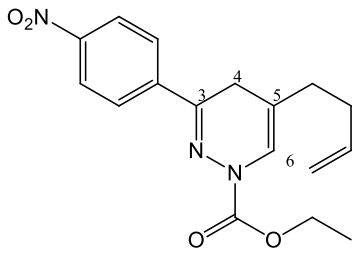
Methyl 5-(but-3-en-1-yl)-3-phenylpyridazine-1(4*H*)-carboxylate (45b):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 69% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C δ = 2.17–2.21 (m, 2H *CH*$_2$CH$_2$CH=CH$_2$), 2.25–2.31 (m, 2H CH$_2$*CH*$_2$CH=CH$_2$), 3.17 (s, 2H, C*CH*$_2$C), 3.92 (s, 3H, O*CH*$_3$), 4.99–5.10 (4m, 2H, CH$_2$CH$_2$CH=*CH*$_2$), 5.77–5.87 (m, 1H, CH$_2$CH$_2$*CH*=CH$_2$), 7.00 (s, 1H, N*CH*), 7.40–7.41 (m, 3H, Ph), 7.81–7.83 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 26.5 (t), 30.7 (t), 33.3 (t), 53.8 (q), 113.9 (s), 115.4 (t), 117.6 (s), 126.0 (d), 128.4 (d), 129.9 (d), 136.4 (s), 137.4 (d), 146.4 (s), 152.6 (s); IR (nujol): ν_{max} = 1717 cm^{-1}; MS *m/z* (ESI): 271.01 (M + H$^+$); anal. calcd. for $C_{16}H_{18}N_2O_2$ (270,33): C, 71.09; H, 6.71; N, 10.36; found: C, 71.22; H, 6.64; N, 10.25;



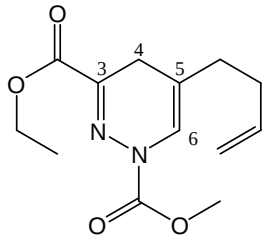
Ethyl 3-(4-bromophenyl)-5-(but-3-en-1-yl)pyridazine-1(4*H*)-carboxylate (45c):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 75% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.38 (t, 3H, OCH$_2$*CH*$_3$), 2.16–2.20 (m, 2H *CH*$_2$CH$_2$CH=CH$_2$), 2.24–2.28 (m, 2H CH$_2$*CH*$_2$CH=CH$_2$), 3.13 (s, 2H, C*CH*$_2$C), 4.34–4.40 (q, 2H, O*CH*$_2$CH$_3$), 5.00–5.09 (4m, 2H, CH$_2$CH$_2$CH=*CH*$_2$), 5.78–5.84 (m, 1H, CH$_2$CH$_2$*CH*=CH$_2$), 6.98 (s, 1H, N*CH*), 7.54 (d, 2H, PhBr), 7.70 (d, 2H, PhBr); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 26.2 (t), 30.6 (t), 33.3 (t), 63.0 (t), 113.6 (s), 115.4 (t), 117.6 (s), 124.1 (d), 127.5 (d), 131.5 (s), 135.3 (s), 137.4 (d), 144.8 (s), 152.6 (s); IR (nujol): ν_{max} = 1723 cm^{-1}; MS *m/z* (ESI): 363.89 (M + H$^+$); anal. calcd. for $C_{17}H_{19}BrN_2O_2$ (363,25): C, 56.21; H, 5.27; Br, 22.00; N, 7.71; found: C, 56.36; H, 5.19; Br, 22.05; N, 7.63



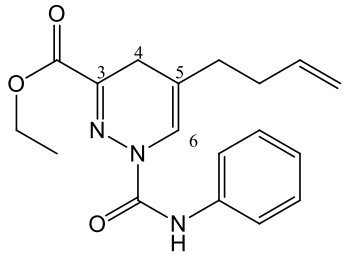
Ethyl 5-(but-3-enyl)-3-(4-nitrophenyl)pyridazine-1(4*H*)-carboxylate (45d):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 79% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.40 (t, 3H, $OCH_2\mathit{CH_3}$), 2.18–2.22 (m, 2H $\mathit{CH_2}CH_2CH{=}CH_2$), 2.26–2.35 (m, 2H $CH_2\mathit{CH_2}CH{=}CH_2$), 3.19 (s, 2H, $C\mathit{CH_2}C$), 4.36–4.40 (q, 2H, $O\mathit{CH_2}CH_3$), 5.01–5.10 (4m, 2H, $CH_2CH_2CH{=}\mathit{CH_2}$), 5.81–5.83 (m, 1H, $CH_2CH_2\mathit{CH}{=}CH_2$), 6.99 (s, 1H, N*CH*), 7.98 (d, 2H, $PhNO_2$), 8.25 (d, 2H, Ph NO_2); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 26.2 (t), 30.5 (t), 33.2 (*t*), 63.3 (t), 114.0 (*s*), 115.6 (t), 117.5 (s), 123.6 (d), 126.7 (d), 136.4 (s), 137.2 (d), 142.3 (s), 148.3 (s), 152.0 (s); IR (nujol): ν_{max} = 1689cm^{-1}; MS *m/z* (ESI): 330.96 (M + H$^+$); anal. calcd. for $C_{17}H_{19}N_3O_4$ (329,35): C, 62.00; H, 5.81; N, 12.76; found: C, 62.14; H, 5.85; N, 12.66



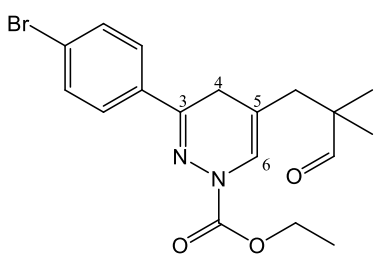
3-Ethyl 1-methyl 5-(but-3-en-1-yl)pyridazine-1,3(4*H*)-dicarboxylate (45e):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 26% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C δ = 1.38 (t, 3H $OCH_2\mathit{CH_3}$), 2.10–2.14 (m, 2H $\mathit{CH_2}CH_2CH{=}CH_2$), 2.20–2.23 (m, 2H $CH_2\mathit{CH_2}CH{=}CH_2$), 3.04 (s, 2H, $C\mathit{CH_2}C$), 3.91 (s, 3H, $O\mathit{CH_3}$), 4.33–4.37 (q, 2H $O\mathit{CH_2}CH_3$) 5.01–5.07 (4m, 2H, $CH_2CH_2CH{=}\mathit{CH_2}$), 5.74–5.81 (m, 1H, $CH_2CH_2\mathit{CH}{=}CH_2$), 6.85 (s, 1H, N*CH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.1 (q), 25.3 (t), 30.3 (t), 33.0 (t), 54.4 (q), 62.2 (t), 115.6 (s), 116.1 (t), 117.0 (s), 137.4 (d), 139.8 (s), 152.4 (s) 164.0 (s); IR (nujol): ν_{max} = 1756, 1723 cm^{-1}; MS *m/z* (ESI): 266.91 (M + H$^+$); anal. calcd. for $C_{13}H_{18}N_2O_4$ (266,29): C, 58.63; H, 6.81; N, 10.52; found: C, 58.74; H, 6.75; N, 10.59;



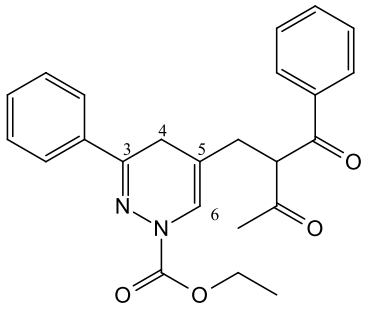
Ethyl 5-(but-3-enyl)-1-(phenylcarbamoyl)-1,4-dihydropyridazine-3-carboxylate (45f):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 31% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C δ = 1.39 (t, 3H $OCH_2\mathit{CH_3}$), 2.12–2.16 (m, 2H $\mathit{CH_2}CH_2CH{=}CH_2$), 2.22–2.27 (m, 2H $CH_2\mathit{CH_2}CH{=}CH_2$), 3.11 (s, 2H, $C\mathit{CH_2}C$), 4.33–4.38 (q, 2H $O\mathit{CH_2}CH_3$) 4.99–5.09 (4m, 2H, $CH_2CH_2CH{=}\mathit{CH_2}$), 5.79–5.80 (m, 1H, $CH_2CH_2\mathit{CH}{=}CH_2$), 7.08–7.12 (m, 2H, N*CH* and NHph), 7.31–7.35 (m, 2H, NHph), 7.51–7.53 (m, 2H, NHph), 8.62 (s, H, N*H*ph);^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.2 (q), 25.5 (t), 30.2 (t), 33.2 (t), 61.9 (t), 114.9 (s), 115.4 (t), 115.5 (s), 119.7 (d), 123.8 (s), 129.0 (d), 136.6 (s), 137.2 (d), 137.5 (s), 149.6 (s) 163.7 (s); IR (nujol): ν_{max} = 3266, 1669, 1624 cm^{-1}; MS *m/z* (ESI): 328.01 (M + H$^+$); anal. calcd. for $C_{18}H_{21}N_3O_3$ (327,38): C, 66.04; H, 6.47; N, 12.84; found: C, 66.19; H, 6.41; N, 12.76



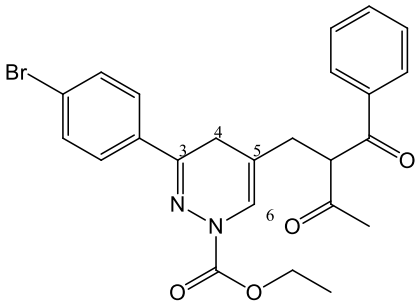
Ethyl 3-(4-bromophenyl)-5-(2,2-dimethyl-3-oxopropyl)pyridazine-1(4*H*)-carboxylate (45g):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 65% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.12 (s, 6H $CH_2C(\mathit{CH_3})_2COH$) 1.39 (t, 3H, $OCH_2\mathit{CH_3}$), 2.32 (s, 2H $\mathit{CH_2}C(CH_3)_2COH$), 3.01 (s, 2H, $C\mathit{CH_2}C$), 4.34–4.39 (q, 2H, $O\mathit{CH_2}CH_3$), 6.98 (s, 1H, N*CH*), 7.52 (d, 2H, PhBr), 7.64 (d, 2H, PhBr), 9.58 (s, 1H $CH_2C(CH_3)_2CO\mathit{H}$); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 22.1 (q), 27.6 (t), 41.6 (t), 46.2 (s), 63.2 (t), 110.2 (s), 120.4 (s), 120.7 (d), 124.4 (s), 127.6 (d), 131.6 (d), 135.0 (s), 139.4 (s), 144.9 (s), 205.6 (d); IR (nujol): ν_{max} = 1742, 1620 cm^{-1}; MS *m/z* (ESI): 538.69 (M + H$^+$); anal. calcd. for $C_{18}H_{21}BrN_2O_3$ (393,27): C, 54.97; H, 5.38; Br, 20.32; N, 7.12; found: C, 54.82; H,



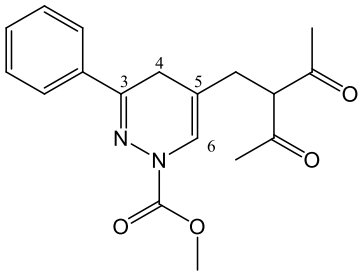
Ethyl 5-(2-benzoyl-3-oxobutyl)-3-phenylpyridazine-1(4*H*)-carboxylate (45h):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 95% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.37 (t, 3H, $OCH_2\mathit{CH_3}$), 2.17 (s, 3H $\mathit{CH_3}CO$), 2.81 (d, 2H $C\mathit{CH_2}CH$), 3.15 (s, 2H, $C\mathit{CH_2}C$), 4.31–4.39 (q, 2H, $O\mathit{CH_2}CH_3$), 4.70 (t, 1H, $CCH_2\mathit{CH}$), 7.01 (s, 1H, $N\mathit{CH}$), 7.49–8.01 (5m, 10H, Ph and COPh); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 26.4 (q), 28.0 (t), 33.0 (t), 61.0 (d), 63.0 (t), 110.5 (d), 119.4 (s), 126.0 (d), 128.4 (d), 128.7 (d), 129.0 (d), 130.0 (d), 134.0 (d), 136.0 (s), 136.1 (s), 145.9 (s), 153.9 (s), 195.4 (s), 203.0 (s); IR (nujol): ν_{max} = 1751, 1744, 1692 cm^{-1}; MS *m/z* (ESI): 405.12 (M + H$^+$); anal. calcd. for $C_{24}H_{24}N_2O_4$ (404,46): C, 71.27; H, 5.98; N, 6.93; found: C, 71.10; H, 5.92; N, 6.8575.



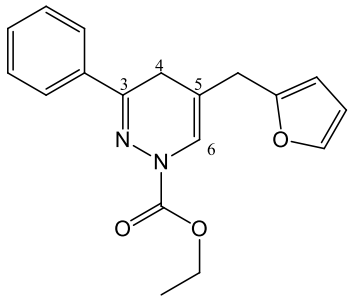
Ethyl 5-(2-benzoyl-3-oxobutyl)-3-(4-bromophenyl)pyridazine-1(4*H*)-carboxylate (45i):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 86% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.36 (t, 3H, $OCH_2\mathit{CH_3}$), 2.17 (s, 3H $\mathit{CH_3}CO$), 2.80 (d, 2H $C\mathit{CH_2}CH$), 3.10 (s, 2H, $C\mathit{CH_2}C$), 4.30–4.38 (q, 2H, $O\mathit{CH_2}CH_3$), 4.69 (t, 1H, $CCH_2\mathit{CH}$), 6.99 (s, 1H, $N\mathit{CH}$), 7.49–8.00 (3m, 9H, PhBr and COPh); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 26.2 (q), 28.0 (t), 32.9 (t), 61.0 (d), 63.1 (t), 110.5 (d), 119.4 (s), 124.3 (d), 127.5 (d), 128.7 (d), 129.0 (d), 131.5 (d), 134.0 (s), 134.9 (s), 136.1 (s), 144.7 (s), 152.2 (s), 195.3 (s), 202.9 (s); IR (nujol): ν_{max} = 1741, 1733, 1679 cm^{-1}; MS *m/z* (ESI): 538.69 (M + H$^+$); anal. calcd. for $C_{24}H_{23}BrN_2O_4$ (483,35): C, 59.64; H, 4.80; Br, 16.53; N, 5.80; found: C, 59.51; H, 4.75; Br, 16.62; N, 5.89.



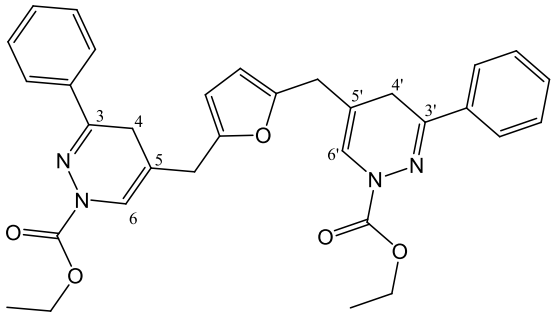
Methyl 5-(2-acetyl-3-oxobutyl)-3-phenylpyridazine-1(4*H*)-carboxylate (45j):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 42% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.14 (2s, 6H, CO*CH*$_3$ enol form), and 2.22 (2s, 6H, CO*CH*$_3$), 2.64 (d 2H C*CH*$_2$CH) 2.99 (s 2H C*CH*$_2$C= enol form), 3.14 (2s, 2H, C*CH*$_2$C), 3.19 (2s, 2H, C*CH*$_2$C enol form), 3.92 (s, 3H, O*CH*$_3$), 3.95–3.99 (m, 1H, CCH$_2$*CH*), 6.89 (2s, 1H, N*CH* enol form) 7.01 (2s, 1H, N*CH*), 7.40–7.43 and 7.80–7.82 (2m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 23.07 (q), 26.4 (t) 26.8 (t, enol form), 29.1 (t, enol form) 29.7 (t), 32.0 (t), 54.0 (t), 66.1 (s), 104.9 (s, enol form) 112.7 (d, enol form), 117.8 (s) 119.4 (s), 125.9 and 126.0 (d), 128.4 (d), 136.0 and 136.1 (s), 145.9 (s), 153.0 (s), 191.8 (s), 202.9 (s); IR (nujol): ν_{max} = 1736, 1672, 1657 cm^{-1}; MS *m/z* (ESI): 328.88 (M + H$^+$); anal. calcd. for $C_{18}H_{20}N_2O_4$ (328,36): C, 65.84; H, 6.14; N, 8.53; found: C, 65.71; H, 6.19; N, 8.47.



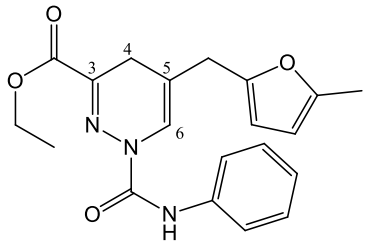
Ethyl 5-(furan-2-ylmethyl)-3-phenylpyridazine-1(4*H*)-carboxylate (45k):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:4) in 58% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.40 (t, 3H, OCH$_2$*CH*$_3$), 3.15 (s, 2H, C*CH*$_2$C), 3.43 (s, 2H, C*CH*$_2$C$_4$H$_4$O), 4.35–4.40 (q, 2H, O*CH*$_2$CH$_3$), 6.12 (dd, 1H, C*CH*CHCHO), 6.31 (dd, 1H, C*C*HC*H*CHO), 7.11 (s, 1H, N*CH*), 7.34 (dd, 1H, C*C*HCHC*H*O), 7.34–7.40 and 7.79–7.81 (2m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 26.0 (t), 32.9 (t), 63.0 (t), 106.9 (d), 110.4(d), 110.8 (s), 118.9 (d), 126.0 (d), 128.3 (d), 129.8 (d), 136.4 (s), 141.8 (d), 146.5 (s), 151.6 (s), 152.6 (s); IR (nujol): ν_{max} = 1673 cm^{-1}; MS *m/z* (ESI): 311.03 (M + H$^+$); anal. calcd. for $C_{18}H_{18}N_2O_3$ (310,35): C, 69.66; H, 5.85; N, 9.03; found: C, 69.73; H, 5.81; N, 9.09.



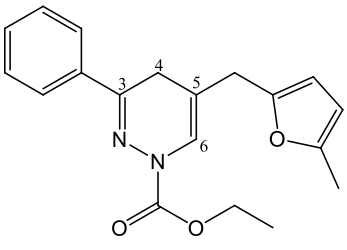
Diethyl 5,5'-[furan-2,5-diylbis(methylene)]bis(3-phenylpyridazine-1(4*H*)-carboxylate) (45l):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 2:3) in 39% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.39 (t, 6H, $OCH_2\mathit{CH_3}$), 3.15 (s, 4H, $C\mathit{CH_2}C$), 3.38 (s, 4H, $C\mathit{CH_2}C_4H_4O$), 4.33–4.38 (q, 4H, $O\mathit{CH_2}CH_3$), 6.03 (s, 2H, -C*CH*C*H*C), 7.09 (s, 2H, N*CH*), 7.37–7.40 and 7.77–7.80 (2m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.5 (q), 26.0 (t), 32.9 (t), 63.0 (t), 106.9 (d), 110.4(d), 110.8 (s), 118.9 (d), 126.0 (d), 128.3 (d), 129.8 (d), 136.4 (s), 141.8 (d), 146.5 (s), 151.6 (s), 152.6 (s); IR (nujol): ν_{max} = 1710, 1688 cm^{-1}; MS *m/z* (ESI): 553.27 ($M + H^+$); anal. calcd. for $C_{32}H_{32}N_4O_5$ (552,62): C, 69.55; H, 5.84; N, 10.14; found: C, 69.49; H, 5.91; N, 10.08.



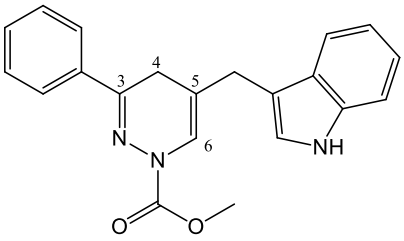
Ethyl 5-((5-methylfuran-2-yl)methyl)-1-(phenylcarbamoyl)-1,4-dihydropyridazine-3-carboxylate (45m):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:4) in 30 % yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.38 (t, 3H, $OCH_2\mathit{CH_3}$), 2.25 (s, 3H, $C\mathit{CH_3}$), 3.09 (s, 2H, $C\mathit{CH_2}C$), 3.31 (s, 2H, $C\mathit{CH_2}C_4H_3O$), 4.31–4.36 (q, 2H, $O\mathit{CH_2}CH_3$), 5.87 (d, 1H, $C\mathit{CH}CHCOCH_3$), 5.98 (d, 1H, $C\mathit{C}HC\mathit{H}COCH_3$), 7.10 (t, 1H NHph),7.17 (s, 1H, N*CH*), 7.34 and 7.52 (t,d, 4H, NHPh), 8.61 (s, 1H N*H*ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.6 (q),14.5 (q), 25.3 (t), 32.9 (t), 61.9 (t), 106.2 (d), 107.9 (d), 112.6 (s), 116.5 (d), 119.7 (d), 123.9 (d), 129.0 (d), 137.0 (s), 137.4 (s), 148.7 (s), 149.6 (s), 151.5 (s), 163.6 (s); IR (nujol): ν_{max} = 3265, 1733, 1645 cm^{-1}; MS *m/z* (ESI): 368.06 ($M + H^+$); anal. calcd. for $C_{20}H_{21}N_3O_4$ (367,40): C, 65.38; H, 5.76; N, 11.44; found: C, 65.25; H, 5.74; N, 11.54.



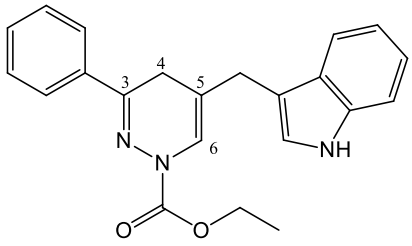
Ethyl 5-((5-methylfuran-2-yl)methyl)-3-phenylpyridazine-1(4*H*)-carboxylate (45n):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:4) in 94% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.40 (t, 3H, OCH_2CH_3), 2.26 (s, 3H, CCH_3), 3.15 (s, 2H, CCH_2C), 3.37 (s, 2H, $CCH_2C_4H_3O$), 4.35–4.40 (q, 2H, OCH_2CH_3), 5.87 (d, 1H, $CCHCHCOCH_3$), 5.98 (d, 1H, $CCHCHCOCH_3$), 7.10 (s, 1H, N*CH*), 7.39–7.40 and 7.79–7.82 (2m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.5 (q) 14.5 (q), 25.9 (t), 33.0 (t), 63.0 (t), 106.1 (d), 107.6 (d), 111.1 (s), 118.8 (d), 126.0 (d), 128.3 (d), 129.8 (d), 136.3 (s), 146.2 (d), 149.5 (s), 151.3 (s), 152.4 (s); IR (nujol): ν_{max} = 1716 cm^{-1}; MS *m/z* (ESI): 324.89 ($M + H^+$); anal. calcd. for $C_{19}H_{20}N_2O_3$ (324,37): C, 70.35; H, 6.21; N, 8.64; found: C, 70.20; H, 6.25; N, 8.58.



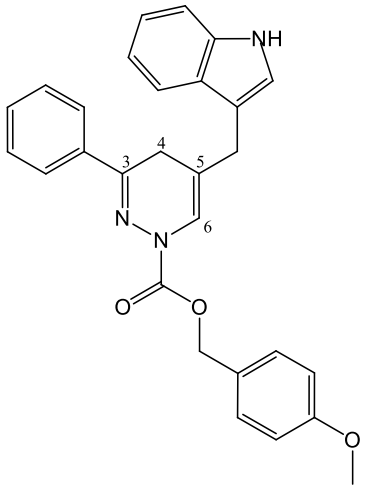
Methyl 5-[(1*H*-indol-3-yl)methyl]-3-phenylpyridazine-1(4*H*)-carboxylate (45o):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 2:3) in 50% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 3.15 (s, 2H, CCH_2C), 3.57 (s, 2H, $CCH_2C_8H_7N$), 3.94 (s, 3H, OCH_3), 7.03 (d, 1H, Indole), 7.13–7.21, (m, 3H, N*CH*, Indole) 7.35–7.37 (m, 4H, Ph, Indole) 7.64 (d, 1H, Indole) 7.73–7.75 (m, 2H, Ph) 8.14 (s, 1H *NH* Indole); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 26.2 (t), 30.1 (t), 53.8 (q), 111.2 (d), 111.4 (s), 114.0 (s), 117.8 (d), 118.8 (d), 119.5 (d), 122.1 (d), 122.5 (d), 126.0 (d), 127.5 (s), 128.3 (d), 129.8 (d), 136.3 (s), 136.4 (s), 146.9 (s), 153.3 (s); IR (nujol): ν_{max} = 3241, 1698 cm^{-1}; MS *m/z* (ESI): 346.01 ($M + H^+$); anal. calcd. for $C_{21}H_{19}N_3O_2$ (345,39): C, 73.03; H, 5.54; N, 12.17; found: C, 73.17; H, 5.58; N, 12.10.



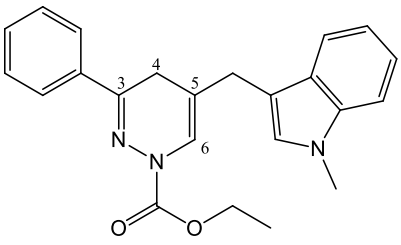
Ethyl 5-[(1*H*-indol-3-yl)methyl]-3-phenylpyridazine-1(4*H*)-carboxylate (45p):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 2:3) in 39% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.41 (s, 3H OCH_2CH_3) 3.15 (s, 2H, CCH_2C), 3.57 (s, 2H, $CCH_2C_8H_7N$), 4.36–4.42 (q, 2H, OCH_2CH_3), 7.04 (d, 1H, Indole), 7.13–7.26, (2m, 3H, N*CH*, Indole) 7.32–7.38 (m, 4H, Ph, Indole) 7.64 (d, 1H, Indole) 7.74–7.76 (m, 2H, Ph) 8.12 (s, 1H *NH* Indole); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 15.5 (q), 26.1 (t), 30.2 (t), 62.9 (t), 111.2 (d), 111.6 (s), 113.7(s), 117.9 (d), 118.9 (d), 119.5 (d), 122.1 (d), 122.5 (d), 126.0 (d), 127.5 (s), 128.2 (d), 129.7 (d), 136.4 (s), 136.4 (s), 146.4 (s), 152.5 (s); IR (nujol): ν_{max} = 3232, 1724, cm^{-1}; MS *m/z* (ESI): 360.07 ($M + H^+$); anal. calcd. for $C_{22}H_{21}N_3O_2$ (359,42): C, 73.52; H, 5.89; N, 11.69; found: C, 73.67; H, 5.96; N, 11.74.



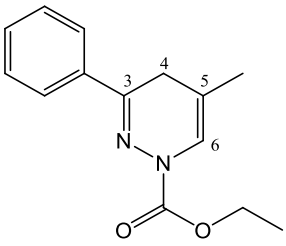
4-Methoxybenyl 5-[(1*H*-indol-3-yl)methyl]-3-phenylpyridazine-1(4*H*)-carboxylate (45r):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 2:3) in 47% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 3.14 (s, 2H, CCH_2C), 3.55 (s, 2H, $CCH_2C_8H_7N$), 3.81 (s, 3H, OCH_3), 5.39 (s, 2H, OCH_2ph), 6.90 (dd, 2H OphO), 7.04 (d, 1H, Indole), 7.10–7.20, (m, 3H, N*CH*, Indole) 7.35–7.30 (m, 6H, Ph, OphO, Indole) 7.62 (d, 1H, Indole) 7.73–7.75 (m, 2H, Ph) 8.07 (s, 1H *NH* Indole); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 26.1 (t), 29.7 (t), 30.1 (t), 55.3 (q), 68.1 (t), 111.2 (d), 111.6 (s), 113.9 (s), 117.9 (d), 118.9 (d), 119.6 (d), 122.2 (d), 122.5 (d), 126.0 (d), 127.5 (s), 128.2 (d), 128.2 (d), 128.6 (s), 129.7 (d), 130.0 (d), 136.3 (s), 136.4 (s), 146.6 (s), 152.7 (s), 159.6 (s); IR (nujol): ν_{max} = 3219, 1685, cm^{-1}; MS *m/z* (ESI): 452.17 ($M + H^+$); anal. calcd. for $C_{28}H_{25}N_3O_3$ (451,52): C, 74.48; H, 5.58; N, 9.31; found: C, 74.31; H, 5.52; N, 9.39.



Ethyl 5-[(1-methyl-1*H*-indol-3-yl)methyl]-3-phenylpyridazine-1(4*H*)-carboxylate (45q):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 2:3) in 38% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.40 (s, 3H $OCH_2\mathit{CH_3}$) 3.15 (s, 2H, $C\mathit{CH_2}C$), 3.56 (s, 2H, $C\mathit{CH_2}C_8H_7N$), 3.76 (s, 3H $N\mathit{CH_3}$) 4.35–4.40 (q, 2H, $O\mathit{CH_2}CH_3$), 6.91 (s, 1H, Indole), 7.09–7.31, (2m, 4H, N*CH*, Indole) 7.35–7.37 (m, 3H, Ph) 7.62 (d, 1H, Indole) 7.74–7.76 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.6 (q), 26.1 (t), 30.1 (t), 32.7 (q), 62.9 (t), 109.2 (d), 110.0 (s), 113.9 (s), 117.8 (d), 118.9 (d), 119.0 (d), 121.7 (d), 126.0 (d), 127.2 (d), 127.9 (s), 128.2 (d), 129.7 (d), 136.4 (s), 137.2 (s), 146.4 (s), 152.7 (s); IR (nujol): ν_{max} = 1732 cm^{-1}; MS *m/z* (ESI): 374.10 (M + H$^+$); anal. calcd. for $C_{23}H_{23}N_3O_2$ (373,45): C, 73.97; H, 6.21; N, 11.25; found: C, 73.79; H, 6.25; N, 11.31.



Ethyl 5-methyl-3-phenylpyridazine-1(4*H*)-carboxylate (45s):

Isolated by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:9) in 53% yield. oil; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.40 (s, 3H $OCH_2\mathit{CH_3}$) 1.78 (s, 3H, $C\mathit{CH_3}$), 3.15 (s, 2H, $C\mathit{CH_2}C$), 4.34–4.39 (q, 2H, $O\mathit{CH_2}CH_3$), 6.97 (s, 1H, N*CH*), 7.40–7.42 (m, 3H, Ph), 7.83 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.6 (q), 19.8 (q), 27.9 (t), 62.9 (t), 110.7 (s), 117.2 (d), 125.9 (d), 128.3 (d), 129.8 (d), 136.4 (s), 145.9 (s), 152.6 (s); IR (nujol): ν_{max} = 1674 cm^{-1}; MS *m/z* (ESI): 245.65 (M + H$^+$); anal. calcd. for $C_{14}H_{16}N_2O_2$ (244,29): C, 68.83; H, 6.60; N, 11.47; found: C, 68.96; H, 6.65; N, 11.40.

CHAPTER 6

1. General experimental details.

All chemicals and solvents were purchased from commercial suppliers and used as received, with the exception of berberine chloride. In fact, commercial berberine contains ca. 15% w/w H_2O; the anhydrous product is obtained by heating it at 50 °C under vacuum for 8 hours. 1,2-Diaza-1,3-dienes were prepared as reported[99] and used as *EE*/*EZ* isomers mixtures. Melting points were determined in open capillary tubes and are uncorrected. FTIR spectra were obtained as Nujol mulls. All ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Proton and carbon spectra were referenced internally to solvent signals, using values of δ = 2.50 ppm for proton (middle peak) and δ = 39.50 ppm for carbon (middle peak) in DMSO-d_6 and δ = 7.27 ppm for proton and δ = 77.00 ppm for carbon (middle peak) in $CDCl_3$. All coupling constants (*J*) are given in Hz. All the NH exchanged with D_2O. Precoated aluminium oxide plates 0.25 mm were employed for analytical thin layer chromatography. All new compounds showed satisfactory elemental analysis. Mass spectra were recorded in the ESI and EI modes. The nomenclature was generated using ACD/IUPAC Name (version 3.50, 5 Apr. 1998), Advanced Chemistry Development Inc., Toronto, ON (Canada).

2. Experimental procedures and spectral data.

General procedure for synthesis of 7,8-dihydroberberine 47.

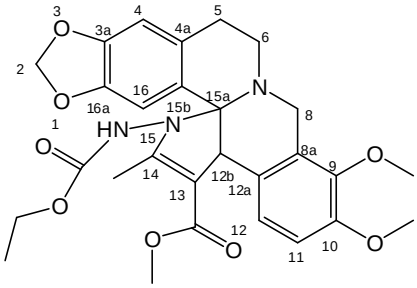
To a solution of berberine chloride (3.718 g, 10 mmol) in pyridine (30 mL) sodium borohydride (450 mg, 12 mmol) was added portion wise and the mixture was stirred at room temperature for 30 min. More sodium borohydride (450 mg, 12 mmol) was added and stirring was continued for 1 h. The reduction reaction was monitored by TLC (elution: dichloromethane-ethyl acetate-methanol 4:4:2). The mixture was poured onto ice water. The precipitate was filtered, the residue washed with water and then dried under vacuum over calcium chloride to give 2.860 g of 7,8-DHB in about 85% yield.

General procedure for synthesis of 8-acetonyl-7,8-dihydroberberine 47'.

To a stirred solution of berberine chloride (3.846 g, 10 mmol) in 5 N Na OH (18 mL) acetone (3.8 mL) was added drop wise. The stirring was continued for 2 h and then filtered and washed with 80% methanol to give 8-acetonyl-7,8-DHB **47'** as dark yellow crystals in about 60% yield.

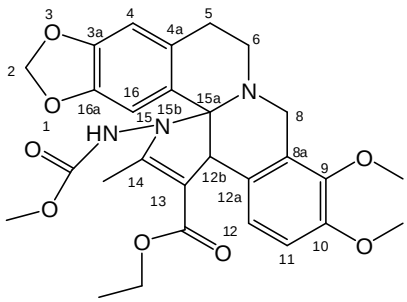
General procedure for synthesis of fused pyrrolino-tetrahydroberberine derivatives 49a–n

A solution of 1,2-diaza-1,3-dienes **1a-n** (0.5 mmol) as a mixture of *EE*/*EZ* isomers[99] and 7,8-dihydroberberines **1**,**2** (0.5 mmol) was stirred in acetonitrile (1.5 mL) at room temperature for 0.25 h, until the disappearance of the reagents and the formation of compounds **49** (TLC monitoring). Products **49d,d'e,e',l** directly precipitated from the reaction medium, while, in the other cases, the solvent was evaporated under reduced pressure at 25 °C and the crude mixtures were then purified by column chromatography on aluminum oxide (elution with cyclohexane-ethyl acetate mixtures) to afford products **49a-d,f-k,m,n**, that were crystallized by concentration under vacuum at 25 °C of the corresponding column fractions.



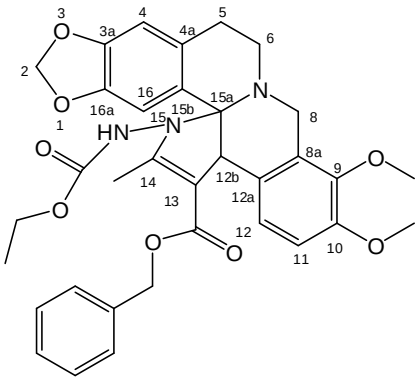
Methyl 15-[(ethoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49a).

49a was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 20:80) in 61% yield. White solid; mp: 120–124 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.151.26 (m, 3H, OCH$_2$*CH$_3$*), 2.12 and 2.16 (2s, 3H, *CH$_3$*), 2.18–2.30 and 2.55–2.65 (2m, 2H, *C(5)H$_2$*), 2.34–2.43 and 2.67–2.78 (2m, 2H, *C(6)H$_2$*), 3.48 and 3.53 (2s, 3H, CO$_2$*CH$_3$*), 3.69 and 3.70 (2s, 3H, O*CH$_3$*), 3.76 (s, 3H, O*CH$_3$*), 3.81–3.88 and 4.42–4.46 (2m, 2H, *C(8)H$_2$*), 4.00–4.09 (m, 2H, O*CH$_2$*CH$_3$), 4.12 and 4.15 (2s, 1H, *C(12b)H*), 5.97 and 5.99 (2s, 2H,O*C(2)H2*O), 6.50 and 6.61 (brs and s, 1H, *C(16)H*), 6.64 and 6.84 (s and brs, 1H, *C(4)H*), 6.78-6.81 (m, 1H, *C(11)H*), 7.04 and 7.10 (2d, 1H, *J* = 8.4 Hz, *J* = 8.4 Hz, *C(12)H*), 8.34, 8.84 and 8.98 (3s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.5 (*CH$_3$*), 14.3 (OCH$_2$*CH$_3$*), 28.5 (*C(5)*), 45.7 (*C(8)*), 46.6 (*C(6)*), 49.7 and 49.8 (*C(12b)*), 59.7 (CO$_2$*CH$_3$*), 55.4 (O*CH$_3$*), 60.3 and 60.5 (O*CH$_3$*), 60.5 and 60.7 (O*CH$_2$*CH$_3$), 86.3 (*C(15a)*), 95.5 and 97.9 (*C(13)*), 100.9 (*C(2)*), 105.3 (*C(16)*), 107.1 (*C(4)*), 109.9 and 109.9 (*C(11)*), 125.3 and 125.6 (*C(12)*), 127.7 (*C(8a)*), 128.3 (*C(12a)*), 128.8 (*C(15b)*), 131.8 (*C(4a)*), 145.1 and 145.4 (*C(9)*), 146.3 (*C(16a)*), 146.5 and 146.7 (*C(3a)*), 150.1 (*C(10)*), 156.6 (NCOO), 160.1 (*C(14)*), 165.5 and 165.6 (*CCOO*); IR (nujol): ν_{max} = 3257, 1749, 1686 cm^{-1}; MS *m/z* (ESI): 538.19 (M + H$^+$); anal. calcd. for $C_{28}H_{31}N_3O_8$ (537.5612): C 62.56, H 5.81, N 7.82; found: C 62.74, H 5.85, N 7.64.



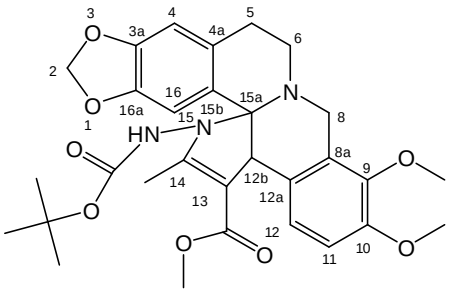
Ethyl 15-[(*meth*oxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49b).

49b was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 40:60) in 83% yield ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.20 (m, 3H, OCH$_2$*CH$_3$*), 2.14 and 2.18 (2s, 3H, *CH$_3$*), 2.22–2.25 and 2.62–2.72 (2m, 2H, *C(5)H$_2$*), 2.38–2.42 and 2.68–2.75 (2m, 2H, *C(6)H$_2$*), 3.61 (s, 3H, O*CH$_3$*), 3.70 (s, 3H, O*CH$_3$*) 3.76 (s, 3H, O*CH$_3$*), 3.84–4.90 and 4.43–4.47 (2m, 2H, *C(8)H$_2$*), 3.91–4.04 (m, 2H, O*CH$_2$*CH$_3$), 4.13 (s, 1H, *C(12b)H*), 5.97 and 6.00 (2s, 2H, ,O*C(2)H2*O), 6.50 and 6.60 (brs and s, 1H, *C(16)H*), 6.65 and 6.86 (s and brs, 1H, *C(4)H*), 6.78–6.81 (m, 1H, *C(11)H*), 7.06 and 7.13 (2d, 1H, *J* = 8.4 Hz, *J* = 8.4 Hz, *C(12)H*), 8.38, 8.88 and 9.07 (3s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.5 (*CH$_3$*), 14.3 (OCH$_2$*CH$_3$*), 28.5 (*C(5)*), 45.7 (*C(8)*), 46.6 (*C(6)*), 50.0 (*C(12b)*), 52.1 (COO*CH$_3$*), 55.4 (O*CH$_3$*), 58.2 and 58.3 (O*CH$_2$*CH$_3$), 60.3 and 60.5 (O*CH$_3$*), 86.3 (*C(15a)*), 97.9 (*C(13)*), 100.9 (*C(2)*), 105.3 (*C(16)*), 107.2 (*C(4)*), 109. 8 (*C(11)*), 125.6 (*C(12)*), 127.7 (*C(8a)*), 128.2 (*C(12a)*), 128.9 (*C(15b)*), 131.8 (*C(4a)*), 145.2 (*C(9)*), 146.3 (*C(16a)*), 146.7 (*C(3a)*), 150.2 (*C(10)*), 157.2 (N*C*OO), 160.0 (*C(14)*), 165.3 (*CCOO*); IR (nujol): ν_{max} = 3235, 1721, 1678 cm^{-1}; MS *m/z* (ESI): 538.03 (M + H$^+$); anal. calcd. for $C_{28}H_{31}N_3O_8$ (537.56): C, 62.56; H, 5.81; N, 7.82; found: C, 62.49; H, 5.86; N, 7.88.



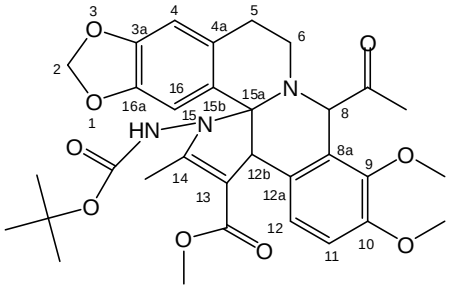
Benzyl 15-[(ethoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49c).

49c was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 40:60) in 92% yield. White solid; mp: 156–159 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.19 (t, *J* = 7.2 Hz, 3H, OCH$_2$*CH$_3$*), 2.14 and 2.17 (2s, 3H, *CH$_3$*), 2.20–2.26 and 2.56–2.64 (2m, 2H, *C(5)H$_2$*), 2.33–2.42 and 2.67–2.89 (2m, 2H, *C(6)H$_2$*), 3.46–3.58 and 4.42–4.46 (2m, 2H, *C(8)H$_2$*), 3.69 and 3.70 (2s, 3H, O*CH$_3$*), 3.76 (s, 3H, O*CH$_3$*), 3.93–4.01 (m, 2H, O*CH$_2$*CH$_3$), 4.16 and 4.18 (2s, 1H, *C(12b)H*), 4.89–5.24 (m, 2H, O*CH$_2$*Ph), 5.90, 5.96, and 5.98 (3s, 2H, ,O*C(2)H2*O), 6.52 and 6.60 (brs and s, 1H, *C(16)H*), 6.67 and 6.89 (s and brs, 1H, *C(4)H*), 6.75–6.82 (m, 1H, *C(11)H*), 7.09 and 7.14 (2d, 1H, *J* = 8.0 Hz, *J* = 8.4 Hz, *C(12)H*), 7.28–7.45 (m, 5H, OCH$_2$*Ph*), 8.49, 9.04 and 9.24 (3s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.5 (*CH$_3$*), 14.2 (OCH$_2$*CH$_3$*), 28.5 (*C(5)*), 45.0 and 45.7 (*C(8)*), 46.7 and 47.0 (*C(6)*), 49.1 (*C(12b)*), 55.4 (O*CH$_3$*), 58.2 and 58.3 (O*CH$_2$*CH$_3$), 60.3 and 60.5 (O*CH$_3$*), 65.9 (O*CH$_2$*Ph), 86.3 (*C(15a)*), 97.8 (*C(13)*), 100.8 and 100.9 (*C(2)*), 105.4 (*C(16)*), 107.1 (*C(4)*), 109.8 (*C(11)*), 125.7 and 126.0 (*C(12)*), 127.1 and 127.4 (OCH$_2$*Ph)*, 127.7 and 127.9 (OCH$_2$*Ph)*, 127.8 (*C(8a)*), 128.2 (*C(12a)*), 128.2 and 128.3 (OCH$_2$*Ph)*, 129.0 (*C(15b)*), 131.8 (*C(4a)*), 136.6 (OCH$_2$*Ph)*, 145.2 and 145.4 (*C(9)*), 146.3 (*C(16a)*), 146.6 and 146.8 (*C(3a)*), 150.2 (*C(10)*), 156.1 and 156.7 (N*C*OO), 159.9 (*C(14)*), 165.2 and 165.3 (*CCOO*); IR (nujol): ν_{max} = 3281, 1758, 1725, 1665 cm^{-1}; MS *m/z* (ESI): 614.22 (M + H$^+$); anal. calcd. for $C_{34}H_{55}N_3O_8$ (613.6571): C 66.55, H 5.75, N 6.85; found: C 66.63, H 5.77, N 6.68.



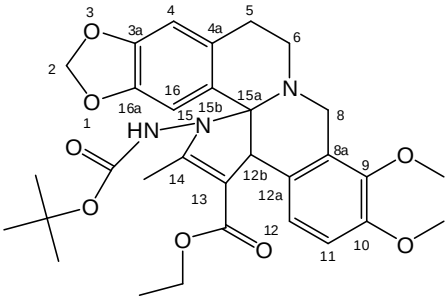
Methyl 15-[(*tert*-butoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49d).

49d was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 40:60) in 60% yield. White solid; mp: 186–188 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.20, 1.40 and 1.46 (3s, 9H, OC(*CH*$_3$)$_3$), 2.03–2.26 and 2.54–2.67 (2m, 2H, *C(5)H*$_2$), 2.12 and 2.15 (2s, 3H, *CH*$_3$), 2.32–2.44 and 2.68–2.80 (2m, 2H, *C(6)H*$_2$), 3.48 and 3.53 (2s, 3H, CO$_2$*CH*$_3$), 3.69 (s, 3H, O*CH*$_3$), 3.76 (s, 3H, O*CH*$_3$), 3.81–3.88 and 4.44–4.48 (2m, 2H, *C(8)H*$_2$), 4.12 and 4.18 (2s, 1H, *C(12b)H*), 5.91–5.99 (m, 2H, O*C(2)H2*O), 6.50 and 6.61 (brs and s, 1H, *C(16)H*), 6.65 and 6.76 (s and brs, 1H, *C(4)H*), 6.78–6.84 (m, 1H, *C(11)H*), 7.03–7.11 (m, 1H, *C(12)H*), 7.95, 8.20 and 8.53 (3s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.4 and 11.6 (*CH*$_3$), 28.0 (OC(*CH*$_3$)$_3$), 28.6 and 28.6 (*C(5)*), 45.7 (*C(8)*), 46.6 (*C(6)*), 49.0 and 49.6 (*C(12b)*), 55.4 and 55.4 (O*CH*$_3$), 60.3 (CO$_2$*CH*$_3$), 60.5 (O*CH*$_3$), 79.4 (O*C*(CH$_3$)$_3$), 86.2 (*C(15a)*), 95.2 and 97.8 (*C(13)*), 100.7 and 100.8 (*C(2)*), 105.4 and 105.4 (*C(16)*), 107.2 (*C(4)*), 109.9 (*C(11)*), 125.2 and 125.7 (*C(12)*), 127.8 (*C(8a)*), 128.4 (*C(12a)*), 128.8 (*C(15b)*), 131.8 (*C(4a)*), 145.0 (*C(9)*), 146.2 (*C(16a)*), 146.5 and 146.7 (*C(3a)*), 150.0 (*C(10)*), 155.0 (N*C*OO), 160.3 (*C(14)*), 165.5 and 165.6 (*CCOO*); IR (nujol): ν_{max} = 3234, 1718, 1673 cm^{-1}; MS *m/z* (ESI): 566.29 (M + H$^+$); anal. calcd. for $C_{30}H_{35}N_3O_8$ (565.6143): C 63.70, H 6.24, N 7.43; found: C 63.58, H 6.19, N 7.52.



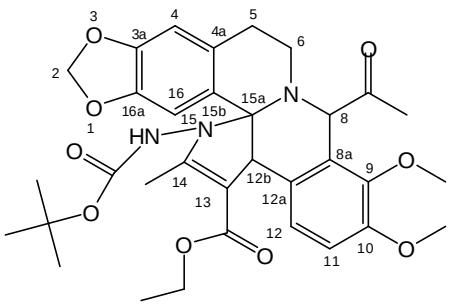
Methyl 15-[(*tert*-butoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-8-(2-oxopropyl)-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49d').

49d' was isolated by precipitation in 49% yield. White solid; mp: 166–168 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.50 (3s, 9H, OC(*CH*$_3$)$_3$), 1.59 (s, 3H, CH$_2$CO*CH*$_3$), 1.98 and 2.00 (2s, 3H, *CH*$_3$), 2.36 (dd, 1H, *J* = 15.6 Hz, *J* = 3.2 Hz, *CH*$_2$COCH$_3$), 2.67–2.69 (m, 2H, *C(5)H*$_2$), 3.06 (dd, 1H, *J* = 15.6 Hz, *J* = 8.0 Hz, *CH*$_2$COCH$_3$), 3.27–3.41 (m, 2H, *C(6)H*$_2$), 3.72 (s, 3H, CO$_2$*CH*$_3$), 3.84 (s, 3H, O*CH*$_3$), 3.88 (s, 3H, O*CH*$_3$), 5.13 (s, 1H, *C(12b)H*), 5.30 (dd, 1H, *J* = 8.0 Hz, *J* = 3.2 Hz, *C(8)H*$_2$), 5.94–5.96 (m, 2H, O*C(2)H2*O), 6.64 (s, 1H, *C(16)H*), 6.77 (d, 1H, *J* = 9.2 Hz, *C(11)H*), 7.00 (s, 1H, *C(4)H*), 7.17 (d, 1H, *J* = 8.8 Hz, *C(12)H*), 7.44 (s, 1H, *NH*); ^{13}C NMR (100 MHz, CDCl$_3$, 25 °C): δ = 13.8 (CH$_2$CO*CH*$_3$), 28.2 and 28.3 (OC(*CH*$_3$)$_3$), 30.9 and 31.0 (*CH*$_3$), 31.7 (*C(5)*), 46.9 and 47.0 (*CH*$_2$COCH$_3$), 49.9 (*C(6)*), 51.9 and 52.0 (CO$_2$*CH*$_3$), 53.5 and 53.6 (*C(8)*), 55.0 and 55.1 (*C(12b)*), 55.7 and 55.8 (O*CH*$_3$), 60.6 and 60.7 (O*CH*$_3$), 80.8 (O*C*(CH$_3$)$_3$), 86.6 (*C(15a)*), 101.0 and 101.1 (*C(2)*), 102.2 (*C(13)*), 107.8 (*C(16)*), 108.8 and 108.9 (*C(4)*), 110.0 (*C(11)*), 119.3 and 119.4 (*C(12)*), 125.5 (*C(8a)*), 125.8 (*C(12a)*), 126.9 (*C(15b)*), 132.7 (*C(4a)*), 139.9 (*C(9)*), 142.2 (*C(16a)*), 145.5 (*C(3a)*), 147.1 (*C(10)*), 150.2 (N*C*OO), 150.8 and 152.3 (*C(14)*), 171.8 (*CCOO*), 207.9 (CH$_2$*C*OCH$_3$); IR (nujol): ν_{max} = 3300, 1716, 1685 cm^{-1}; MS (EI) *m/z* (%): 621 (M$^+$) (3), 564 (100), 508 (55), 432 (71); anal. calcd. for C$_{33}$H$_{39}$N$_3$O$_9$ (621.6776): C 63.76, H 6.32, N 6.76; found: C 63.67, H 6.29, N 6.79.



Ethyl 15-[(*tert*-butoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49e).

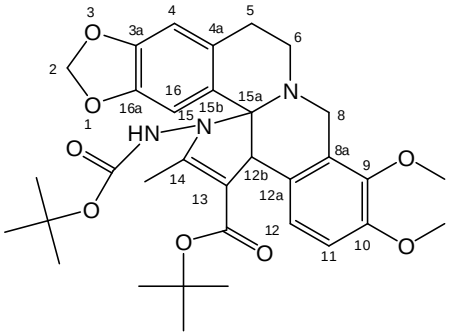
49e was isolated by precipitation in 87% yield. White solid; mp: 170–172 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.16 (t, *J* = 6.8 Hz, 3H, OCH$_2$*CH$_3$*), 1.20, 1.40 and 1.46 (3s, 9H, OC(*CH$_3$*)$_3$), 2.14 and 2.18 (2s, 3H, *CH$_3$*), 2.25–2.29 and 2.59–2.70 (2m, 2H, *C(5)H$_2$*), 2.35–2.45 and 2.70–2.81 (2m, 2H, *C(6)H$_2$*), 3.70 (s, 3H, O*CH$_3$*), 3.76 (s, 3H, O*CH$_3$*), 3.81–3.89 and 4.44–4.48 (2m, 2H, *C(8)H$_2$*), 3.91–4.04 (m, 2H, O*CH$_2$*CH$_3$), 4.13 and 4.19 (2s, 1H, *C(12b)H*), 5.94, 5.97 and 5.99 (3s, 2H, ,O*C(2)H2*O), 6.52 and 6.60 (brs and s, 1H, *C(16)H*), 6.66 and 6.84 (s and brs, 1H, *C(4)H*), 6.78–6.81 (m, 1H, *C(11)H*), 7.06 and 7.10 (2d, 1H, *J* = 8.0 Hz, *J* = 8.4 Hz, *C(12)H*), 7.87, 8.11 and 8.46 (3s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.4 and 11.5 (*CH$_3$*), 14.1 and 14.2 (OCH$_2$*CH$_3$*), 27.4 and 27.9 (OC(*CH$_3$*)$_3$), 28.5 and 28.6 (*C(5)*), 44.8 and 45.7 (*C(8)*), 46.6 and 46.9 (*C(6)*), 49.0 (*C(12b)*), 55.4 (O*CH$_3$*), 58.0 and 58.1 (O*CH$_2$*CH$_3$), 60.2 and 60.4 (O*CH$_3$*), 79.3 (O*C*(CH$_3$)$_3$), 86.2 (*C(15a)*), 95.2 and 97.7 (*C(13)*), 100.8 (*C(2)*), 105.4 (*C(16)*), 107.1 (*C(4)*), 109.8 and 109.8 (*C(11)*), 125.4 and 126.0 (*C(12)*), 127.6 and 127.8 (*C(8a)*), 128.4 and 128.4 (*C(12a)*), 128.8 (*C(15b)*), 131.8 (*C(4a)*), 145.0 and 145.2 (*C(9)*), 145.4 and 146.2 (*C(16a)*), 146.5 and 146.7 (*C(3a)*), 150.0 and 150.1 (*C(10)*), 155.0 and 155.7 (N*C*OO), 159.9 and 160.2 (*C(14)*), 165.2 and 165.2 (*CCOO*); IR (nujol): ν_{max} = 3226, 1713, 1667 cm^{-1}; MS *m/z* (ESI): 580.21 (M + H$^+$); anal. calcd. for C$_{31}$H$_{37}$N$_3$O$_8$ (579.6409): C 64.23, H 6.43, N 7.25; found: C 64.38, H 6.56, N 7.13.



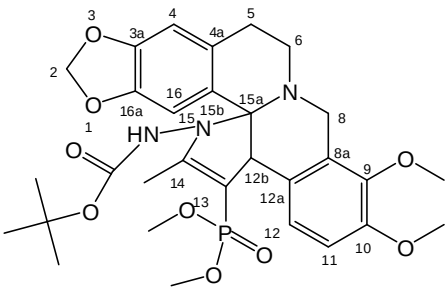
Ethyl 15-[(*tert*-butoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-8-(2-oxopropyl)-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49e').

49e' was isolated by precipitation in 72% yield. White solid; mp: 168–170 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.25 (t, *J* = 6.4 Hz, 3H, OCH_2CH_3), 1.50 (s, 9H, $OC(CH_3)_3$), 1.61 (s, 3H, CH_2COCH_3), 1.99 and 2.01 (2s, 3H, CH_3), 2.24–2.25 (m, 1H, CH_2COCH_3), 2.67–2.69 (m, 2H, *C(5)H*$_2$), 3.07–3.11 (m, 1H, CH_2COCH_3), 3.30–3.40 (m, 2H, *C(6)H*$_2$), 3.84 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.15–4.20 (m, 2H, OCH_2CH_3), 5.09 (s, 1H, *C(12b)H*), 5.29–5.31 (m, H, *C(8)H*$_2$), 5.95 and 5.96 (2s, 2H, ,O*C(2)H2*O), 6.64 (s, 1H, *C(16)H*), 6.76 (d, 1H, *J* = 9.2 Hz, *C(11)H*), 7.03 (s, 1H, *C(4)H*), 7.19 (d, 1H, *J* = 8.8 Hz, *C(12)H*), 7.40 (s, 1H, *NH*); ^{1}H NMR (400 MHz, $DMSO_{d6}$, 25 °C): δ = 1.06 (t, *J* = 6.8 Hz, 3H, OCH_2CH_3), 1.37, 1.41 and 1.43 (3s, 9H, $OC(CH_3)_3$), 1.77 and 1.81 (2s, 3H, CH_2COCH_3), 1.99 and 2.07 (2s, 3H, CH_3), 2.25–2.29 (m, 1H, CH_2COCH_3), 2.54–2.58 and 2.67–2.72 2(m, 2H, *C(5)H*$_2$), 3.06–3.11 (m, 1H, CH_2COCH_3), 3.28–3.30 (m, 2H, *C(6)H*$_2$), 3.75 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.80–□□□□ (m, 2H, OCH_2CH_3), 4.82 (s, 1H, *C(12b)H*), 5.10–5.20 (m, 1H, *C(8)H*$_2$), 5.93–6.04 (m, 2H, ,O*C(2)H2*O), 6.80 (d, 1H, *J* = 8.8 Hz, *C(11)H*), 6.84 (s, 1H, *C(16)H*), 6.91 (brs, 1H, *C(4)H*), 7.03 (d, 1H, *J* = 8.8 Hz, *C(12)H*), 9.61 (s, 1H, *NH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 13.9 (OCH_2CH_3), 14.2 (CH_2COCH_3), 28.2 ($OC(CH_3)_3$), 30.9 (CH_3), 31.6 (*C(5)*), 47.0 (CH_2COCH_3), 49.8 (*C(6)*), 53.4 and 53.5 (*C(8)*), 55.1 and 55.2 (*C(12b)*), 55.7 and 55.8 (OCH_3), 60.0 (OCH_3), 60.8 (OCH_2CH_3), 80.8 ($OC(CH_3)_3$), 86.0 (*C(15a)*), 100.1 (*C(2)*), 101.1 (*C(13)*), 107.7 (*C(16)*), 108.8 and 108.9 (*C(4)*), 110.9 (*C(11)*), 119.7 (*C(12)*), 125.6 (*C(8a)*), 125.8 (*C(12a)*), 126.9 (*C(15b)*), 132.6 (*C(4a)*), 139.7 (*C(9)*), 142.2 (*C(16a)*), 145.5 (*C(3a)*), 147.1 (*C(10)*), 150.1 (N*C*OO), 150.8 and 152.5 (*C(14)*), 171.2 (*CCOO*), 207.9 (CH_2COCH_3); IR (nujol): ν_{max} = 3312, 1718 cm^{-1}; MS (EI) *m/z* (%): 635 (M^+) (4), 578 (100), 522 (62), 432 (75); anal. calcd. for $C_{34}H_{41}N_3O_9$ (635.7042): C 64.24, H 6.50, N 6.61; found: C 64.38, H 6.54, N 6.54.

***Tert*-butyl 15-[(*tert*-butoxycarbonyl)amino]-9,10-dimethoxy-14-methyl -5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49f).**

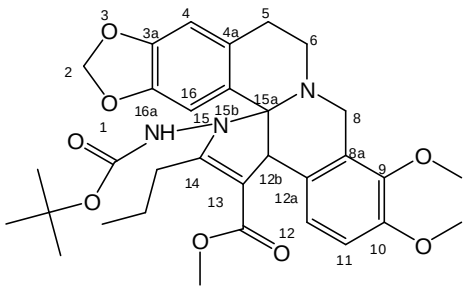


49f was isolated by column chromatography on alumina oxide (acetate/cyclohexane 20:80) in 96% yield. White solid; mp: 98–100 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.21, 1,35, 1.39, 1.40 and 1,46 (5s, 18H, 2 OC(*CH*$_3$)$_3$), 2.09 and 2.11 (2s, 3H, *CH*$_3$), 2.20–2.33 and 2.53–2.60 (2m, 2H, *C(5)H*$_2$), 2.37–2.44 and 2.67–2.75 (2m, 2H, *C(6)H*$_2$), 3.70 and 3.70 (2s, 3H, O*CH*$_3$), 3.75 (s, 3H, O*CH*$_3$), 3.79–3.86 and 4.27–4.52 (2m, 2H, *C(8)H*$_2$), 4.07 and 4.14 (2s, 1H, *C(12b)H*), 5.90–6.00 (m, 2H, ,O*C(2)H2*O), 6.48–6.76 (m, 2H, *C(16)H* and *C(4)H*), 6.79–7.16 (m, 2H, *C(11)H* and *C(12)H*), 7.87, 8.09 and 8.46 (3s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.4 and 11,5 (*CH*$_3$), 27.4, 27.7, 28.0 and 28.3 (2 OC(*CH*$_3$)$_3$), 28.7 (*C(5)*), 45.0 and 45.9 (*C(8)*), 46.6 and 47.0 (*C(6)*), 49.0 (*C(12b)*), 55.4 (O*CH*$_3$), 60.2 and 60.4 (O*CH*$_3$), 78.1 (O*C*(CH$_3$)$_3$), 79.3 (O*C*(CH$_3$)$_3$), 86.0 (*C(15a)*), 96.8 and 99.7 (*C(13)*), 100.9 (*C(2)*), 105.3 (*C(16)*), 107.2 (*C(4)*), 109.6 (*C(11)*), 125.4 (*C(12)*), 126.1 (*C(8a)*), 127.8 (*C(12a)*), 128.6 and 128.8 (*C(15b)*), 132.0 (*C(4a)*), 145.0 and 145.4 (*C(9)*), 146.2 (*C(16a)*), 146.5 and 146.7 (*C(3a)*), 150.1 and 150.2 (*C(10)*), 155.1 and 155.8 (N*C*OO), 159.3 and 159.4 (*C(14)*), 165.0 (C*C*OO); IR (nujol): ν_{max} = 3237, 1756, 1679 cm^{-1}; MS *m/z* (ESI): 608.24 (M + H^{+}); anal. calcd. for $C_{33}H_{41}N_3O_8$ (607.6941): C 65.22, H 6.80, N 6.91; found: C 65.04, H 6.76, N 7.03.



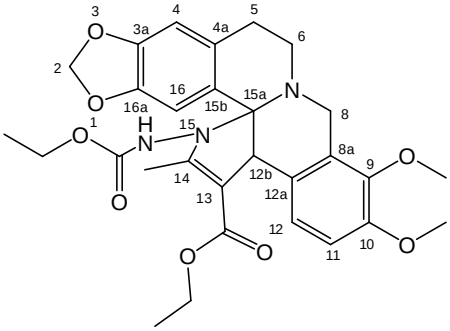
Dimethyl {15-[(*tert*-butoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinolin-13-yl}phosphonate (49g).

49g was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 40:60) in 22% yield. White solid; mp: 101–104 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.16 (t, *J* = 6.8 Hz, 3H, OCH$_2$*CH$_3$*), 1.20, 1,40 and 1.44 (3s, 9H, OC(*CH$_3$*)$_3$), 2.06 and 2.08 (s and brs, 3H, *CH$_3$*), 2.13–2.23 and 2.54–2.64 (2m, 2H, *C(5)H$_2$*), 2.31–2.45 and 2.65–2.79 (2m, 2H, *C(4)H$_2$*), 2.92 and 2.96 (2d, 3H, *J* = 11.2 Hz, *J* = 11.6 Hz, PO*CH$_3$*), 3.70, (s, 3H, O*CH$_3$*), 3.44 and 3.46 (2d, 3H, *J* = 11.2 Hz, *J* = 11.2 Hz, PO*CH$_3$*), 3.68, (s, 3H, O*CH$_3$*),3.76 (s, 3H, O*CH$_3$*), 3.78–3.90 and 4.41–4.45 (2m, 2H, *C(8)H$_2$*), 3.98–4.07 (m, 1H, *C(12b)H*), 5.95–6.02, (m, 2H, ,O*C(2)H2*O), 6.48, 6.55, 6.60 and 6.67 (2brs and 2s, 2H, *C(16)H* and *C(4)H*), 6.76–6.88 (m, 2H, *C(11)H* and *C(12)H*), 7.08, 8.41 and 9.54 (brs, s, brs 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.9 and 12,1 (*CH$_3$*), 28.0 and 28.1 (OC(*CH$_3$*)$_3$), 28.6 and 28.6 (*C(5)*), 45.1 and 45.8 (*C(8)*), 46.7 and 47.2 (*C(6)*), 50.1 ($^{2}J_{CP}$ = 3.9 Hz (*C(12b)*), 50.7 ($^{2}J_{CP}$ = 5.2 Hz PO*CH$_3$*), 50.8 ($^{2}J_{CP}$ = 4.9 Hz PO*CH$_3$*) 55.5 (O*CH$_3$*), 60.4 and 60.6 (O*CH$_3$*), 79.3 and 79.3 (O*C*(CH$_3$)$_3$), 85.7 (*C(15a)*), 100.8 (*C(13)*), 105.6 (*C(2)*), 107.0 (*C(16)*), 109.9 (*C(4)*), 110.1 (*C(11)*), 124.8 and 124.9 (*C(12)*), 127.8 (*C(8a)*), 128.1 and 128.1 (*C(12a)*), 129.1 (*C(15b)*), 132.2 (*C(4a)*), 145.3 (*C(9)*), 145.7 (*C(16a)*), 146.3 (*C(3a)*), 146.5 and 146.6 (*C(10)*), 150.3 and 150.4 (*NCOO*) 155.2 and 156.0 (*C(14)*); IR (nujol): ν_{max} = 3268, 1751, 1698 cm^{-1}; MS *m/z* (ESI): 616.13 (M + H^{+}); anal. calcd. for $C_{30}H_{38}N_3O_9P$ (615.6113): C 58.53, H 6.22, N 6.83; found: C 58.33, H 6.17, N 6.94.



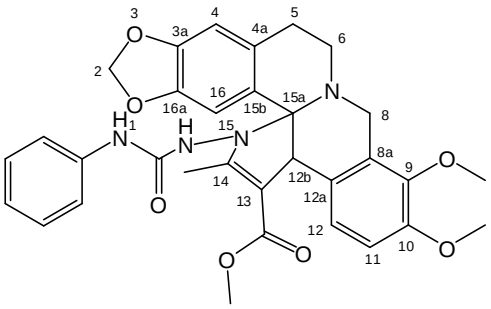
Methyl 15-[(*tert*-butoxycarbonyl)amino]-9,10-dimethoxy-14-propyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49h).

49h was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 40:60) in 73% yield. White solid; mp: 98–100 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 0.87 (t, *J* = 7.2 Hz, 3H, $CH_2CH_2\mathit{CH_3}$), 1.10–1.16 (m, 2H, $CH_2\mathit{CH_2}CH_3$), 1.20, 1.39, 1.40 and 1.47 (4s, 9H, $OC(\mathit{CH_3})_3$), 2.08–2.42 and 2.49–2.78 (2m, 6H, $\mathit{C(5)H_2}$, $\mathit{C(6)H_2}$ and $\mathit{CH_2}CH_2CH_3$), 3.47 and 3.52 (2s, 3H, CO_2CH_3), 3.69 and 3.69 (2s, 3H, $O\mathit{CH_3}$), 3.75 (s, 3H, $O\mathit{CH_3}$), 3.79–3.94 and 4.45–4.49 (2m, 2H, $\mathit{C(8)H_2}$), 4.11 and 4.19 (2s, 1H, *C(12b)H*), 5.91–6.00 (m, 2H, O*C(2)H2*O), 6.48–6.68 (m, 2H, *C(16)H* and *C(4)H*), 6.77–7.10 (m, 2H, *C(11)H* and *C(12)H*), 7.89, 8.22, 8.55 and 8.60 (4s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 14.0 ($CH_2CH_2\mathit{CH_3}$), 14.1 ($CH_2\mathit{CH_2}CH_3$), 20.5 ($\mathit{CH_2}CH_2CH_3$), 27.3 and 28.0 ($OC(\mathit{CH_3})_3$), 28.7 (*C(5)*), 44.8 (*C(8)*), 46.6 (*C(6)*), 49.1 (*C(12b)*), 49.6 and 49.7 ($CO_2\mathit{CH_3}$), 55.4 ($O\mathit{CH_3}$), 60.3 and 60.5 ($O\mathit{CH_3}$), 79.3 ($OC(CH_3)_3$), 86.4 (*C(15a)*), 97.5 (*C(13)*), 100.9 (*C(2)*), 105.3 (*C(16)*), 107.1 (*C(4)*), 109.8 (*C(11)*), 125.4 (*C(12)*), 125.7 (*C(8a)*), 128.4 (*C(12a)*), 128.9 (*C(15b)*), 132.0 (*C(4a)*), 145.1 (*C(9)*), 146.3 (*C(16a)*), 146.8 (*C(3a)*), 150.1 (*C(10)*), 155.7 (NCOO), 164.1 and 160.8 (*C(14)*), 165.3 and 165.4 (*CCOO*); IR (nujol): ν_{max} = 3271, 1749, 1732 cm^{-1}; MS *m/z* (ESI): 594.22 (M + H$^+$); anal. calcd. for $C_{32}H_{39}N_3O_8$ (593.6675): C 64.74, H 6.62, N 7.08; found: C 64.90, H 6.67, N 6.96.



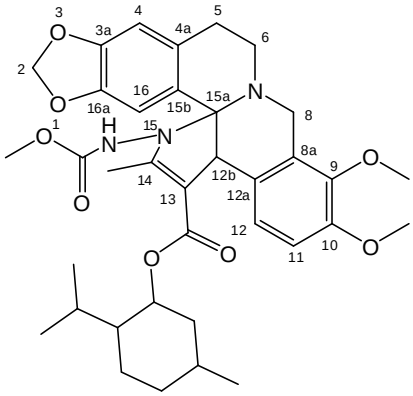
Ethyl 15-[(ethoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49i).

49i was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 30:70) in 62% yield. White solid; mp: 134–136 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.19 (t, *J* = 6.8 Hz, 3H, OCH$_2$*CH$_3$*), 2.13 and 2.16 (2s, 3H, *CH$_3$*), 2.18–2.24 and 2.56–2.64 (2m, 2H, *C(5)H$_2$*), 2.33–2.41 and 2.68–2.75 (2m, 2H, *C(6)H$_2$*), 3.61 (s, 3H, CO$_2$*CH$_3$*), 3.69 (s, 3H, O*CH$_3$*), 3.76 (s, 3H, O*CH$_3$*), 3.81–3.88 and 4.41–4.46 (2m, 2H, *C(8)H$_2$*), 3.92–4.02 (m, 2H, O*CH$_2$*CH$_3$), 4.13 and 4.17 (2s, 1H, *C(12b)H*), 5.92, 5.97 and 5.99 (3s, 2H, O*C(2)H2*O), 6.50 and 6.60 (brs and s, 1H, *C(16)H*), 6.65 and 6.84 (s and brs, 1H, *C(4)H*), 6.78-6.80 (m, 1H, *C(11)H*), 7.05 and 7.12 (2d, 1H, *J* = 8.4 Hz, *J* = 8.4 Hz, *C(12)H*), 8.37, 8.88 and 9.05 (3s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.5 (*CH$_3$*), 14.3 (OCH$_2$*CH$_3$*), 28.5 (*C(5)*), 45.7 (*C(8)*), 46.1 (*C(6)*), 49.0 (*C(12b)*), 52.1 (CO$_2$*CH$_3$*), 55.4 (O*CH$_3$*), 58.2 and 58.3 (O*CH$_2$*CH$_3$), 60.3 and 60.5 (O*CH$_3$*), 86.3 (*C(15a)*), 97.9 (*C(13)*), 100.9 (*C(2)*), 105.3 (*C(16)*), 107.1 (*C(4)*), 109.8 (*C(11)*), 125.6 and 126.0 (*C(12)*), 127.7 (*C(8a)*), 128.2 (*C(12a)*), 128.9 (*C(15b)*), 131.8 (*C(4a)*), 145.2 and 145.4 (*C(9)*), 146.3 (*C(16a)*), 146.5 and 146.8 (*C(3a)*), 150.2 (*C(10)*), 155.2 (N*C*OO), 160.0 (*C(14)*), 165.3 (*CCOO*); IR (nujol): ν_{max} = 3279, 1756, 1720, cm^{-1}; MS *m/z* (ESI): 538.19 (M + H$^+$); anal. calcd. for $C_{28}H_{31}N_3O_8$ (537.5612): C 62.56, H 5.81, N 7.82; found: C 62.80, H 5.87, N 7.67.



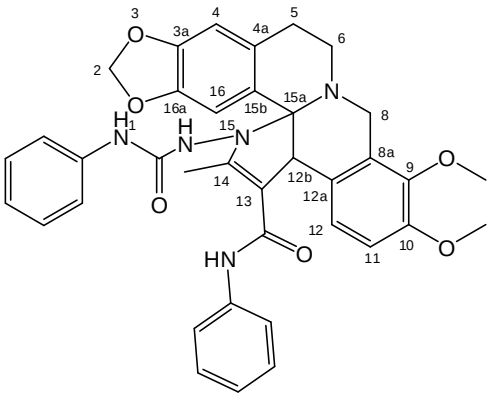
Methyl 15-[(anilinocarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49j).

49j was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 50:50) in 61% yield. White solid; mp: 130–134 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ =, 2.20 and 2.24 (2s, 3H, *CH*$_3$), 2.32–2.36 and 2.55–2.66 (2m, 2H, *C(5)H*$_2$), 2.44–2.48 and 2.68–2.77 (2m, 2H, *C(6)H*$_2$), 3.49 and 3.54 (2s, 3H, CO$_2$*CH*$_3$), 3.69 and 3. 71 (2s, 3H, O*CH*$_3$), 3.77 and 3.78 (2s, 3H, O*CH*$_3$), 3.81–3.88 and 4.48–4.51 (2m, 2H, *C(8)H*$_2$), 4.15 and 4.26 (2s, 1H, *C(12b)H*), 5.92, 6.02,6.04 and 5.42 (4s, 2H, O*C(2)H2*O), 6.67–7.40 (*C(16)H*), *C(4)H*), *C(11)H*, *C(12)H* NH*ph*) 7.73 (s, 1H, N*H*ph) 8.86 (s, 1H, *NH*); IR (nujol): ν_{max} = 3312, 3245, 1746, 1682, cm^{-1}; MS *m/z* (ESI): 585.27 (M + H$^+$); anal. calcd. for $C_{32}H_{32}N_4O_7$ (584.62): C, 65.74; H, 5.52; N, 9.58; found: C, 65.59; H, 5.56; N, 9.5851.



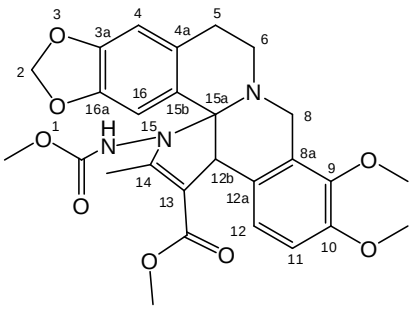
Menthol 15-[(methoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49k).

49k was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 70:30) in 55% yield. White solid; mp: 112–116 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 0.51–1.12(m, 14H, *Menthol*), 1.58–1.77(2m, 4H, *Menthol*), 2.10, 2.15 and 2.18 (2s, 3H, *CH*$_3$), 2.27–2.30 and 2.56–2.64 (2m, 2H, *C(5)H*$_2$), 2.35–2.43 and 2.66–2.75 (2m, 2H, *C(6)H*$_2$), 3.61 (s, 3H, CO$_2$*CH*$_3$), 3.69 (s, 3H, O*CH*$_3$), 3.74–3.75 (2s, 3H, O*CH*$_3$), 4.13 and 4.17 (2s, 1H, *C(12b)H*), 4.36–4.68 (2m, 2H, *C(8)H*$_2$), 5.91, 5.97 and 5.99 (3s, 2H, O*C(2)H2*O), 6.48–7.11 (m 4H, *C(16)H*), *C(4)H*), *C(11)H*), *C(12)H*), 8.38, 8.87, 8.89 and 9.06 (4s, 1H, *NH*); IR (nujol): ν_{max} = 3289, 1732, 1698, cm^{-1}; MS *m/z* (ESI): 648.39 (M + H$^+$); anal. calcd. for $C_{36}H_{45}N_3O_8$ (647.76): C, 66.75; H, 7.00; N, 6.49; found: C, 66.89; H, 7.07; N, 6.43



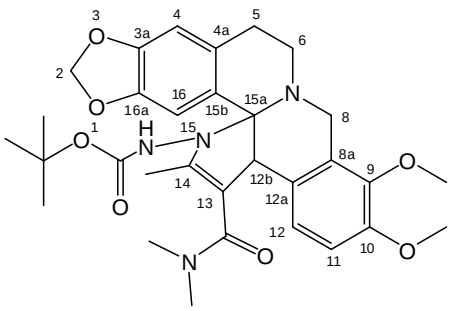
Anilino 15-[(anilinocarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49l).

49l was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 50:50) in 61% yield. White solid; mp: 173–137 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 2.07 and 2.12 (2s, 3H, *CH*$_3$), 2.17–2.21 and 2.55–2.61 (2m, 2H, *C(5)H*$_2$), 2.29–2.38 and 2.63–2.69 (2m, 2H, *C(6)H*$_2$), 3.61 (s, 3H, CO$_2$*CH*$_3$), 3.76 (s, 3H, O*CH*$_3$), 3.77 (s, 3H, O*CH*$_3$), 4.02–4.13 (m, 2H, *C(8)H*$_2$), 4.45 and 4.47 (2s, 1H, *C(12b)H*), 5.67 and 5.97 (2s, 2H, O*C(2)H2*O), 6.46– 7.60 (s and m 16H, *C(16)H*), *C(4)H*, *C(11)H*, *C(12)H*, *2NHph*), 8.57 9.97 (2s, 1H, *NH*); IR (nujol): ν_{max} = 3313, 3289,3279, 1731, 1719, cm^{-1}; MS *m/z* (ESI): 646.27 (M + H$^+$); anal. calcd. for $C_{37}H_{35}N_5O_6$ (645.70): C, 68.82; H, 5.46; N, 10.85; found: C, 68.75; H, 5.42; N, 10.91.



Methyl 15-[(methoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49m).

49m was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 70:30) in 85% yield. White solid; mp: 107–111 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C):, 2.12 and 2.15 (2s, 3H, *CH*$_3$), 2.18–2.25 and 2.56–2.63 (2m, 2H, *C(5)H*$_2$), 2.34–2.45 and 2.64–2.76 (2m, 2H, *C(6)H*$_2$), 3.48 and 3.53 (2s, 3H, CO$_2$*CH*$_3$), 3.60 (2s, 3H, CO$_2$*CH*$_3$), 3.69 (s, 3H, O*CH*$_3$), 3.76 (s, 3H, O*CH*$_3$), 3.82–3.88 and 4.41–4.46 (2m, 2H, *C(8)H*$_2$), 4.12 (brs, 1H, *C(12b)H*), 5.97 and 6.00 (3s, 2H, O*C(2)H2*O), 6.50 and 6.60 (brs and s, 1H, *C(16)H*), 6.64 and 6.83 (s and brs, 1H, *C(4)H*), 6.78-6.80 (m, 1H, *C(11)H*), 7.04 and 7.10 (2d, 1H, *J* = 8.4 Hz, *J* = 8.4 Hz, *C(12)H*), 8.89 and 9.07 (3s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.5 (*CH*$_3$), 28.5 (*C(5)*), 45.7 (*C(8)*), 46.6 (*C(6)*), 48.9 (*C(12b)*), 49.8 (CO$_2$*CH*$_3$), 52.1 (CO$_2$*CH*$_3$), 55.4 (O*CH*$_3$), 60.3 (O*CH*$_3$), 86.3 (*C(15a)*), 97.8 (*C(13)*), 100.9 (*C(2)*), 105.2 (*C(16)*), 107.2 (*C(4)*), 110.0 (*C(11)*), 125.3 and 125.6 (*C(12)*), 127.7 (*C(8a)*), 128.2 (*C(12a)*), 128.9 (*C(15b)*), 131.7 (*C(4a)*), 145.2 (*C(9)*), 146.3 (*C(16a)*), 146.8 (*C(3a)*), 150.2 (*C(10)*), 157.2 (N*C*OO), 160.0 (*C(14)*), 165.6 (*CCOO*); IR (nujol): ν_{max} = 3288, 1750, 1736, cm^{-1}; MS *m/z* (ESI): 524.11 (M + H$^+$); anal. calcd. for $C_{27}H_{29}N_3O_8$ (523.53): C, 61.94; H, 5.58; N, 8.03; found: C, 61.78; H, 5.53; N, 8.10



N-dimethyl 15-[(*tert*-butoxycarbonyl)amino]-9,10-dimethoxy-14-methyl-5,6,12*b*,15-tetrahydro-8*H*-[1,3]dioxo[4,5-*g*]pyrrolo[2',3':3,4]isoquino[3,2-*a*]isoquinoline-13-carboxylate (49n).

49n was isolated by column chromatography on aluminum oxide (acetate/cyclohexane 50:50) in 63% yield. White solid; mp: 145–148 °C; ^{1}H NMR (400 MHz, DMSO$_{d6}$, 25 °C): δ = 1.21 and 1.23 (2s, 6H, N(*CH*$_3$)$_3$), 1.34 and 1.39 (2s, 9H, OC(*CH*$_3$)$_3$), 2.08–2.15 (m, 3H, *CH*$_3$), 2.24–2.30 and 2.56–2.60 (2m, 2H, *C(5)H*$_2$), 2.33–2.44 and 2.67–2.75 (2m, 2H, *C(6)H*$_2$), 3.69 and 3.70 (2s, 3H, O*CH*$_3$), 3.75 and 3.77 (2s, 3H, O*CH*$_3$), 3.81–3.87 and 4.48–4.52 (2m, 2H, *C(8)H*$_2$), 4.07 and 4.13 (2s, 1H, *C(12b)H*), 5.94, 5.97 and 6.00 (3s, 2H, O*C(2)H2*O), 6.477.16 (m, 4H, *C(16)H*, *C(4)H*, *C(11)H*, *C(12)H*), 7.87, 8.09 and 8.46 (3s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO$_{d6}$, 25 °C): δ = 11.4 and 11.5 (*CH*$_3$), 26.3 (OC(*CH*$_3$)$_3$), 28.0 and 28.3 (*C(5)*), 45.0 (*C(8)*), 45.8 (*C(6)*), 46.5 (N(*CH*$_3$)$_2$), 47.0 (N(*CH*$_3$)$_2$) 49.0 (*C(12b)*), 55.4 (O*CH*$_3$), 60.2 and 60.5 (O*CH*$_3$), 78.1 and 79.4 (OC(*CH*$_3$)$_3$), 86.0 (*C(15a)*), 97.8 (*C(13)*), 99.7 and 100.8 (*C(2)*), 105.3 (*C(16)*), 107.1 (*C(4)*), 109.6 (*C(11)*), 125.3 and 126.0 (*C(12)*), 127.8 (*C(8a)*), 128.6 (*C(12a)*), 128.8 (*C(15b)*), 132.0 (*C(4a)*), 145.0 (*C(9)*), 146.2 (*C(16a)*), 146.5 and 146.7 (*C(3a)*), 150.1 (*C(10)*), 155.1 and 155.8 (N*C*OO), 159.4 (*C(14)*), 165.0 (*CCOO*); IR (nujol): ν_{max} = 3293, 1713, 1689, cm^{-1}; MS *m/z* (ESI): 579.22 (M + H^{+}); anal. calcd. for $C_{31}H_{38}N_4O_7$ (578.66): C, 64.34; H, 6.62; N, 9.68; found: C, 64.59; H, 6.58; N, 9.61.

Experimental Section

Oxygen Radical Absorbance Capacity (ORAC) assay. The determination of the antioxidant activity of the compounds **49a-n** was made by the ORAC assay,[100] using a Fluostar Optima plate reader fluorimeter (BMG Labtech, Offenburgh, Germany) equipped with a temperature-controlled incubation chamber and automatic injection pump. Incubator temperature was set at 37 °C. The reaction mixture for the assay was the following: 200 μL of 0.096 μM fluorescein sodium salt in 0.075 M sodium-phosphate buffer (pH 7.0), and 20 μL of sample or 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox). The reaction was initiated with 80 μL of 0.33 M 2,2´-azobis(2-amidinopropane) dihydrochloride (AAPH). A calibration curve was made each time with the standard Trolox (500, 300, 100, 50, 25 μM). The blank was 80% acetone diluted 1:10 with 0.075 M sodium-phosphate buffer (pH 7.0). Samples were dissolved in 80% acetone (1 mg/ml) and diluted 1:10 with 0.075 M sodium-phosphate buffer (pH 7.0) before ORAC assay. Fluorescence was read at 485 nm ex. and 520 nm em. until complete extinction. ORAC values were expressed as μmol Trolox Equivalents (TE)/mmol.

Cell culture. Human keratinocytes (NCTC-2544) were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2% L-glutamine, and 1% penicillin/streptomycin 100 U/ml. Cells were maintained in a CO2 incubator at 37°C and 5% CO2. Cell culture materials and reagents were from Sigma-Aldrich (Milan, Italy).

2′,7′-dichlorofluorescin diacetate (**DCFH-DA) assay.** The antioxidant properties of THB derivatives were analyzed in NCTC-2544 cells by DCFH-DA (Sigma-Aldrich, Milan, Italy)[101,102]. DCFH-DA is a cell-permeable non-fluorescent probe which turns to highly fluorescent 2′,7′-dichlorofluorescein upon oxidation. Briefly, cells (4000/well) were incubated in black 96-well plates with THB derivatives (30 μM) or vehicle (0.1% DMSO). After 1 and 3 hours of incubation, test compounds were removed and DCFH-DA (5 μM) was added to each well for 30 min at 37°C. After excess probe removal, the basal intracellular fluorescence emission was recorded. Cells were then treated with hydrogen peroxide (H2O2, 100 μM) and fluorescence emission upon probe oxidation was monitored for 30 min. at Ex/Em 485/520 nm in the multiwell plate reader FluoStar Optima (BMG Labtech, Germany). Data were expressed as relative oxidation versus non-oxidized cells.

Cell viability assay. The effect of THB derivatives on NCTC-2544 cell viability was analyzed by WST-8 reagent [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] (Sigma-Aldrich, Milan, Italy)][102,103]. The assay was based on the cleavage of the tetrazolium salt WST-8 by cellular dehydrogenases in viable cells. Briefly, cells (1500/well) were incubated in clear 96-well plates with test compounds (30 μM) or vehicle (0.1% DMSO, untreated control). After 24, 48, and 72 hours of incubation, WST-8 was added

to each well, and cells were further incubated at 37°C up to 4 hours. Color development was monitored at 450 nm in a multiwell plate reader (Thermo Fisher Scientific, Milan, Italy).

Statistical analysis. Antioxidant capacity (ORAC) was established by six independent determinations for each sample; each value was the mean ± SD. Statistical significance was assessed by one-way ANOVA, using PRISM 5.1 (GraphPad Software, La Jolla, USA). The results were considered statistically significant when $p < 0.05$. Data on DCFH-DA assay and cell viability assay were presented as mean ± standard deviation (SD) of at least three independent experiments and analyzed by Student's t-test to compare treated vs. untreated cells. Significance level was set at $p<0.05$ for all analysis.

9. BIBLIOGRAPHY

CHAPTER 9

BIBLIOGRAFIA

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10. AKNOWLEGMENT

CHAPTER 10

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