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REVIEW ARTICLE

Chirality WILEY

A recent update on new synthetic chiral compounds with antileishmanial activity

Michele Verboni | **Diego Olivieri** | **Simone Lucarini**

Department of Biomolecular Science, University of Urbino Carlo Bo, Urbino, Italy

Correspondence
Simone Lucarini, Department of Biomolecular Science, University of Urbino Carlo Bo, piazza RInascimento 6, 61029 Urbino (PU), Italy.
Email: simone.lucarini@uniurb.it

Abstract

Parasitic diseases, including malaria, leishmaniasis, and trypanosomiasis, affect billions of people and are responsible for almost 500,000 deaths/year. In particular, leishmaniasis, a neglected tropical disease, is considered a global public health problem because current drugs have several drawbacks including to toxicity, high cost, and drug resistance, which result in a lack of effective and readily available therapies. Therefore, the synthesis of new, safe, and effective molecules still requires the attention of the scientific community. Moreover, it is well known that chirality plays a crucial role in the antiparasitic activity of molecules, driving the design of their synthesis. Therefore, in this review we report a recent update on new chiral compounds with promising antileishmanial activity, focusing on synthetic approaches. Where reported, in most cases the enantiopure compound has shown better potency against the protozoa than its enantiomer or corresponding racemic mixture.

KEYWORDS

chiral catalysis, chiral pool, chiral resolution, imidazoxazines, imidazoxazoles, kinetic resolution, leishmania, medicinal chemistry, natural products, peptides

1 | INTRODUCTION

Leishmaniasis is one of the most dangerous neglected diseases, second only to malaria in parasitic causes of death[1]; it is endemic in over 90 countries (in particular, South-East Asia, East and North Africa, and Central America), involves more than 350 million people, and presents more than a million new cases per year.[2,3]

MV and DO equally contributed to the writing of the review.

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The infection is caused by different species of *leishmania* protozoan parasite, which are transmitted to humans through the bite of infected sandflies. A specific sandfly, called phlebotominae, inoculates promastigotes into the skin of the host. In humans, these are taken up by macrophages or dendritic cells and transformed into flagellar amastigotes.[1] The future course of the infection depends upon the strain of *leishmania*, and the type of immune response mounted by the host.[4–6] There are three forms of Leishmaniasis: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL).[2] VL, the deadliest form, causes 20,000–40,000 casualties every year.[3]

No vaccine is available to efficaciously prevent the disease. Moreover, commercial drugs (Figure 1) suffer

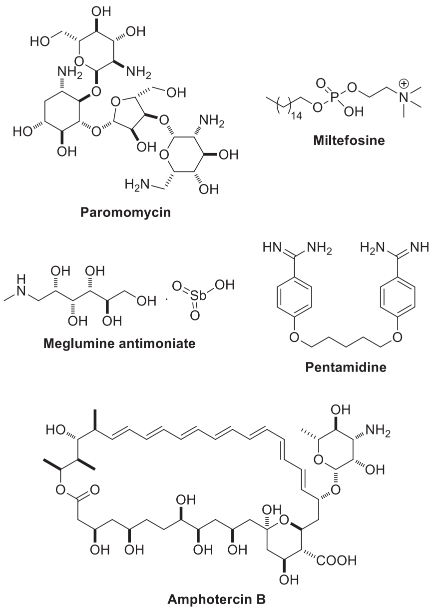


FIGURE 1 Main commercial drugs against Leishmaniasis

various limitations including lack of safety and efficacy, high costs, high toxicity, difficulty in administration, long treatment duration, and drug resistance.[7,8] Current drug therapies cannot eliminate leishmaniasis completely.[9]

Therefore, the discovery and development of new efficient, and safe molecules is still receiving great attention.[10,11] In recent years, several groups have come up with promising antileishmanial agents, some of them being chiral.[12–14]

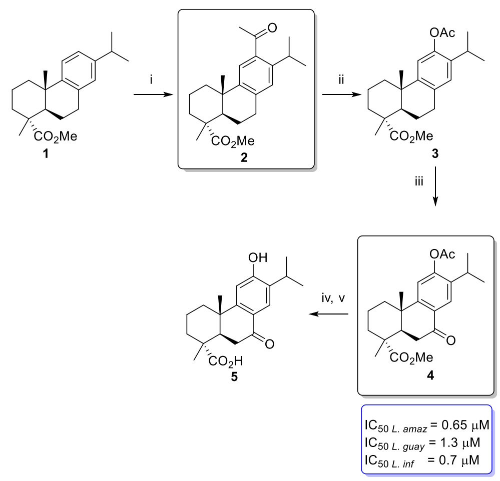
In this review, we report recent (i.e., from 2017) syntheses of chiral compounds that show promising activity against *leishmania*. Different synthetic strategies to achieve the desired enantiopure compounds were utilized. From our investigation, the chiral pool approach, which essentially foresees the use of enantiopure reagents (e.g., amino acid), is highly preferred over asymmetric synthesis, in which the installation of the stereogenic center is generally driven by chiral catalysis. Moreover, other approaches, such as chiral resolution, and kinetic resolution were also utilized. In this update, only the most promising chiral molecules, in terms of showing good or excellent IC_{50}/EC_{50} against several *Leishmania* strains, are reported. The review is ultimately divided by classifying the products. Initially, some classes of natural products (NPs) and their promising analogues are discussed, followed by amino acids containing compounds, with a particular focus on peptides. The last chapter is dedicated to nitroimidazoxazines and -oxazoles, which are promising agents against *leishmania*, some of them already drug candidates.

2 | NATURAL PRODUCTS AND ANALOGUES

NPs are substances that are produced by a living organism and can be found in nature. Although they can be obtained by extraction, generally from plants, the possibility to develop new synthetic strategies for the formation of such molecules enables the selective modification of some of their properties to better respond to the desired requirements. Therefore, in this chapter, the synthesis of various NPs and their analogues that have shown interesting activity against *leishmania* are described.

Besides all, abietane-type diterpenoids are characterized by a tricyclic ring system and have shown a wide range of chemical diversity and biological activity, including antitumor and anti-infective properties.[15,16] Encouraged by these considerations, in 2019, González-Cardenete et al. reported the first semisynthesis of the abietane diterpenoid (+)-liquiditerpenoic acid A (abietopinoic acid) (**5**) together with several analogues with different C-18 functional groups and oxidation pattern at C-7. (Scheme 1).[17] The compounds were synthesized starting from the methyl dehydroabietate (**1**), obtainable from commercially available (−)-abietic acid.[18]

A Friedel–Crafts acetylation followed by Baeyer–Villiger oxidation afforded the key intermediate **3** (80% overall yield, two steps). From **3**, the NP **5** is obtained through oxidation achieved by CrO_3 at the benzylic C-7 position, affording the ketone **4**, followed by



SCHEME 1 Reagents and conditions: (i) acetyl chloride, anhydrous $AlCl_3$, 1,2-dichloroethane, 0°C to rt, 24 h, under Ar; (ii) *m*-CPBA, TFA, CH_2Cl_2, 0°C to rt, 22 h; (iii) CrO_3, AcOH, 0°C to rt, 20 h; (iv) K_2CO_3, MeOH, rt, 3.5 h; (v) LiI, 2,4,6-collidine, reflux, 3 h, under argon, then H_2O (0°C) and 6 N HCl (to pH = 1)

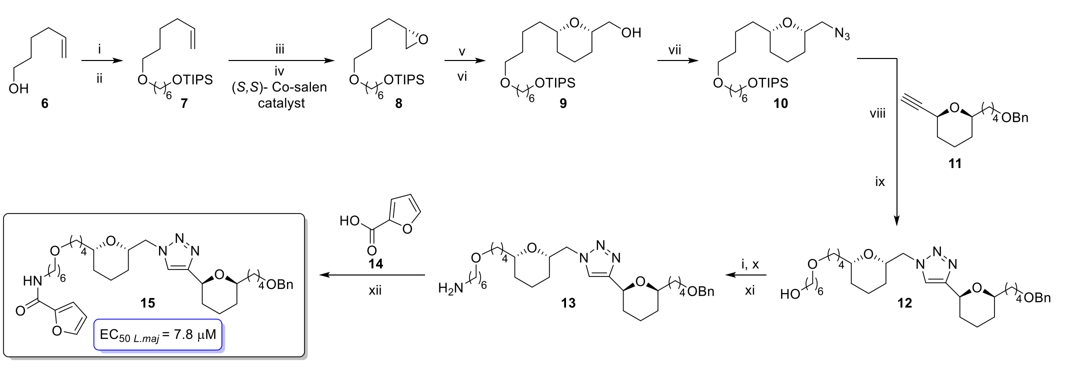
deacetylation and nucleophilic methyl ester cleavage, with 34% isolated yield (over 5 steps).

The antileishmanial activity of these molecules was evaluated against four promastigote *Leishmania* strains (*Leishmania infantum*, *Leishmania donovani*, *Leishmania amazonensis*, and *Leishmania guyanensis*) together with the cytotoxicity against J774 macrophages. The best results were obtained with **4**, which showed a submicromolar IC_{50}, 73-fold more potent than miltefosine (0.65 μM vs. 47.7 μM) for *L. amazonensis*, 13 times (1.3 μM vs. 18.2 μM) for *L. guyanensis*. For *L. infantum*, **4** was also the most potent among the tested analogues, with an IC_{50} of 0.70 μM. However, the best balance of activity−selectivity was exhibited by compound **2** (Selectivity Index [SI] between 8.76 and 52.49) with reduced toxicity (CC_{50} = 129.6 μM) as compared to **4**. Hence, analogue **2** was tested against amastigotes, showing IC_{50} values of 31.4 μM (*L. amazonensis)* and 37.2 μM *(L. infantum*). Moreover, molecular docking studies showed a comparatively good correlation between the in vitro activity of this compound in three *L.* pathogens with UDP-glucose pyrophosphorylase ($r^2 > 0.71$) as potential targets for antileishmaniasis derivatives.

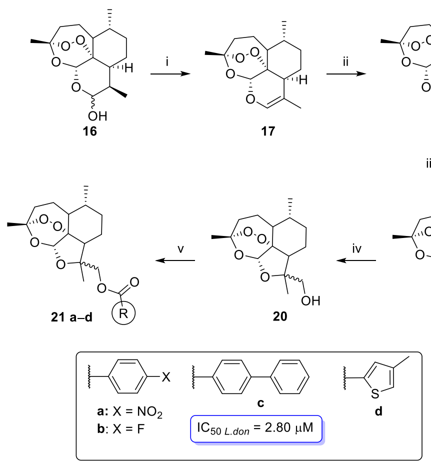
Florence et al., inspired by the activity of chamuvarinin against *Trypanosoma brucei*,[19,20] synthesized a series of triazole-containing compounds, essentially by replacing the tetrahydrofuran ring with a 1,4-triazole.[21,22] More recently, in 2017, they further continued this study introducing (hetero)aromatic rings on the side chain of these molecules and testing them also against *Leishmania major*.[23]

An S_N2 reaction of **6** with 6-([triisopropylsilyl]oxy) hexan-1-ol on the mesylated 5-hexen-1-ol afforded the olefin **7**. The (*S*)-epoxide **8** was obtained by kinetic resolution, with Jacobsen salen catalyst,[24] of the racemic epoxide, synthesized via classical epoxidation with *m*-CPBA. Successive treatment with homoallyl magnesium bromide and epoxidation of the double bond, generated, through an in situ cyclization, both diastereoisomers of the alcohol **9**, which were then separated by chromatography column. "Click" reaction of the azide derived from the *syn* product **9** with the alkyne **11**,[22] furnished, after TIPS deprotection, the key intermediate **12**. The latter was then functionalized to obtain different compounds showing the same scaffold. In particular, a coupling reaction of the primary amine **13** with aryl carboxylic acids, allowed the generation of the final desired amides bearing various five-membered heteroaromatic rings. In Scheme 2, the synthesis for the most active furan compound **15** is reported. This was tested against *L. major* exhibiting micromolar activity (EC_{50} = 7.8 μM) with outstanding selectivity on human cell line.

N'Da and coworkers recently reported the in vitro anti-infective potential of artemisinin derivatives, which are biologically active through the generation of reactive oxygen species (ROS).[25] Similarly, the antileishmanial antimonials owe their pharmacological effects to the generation of ROS, resulting in oxidative stress and ultimately in parasite death.[26] Starting from dihydroartemisinin (DHA), the authors synthesized and tested a plethora of artemisinin-acridine hybrids[27] and non-hemiacetal artemisinin derivatives.[28] Focusing on the latter, using commercially available DHA **16**, the key intermediate aldehyde **19** was synthesized and reduced to the corresponding primary alcohol, which is esterified with various acyl chlorides obtaining the desired contracted artemisinin ester analogues **21a-d** (Scheme 3).[29] It is worth mentioning that the stereochemistry of the new stereocenter, generated by the contraction of the six-membered ring, is not controlled, being the products isolated as a mixture of epimers.



SCHEME 2 Reagents and conditions: (i) MsCl, Et_3N, dry CH_2Cl_2, 0°C to rt, 3 h; (ii) 6-([triisopropylsilyl]oxy)hexan-1-ol, NaH (60% in mineral oil), dry THF, reflux, overnight; (iii) *m*-CPBA, CH_2Cl_2, 0°C to rt, overnight; (iv) (*S*,*S*)-Co-salen catalyst, AcOH, THF, H_2O, 0°C to rt, 16 h; (v) 3-butenylmagnesium bromide, CuI, dry THF, −78°C to rt, 2 h; (vi) *m*-CPBA, CH_2Cl_2, 0°C to rt, 2 h, then (±)-CSA, 0°C to rt, 5 h (*syn* alcohol by flash column chromatography); (vii) $(PhO)_2P(O)N_3$, DIAD, PPh_3, Et_3N, 0°C to rt, overnight; (viii) **11**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-BuOH:H_2O = 1:1, rt, 16 h; (ix) (±)-CSA, CH_2Cl_2,MeOH, rt; (x) NaN_3, DMF, 40°C, overnight; (xi) PPh_3, THF, rt, overnight then H_2O; (xii) **14**, EDC hydrochloride, DMAP, CH_2Cl_2, rt, overnight

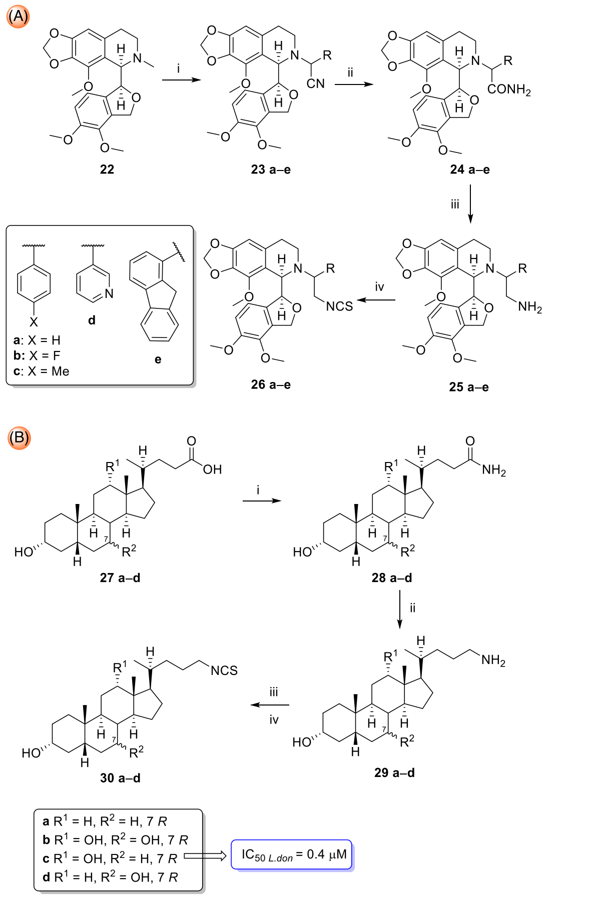


SCHEME 3 Reagents and conditions: (i) $BF_3 \cdot Et_2O$, Et_2O, 0°C to r.t, 24–48 h; (ii) Br_2, acetone, H_2O, rt, 3 h; (iii) Et_3N, CH_2Cl_2, rt, 3 h; (iv) $NaBH_4$, dry MeOH, 0°C to rt, 2 h; (v) acyl chloride [i.e., 4-nitrobenzoyl chloride, 4-fluorobenzoyl chloride, (1,1′-biphenyl)-4-carbonyl chloride, 4-methylthiophene-2-carbonyl chloride], Et_3N, CH_2Cl_2 dry, 0°C, 1 h, then rt, 18 h

The *Leishmania* antipromastigote activity of artemisinin analogues were studied in vitro in *L. donovani* and *L. major* stains.[29] These molecules possessed high intrinsic activities ($IC_{50} \leq 10$ μM), without a toxic profile and a selective action (SI ≥ 10) toward *Leishmania* pathogens. Regarding *L. donovani* strain, the best result was achieved with the ester containing biphenyl moiety **21c** ($IC_{50} = 2.80$ μM), which was as much as 30-fold more potent than clinical artemisinins. This molecule was therefore indicated as suitable for further investigation as possible intracellular antiamastigote hit.[13]

Among NPs, both noscapine,[30–33] a natural phthalideisoquinoline alkaloid, and bile acids,[34–37] natural steroids produced by mammals, have shown interesting antiparasitic activity.

Starting from these scaffolds, Salehi and coworkers semi-synthesized novel isothiocyanate derivatives[38] (Scheme 4), considering that the SCN moiety was suggested as an interesting pharmacophore in inhibiting the Trypanothione reductase.[39] Despite the differences in the starting materials, the authors pointed out the necessity to install a primary amine moiety. In particular, through a three-component Strecker reaction, α-amino nitrile derivatives **23** of noscapine were synthesized under acidic conditions. Then, the nitrile group was transformed into an amide, which was converted into the crucial primary amine **25** by utilizing $NaBH_4$ and $BF_3 \cdot Et_2O$. Later, treatment of these molecules with CS_2, followed by the use of Boc_2O and catalytic DMAP, afforded the desired isothiocyanate derivatives **26a-e**.[40] Regarding the bile acids derivatives **30a-d**, an amidation, using NH_4Cl and TBTU, allows the obtainment of products **28a-d**, and the

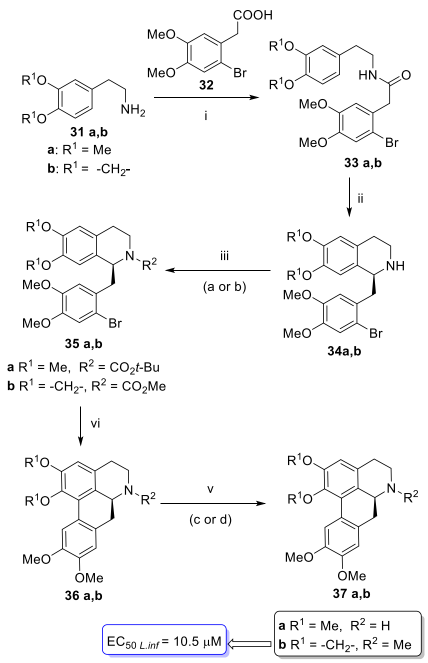


SCHEME 4 Reagents and conditions: (A) (i) R-CHO, AcOH, rt, 30 min, then KCN, rt, TLC monitored; (ii) H_2O_2 (30% v/v), K_2CO_3, MeOH:DMSO = 10:1, rt, 5–24 h; (iii) $NaBH_4$, $BF_3 \cdot EtO_2$, dry THF, −5°C, 1 h, then rt, overnight; (iv) 1. CS_2, Et_3N, EtOH, rt, TLC monitored; 2. Boc_2O, DMAP, EtOH, −5°C, 5 min, then rt, 2 h. (B) (i) NH_4Cl, TBTU, Et_3N, CH_3CN, rt, 1–3 h. (ii) $NaBH_4$, $BF_3 \cdot EtO_2$, dry THF, −5°C, 1 h, then rt, overnight; (iii) 1. CS_2, Et_3N, EtOH, rt, TLC monitored; 2. Boc_2O, DMAP, EtOH, −5°C, 5 min, then rt, 2 h

isothiocyanate derivatives were obtained according to the methods described above, again passing through formation of a primary amine.

The isothiocyanate compounds reported in Scheme 4 demonstrated excellent antileishmanial activities against *L. donovani* compared to miltefosine. In detail, **26a**, **26b**, **26c**, **26e**, **30b**, **30c** displayed IC_{50} between 0.4–1.0 μM and SI values varying from 1.7 to 18.4. The best activity was shown by isothiocyanate bile acid **30c** ($IC_{50} = 0.4$ μM), which was two-fold more active than miltefosine ($IC_{50} = 0.7$ μM). Isothiocyanates with noscapine scaffold **26a**, **26b**, were the most selective with SI of 18.4 and 17.5, respectively. Importantly, the introduction of the isothiocyanate group is crucial for the antileishmanial activity; in fact, no activity was observed in compound **25a-e**.

An enantioselective, modular, and convergent strategy for the synthesis of aporphines, another class of interesting natural alkaloids with interesting biological



SCHEME 5 Reagents and conditions: (i) **32**, EDC hydrochloride, HOBt, NMM, DMF, 0°C to rt, 4 h; (ii) 1. Tf_2O, 2-chloropyridine, DCM, −78°C to 0°C, 15 min. 2. RuCl[(*R*,*R*)-TsDPEN](*p*-cymene), Et_3N/HCO_2H (2:5), 0°C to rt, 10 h; (iii) (a) Boc_2O, (*i*-Pr_2)EtN, DMAP, CH_2Cl_2, rt, 2 h (**34a** to **35a**) or (b) MeOCOCl, (*i*-Pr_2)EtN, DMAP, CH_2Cl_2, rt, 10 h (**34b** to **35b**); (iv) Pd $(OAc)_2$, $(t\text{-Bu})_2PMeHBF_4$, K_2CO_3, DMA, 130°C; (v) (c) $ZnBr_2$, CH_2Cl_2, rt, 8 h (**36a** to **37a**) or (d) $LiAlH_4$, THF, 0°C to rt, 20 h (**36b** to **37b**)

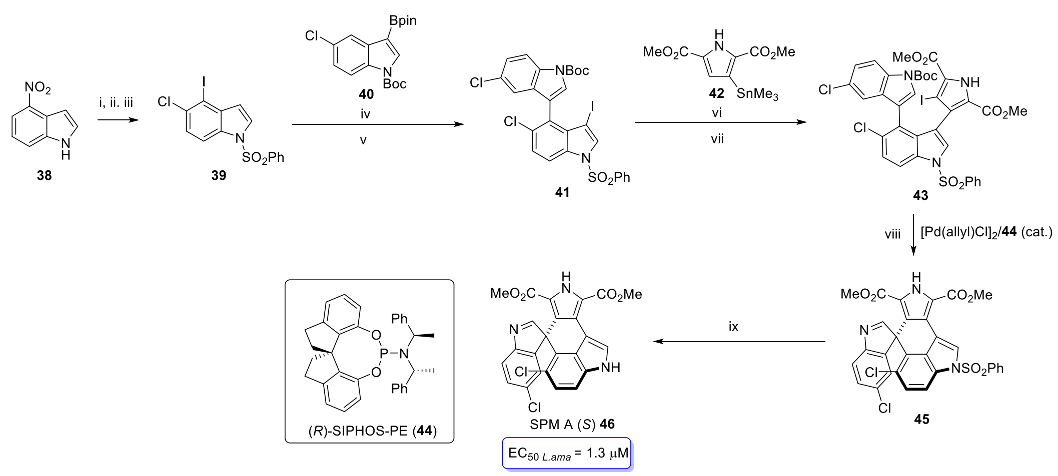
activities,[41–46] has been reported by Anderson et al. as described in Scheme 5.[47]

Phenylethylamines **31a,b** are obtained from veratraldehyde and piperonal, through the respective β-nitrostyrenes. A coupling with 2-(2-bromo-4,5-dimethoxyphenyl) acetic acid **32**[48] afforded the amides **33a,b**. Here, a Bischler-Napieralsky cyclodehydratation in presence of Tf_2O and 2-chloropyridine, afforded the bezyldihydroisoquiniles, which were directly reduced by Noyori asymmetric transfer hydrogenation (AHT), obtaining the tetrahydroisoquinolines **34a** and **34b** in good yields with 93% *ee* and 94% *ee* respectively (*S* isomer). The reaction foresees the use of RuCl(*p*-cymene)[(*R*,*R*)-Ts-DPEN] as catalyst and formic acid as the hydrogen source.

The amine moieties of these chiral molecules were then protected and a direct palladium-catalyzed ortho-arylation was next achieved using Pd $(OAc)_2$ with bis(*tert*-butyl)methylphosphine obtaining the aporphine carbamates **36a,b** in moderate to good yields. The authors pointed out how the nature of the ligand is crucial in achieving such good results. Subsequently, deprotection of the Boc-protected amines or reduction of the methyl carbamate formed the tested products **37a,b**.

Key steps for achieving the reported transformation are therefore the Bischler-Napieralski cyclization/Noyori asymmetric reduction to construct the (*S*)-tetrahydroisoquinoline isomers and the successive Pd-catalyzed arylation for ring closure.

These aporphine alkaloids were tested versus amastigote *L. infantum*. This study reported a reduction of toxicity in murine conjunctive cells (NCTC) with norglaucine **37a** ($CC_{50} = 71.3$ μM) related to miltefosine ($CC_{50} = 119.7$ μM). On the other hand, dicentrine **37b** was more bioactive ($EC_{50} = 10.5$ μM) but more toxic than **37a**.



SCHEME 6 Reagents and conditions: (i) $PhSO_2Cl$, (*i*-Pr)$_2$EtN, CH_3CN, 80°C, 5 h, then Zn, AcOH, CH_2Cl_2, 0°C to rt, 2 h; (ii) NCS, DMF, 0°C, 1 h, then rt, 1 h; (iii) $NaNO_2$, aq. HCl, H_2O, 0°C, 2 h, then KI, H_2O, 0°C, 2 h; (iv) **40**, Pd $(OAc)_2$/SPhos, K_2CO_3, THF, H_2O, 75°C, 6 h; (v) *N*-iodosuccinimide, *p*-TsOH, CH_2Cl_2, 40°C, 6 h; (vi) **42**, Pd-PEPPSI-IPr, Cs_2CO_3, 4 Å molecular sieves, dioxane, 90°C, 14 h; (vii) I_2, KOH, DMF, rt, 6 h; (viii) neat, 150°C, 3 h, under Ar, then [Pd (allyl)Cl]$_2$/**44**, Ag_2CO_3, Cs_2CO_3, toluene, 70°C, 12 h; (xi) Bu_4NOH, THF, 80°C, 8 h

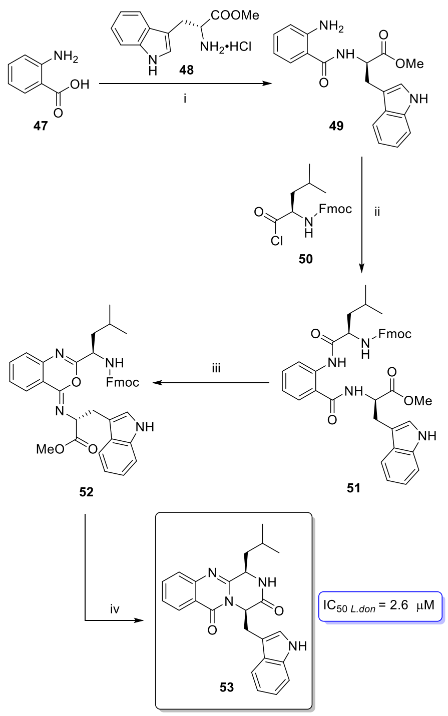
Dimeric tryptophan NPs were proofed as medicinally important molecules.[49,50] Among these, spiroindimicins are a class of chlorinated indole alkaloids characterized by three heteroaromatic rings structured around a congested spirocyclic stereocenter. In 2021, Smith et al. reported the first total synthesis of (+)-spiroindimicin A.[51] The main challenge associated with this total synthesis was the construction of a core quaternary spirocenter in an enantiocontrolled fashion. Hence, a synthetic route of nine steps starting from the commercially available 4-nitroindole **38** was developed (Scheme 6). This, through a series of transformations, including Suzuki and Stille couplings with **40**[51] and **42**,[51] respectively, was converted to the key triaryl compound **43**. Then, after a screening on ligands and reaction conditions, the authors were able to reach the desired Pd-catalyzed asymmetric spirocyclization using the chiral phosphoramidate ligand **44** attaining product **45** with high enantioselectivity. It is worth noting that the Boc deprotection and spirocyclization could be conducted as a one-pot procedure.[51] Finally, removal of the protecting benzenesulfonyl group on the starting indole nitrogen led to the spiroindimicin A (**46**). *S*-**46** and *R*-**46** antiparasitic activity were evaluated in *L. amazonesis*. Notably, both enantiomers did not exhibit significant cytotoxicity in RAW cells (CC_{50} > 10 μM), with respect to the racemic mixture. Regarding efficacy,**46**-(*S*) showed lower EC_{50} than **46**-(*R*) (1.3 and 5.3 μM, respectively).

3 | AMINO ACID-BASED COMPOUNDS

Amino acids are preferred reagents for the synthesis of chiral compounds, due to the already present stereogenic center. Starting from natural (L)- or unnatural (D)-amino acids, chiral pool strategies can be then easily projected. Moreover, due to their biological function, their presence can facilitate the interaction against a specific site of different enzymes.

Moreover, as shown with (+)-spiroindimicin A, indole containing compounds have been widely studied against *leishmania* for their antiparasitic activities.[52,53] Recently, Nogueira, Sousa and co-workers studied the activity of indoles having a pyrazino[2,1-*b*]quinazoline-3,6-dione functionality against *Plasmodium falciparum*, *T. brucei*, and *L. infantum*.[54]

The synthesis of the most active compound **53** is reported in Scheme 7[55] and essentially followed a Mazurkiewicz–Ganesan approach.[56] The reaction of 2-aminobenzoic acid **47** with methyl D-tryptophanate **48**, followed by a two-phase Schotten–Baumann reaction with D-leucine chloride **50**[57] yielded the tripeptides **51**.



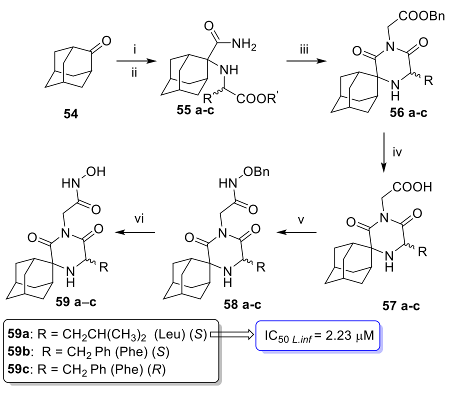
SCHEME 7 Reagents and conditions: (i) **48**, CH_3CN, TBTU, Et_3N, rt, 5 h; (ii) **50**, CH_2Cl_2/aq. Na_2CO_3, rt, 3 h; (iii) CH_2Cl_2 dry, Ph_3P, I_2, (*i*-Pr_2)EtN, rt, overnight; (iv) piperidine in CH_2CH_2, rt, 12 min, then CH_3CN, DMAP, reflux 19 h

The addition of a dehydrating agent (i.e., Ph_3P) led to the oxazoles **52** and the desired *syn* product **53** was obtained by cyclization after Fmoc-deprotection using piperidine. Interestingly, employing a microwave-assisted one-pot strategy, similar to that reported by Liu et al.,[58] only the *anti*-enantiomer was isolated.[55]

Concerning the antileishmanial activity, compound **53** could represent an interesting hit against *L. infantum* with an IC_{50} of 2.6 μM. Notably, its configuration (1*R*,4*R*) is crucial for the activity; indeed the other stereoisomers were not biologically active. Moreover, in silico studies emphasized these molecules as possible inhibitors for prolyl-tRNA synthetase. In particular, the indole scaffold is fundamental for the polar interactions with Ser323. Importantly, the appropriate geometry and chirality are essential for the interaction of this enzyme due to the tight recesses of the target active site.

Fytas and co-workers studied the antileishmanial activity of lipophilic conformationally constrained spiro carbocyclic 2,6-diketopiperazine-1-acetohydroxamic acid analogues,[59] since similar compounds, reported by them, showed antitrypanosome activity.[60] Besides all the tested molecules, chiral acetohydroxamic acids bearing the adamantane scaffold were the most active. Strecker reaction on 2-adamantanone **54** in presence of the selected α-amino acid (i.e., L-alanine, L-leucine, L-methionine, L-phenylalanine, and D-phenylalanine) and successive amidation with NH_3 of the formed carboxylic acid, afforded compounds **55a-c**. The spyrane, containing the piperazindione scaffold **56a-c**, was formed by cyclization reaction, followed by nucleophilic substitution on the benzyl 2-bromoacetate.[61] The successive formation of the acid **57a-c** allowed the coupling with the *O*-benzylhydroxylamine to form *O*-benzyl hydroxamates **58a-c**. Hydrogenolysis of the latter compounds led to the acetohydroxamic acid analogues **59a-c** (Scheme 8).[59,60]

These compounds demonstrated important activities against *L. infantum* promastigotes and intracellular amastigotes, with low micromolar IC_{50} values. *C*-isobutyl analogue **59a**, was active versus *L. infantum* promastigotes with IC_{50} of 7.23 μM. Nevertheless, *L. infantum* amastigote is more sensitive to this compound; in fact, it possesses higher efficiency (2.23 μM). An interesting activity was also shown with cyclooctenone scaffold instead of adamantone for this analogue. The *C*-benzylated analogues **59b** and **59c** and racemate were efficient growth inhibitors on both forms of *L. infantum*, with low micromolar activity. Regarding these latter compounds, the (*S*)-enantiomer **59b** was the most potent and selective against *L. infantum* with similar antipromastigote ($IC_{50} = 2.67$ μM) and antiamastigote ($IC_{50} = 2.60$ μM) activities, being 1.4-and 1.7-fold more active than the (*R*)-enantiomer **59c.** Remarkably, **59c** and its racemate were the only derivatives active against *L. donovani* promastigote ($IC_{50} = 8.35$ μM and $IC_{50} = 9.73$ μM). **59c** also displayed an activity for the amastigotes ($IC_{50} = 15.0$ μM). Hence, it appears that



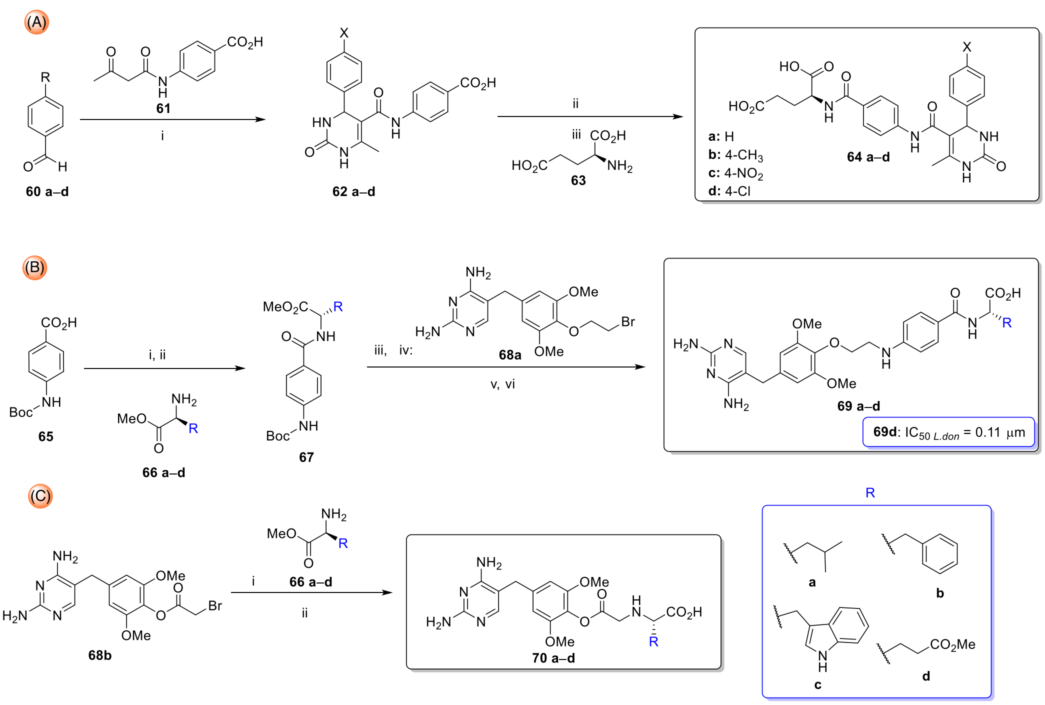
SCHEME 8 Reagents and conditions: (i) NaCN, α-amino acid alkyl ester hydrochloride, DMSO/H_2O 29:1 (v/v), rt, 48 h; (ii) 1. H_2SO_4 97%, CH_2Cl_2, rt, 24 to 48 h. 2. ice and then aq. NH_3 26% to pH 7–8; (iii) 1. $(Me_3Si)_2NK$, THF, 0–5°C, then rt, 1 h, under Ar. 2. benzyl 2-bromoacetate, DMF, rt, 48 h, under Ar; (iv) H_2/Pd-C 10%, EtOH-EtOAc 3:2 (v/v), 50 psi, rt, 3 h; (v) 1. CDI, THF, 28°C, 1 h, under Ar. 2. *O*-benzylhydroxylamine hydrochloride, Et_3N, 28°C, 25 h, under Ar; (vi) H_2/Pd-C 10%, EtOH, 50 psi, rt, 3 h

efficacy against *L. donovani* of the (*R*)-stereochemistry is favored over the (*S*) one. Importantly, these molecules show very low cytotoxicity against mammalian cells ($CC_{50} > 200$ μM), with the exception of **59b** ($CC_{50} = 29.2$ μM), implying significant selectivity.

Dihydrofolate reductase is considered a key target; therefore, based on their previous work,[62] Rashid et al. investigated the ability of dihydropyrimidine-5-carboxamide and 5-benzyl-2,4-diaminopyrimidine-based derivates to inhibit this enzyme for *L. major*.[63] Compounds **64a-d** were synthesized through a Biginelli approach,[64] utilizing differently *p*-substituted benzaldehydes (**60a-d**), urea, and compound **61**.[63] This step allows the formation of the 2-oxo-3,4-dihydropyrimidine-5-carboxylates **62a-d**, which are converted into the respective acyl chloride. *N*-Acylation with L-glutamic acid **63** led to expected compounds **64a-d** (Scheme 9A). In Scheme 9B, a coupling with the Boc-protected 4-aminobenzoic acid **65**, via acyl chloride formation, with the amino acid methyl esters **66a-d**, is followed by Boc-deprotection. The subsequent reaction with product **68a**, followed by hydrolysis yielded **69a-d**. Similarly, the trimethoprim-based derivatives **70a-d** were essentially obtained by *N*-alkylation of the opportune amino acid methyl ester **66a-d** with **68b** and successive hydrolysis (Scheme 9C) Finally, **68a,b** were previously synthesized by reaction of the hydroxy trimethoprim derivative[64–67] with 1,2-dibromoethane or bromoacetyl bromide respectively.

L. major DHFR inhibition activities of these compounds were evaluated. All compounds (except **69b**) showed low micromolar and submicromolar inhibition of *lm*DHFR, emerging **70d** as the more potent, with the $IC_{50} = 0.10$ μM. In addition, selectivity for *lm*DHFR over human DHFR was also measured. Analogues **70a-d** exhibited outstanding results for *lm*DHFR, resulting compounds **70a** (SI = 84.5) and **70b** (SI = 87.5) the most selective. Regarding antipromastigote activity, **64a-d** showed low micromolar concentration. Compounds **69a-d** demonstrated superb inhibitory effect against *L. major* and *L. donovani* strains submicromolar concentration, the best result obtained with **69d** ($IC_{50} = 0.19$ μM vs. *L. major* and $IC_{50} = 0.11$ μM vs. *L. donovani*). Unfortunately, compounds **70a-d** do not show high potency against these pathogens.

Thiohydantoins have emerged as an important class of compounds due to their biological activity,[68–74] with particular focus on the antiparasitic behavior.[75–78] Wowk and Pavanelli explored the antileishmanial (*L. amazonensis*) effect, determining the key mechanism presented in parasite death, of various thiohydantoins bearing an acetyl group at the *N*1-position of the heterocyclic ring.[79] Five L-amino acids **71** (i.e., tryptophan, glutamine, methionine, leucine, and phenylalanine), in the presence of an equimolar amount of ammonium thiocyanate, were treated with

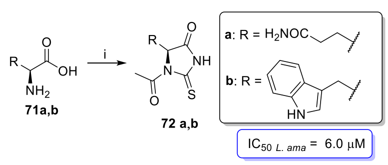


SCHEME 9 Reagents and conditions: (A) (reaction time monitored by TLC) (i) **61**, $(NH_2)_2CO$, $SnCl_2 \cdot 2H_2O$. CH_3CN, reflux; (ii) $SOCl_2$, DMF (1 drop), CH_2Cl_2, reflux; (iii) **63**, NMM, DMF, reflux. (B) (i) **65**, $SOCl_2$, DMF (1 drop), CH_2Cl_2, reflux, 18 h, under N_2; (ii) *L*-amino acid methyl esters **66a-d**, Et_3N, DCM, 4 h; (iii) TFA:H_2O = 1:1, CH_2Cl_2, 0°C, 2 h; (iv) **68a**, CH_2Cl_2, reflux, 16 h; (v) 1-M NaOH, MeOH, rt, overnight; (vi) 1-M HCl (to pH 2–3). (C) (i) L-amino acid methyl esters **66a-d**, CH_2Cl_2, reflux, 16 h; (ii) 1-M NaOH, MeOH, rt, overnight then 1-M HCl (to pH 2)

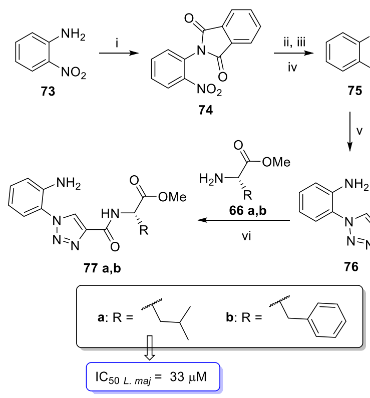
acetic anhydride, affording the desired products with moderate to excellent yields (Scheme 10).

Regarding the activity, acetyl-thiohydantoin **72a** and **72b** inhibited the proliferation of promastigote form with an IC_{50} of 8 and 6 μM, respectively. Moreover, both induced cell cycle arrest at the G2/M phase, a reduction in the cell volume of the parasite, and caused morphological and ultrastructural changes, such as rounded shapes, reduced cell body size, cell surface roughness, plasma membrane damage, cytoplasmic content leakage, mitochondrial swelling, irregular flagellation, and nuclei alteration. Thiohydantoins exercised an apoptosis-like mechanism on promastigote cells due to an increasing ROS production, phosphatidylserine revelation, plasma membrane permeabilization, and a loss of mitochondrial membrane potential, causing an accretion of lipid bodies and the formation of autophagic vacuoles on the pathogen. In intracellular amastigotes, a decreased of number of infected macrophages by enhancing ROS formation and decreasing TNF-*α* amount were observed. Additionally, low cytotoxicity was measured in human monocytes (THP-1), murine macrophages (J774), and sheep erythrocytes. Finally, molecular docking analyses of acetyl-thiohydantoins were carried out on two important targets of *L. amazonensis*: arginase and TNF-α converting enzyme. The results proposed the acetyl group and the thiohydantoin as plausible pharmacophoric groups thanks to possible hydrogen bond interactions with amino acid residues at the active site of these enzymes.

Amino acid derived compounds have also been synthesized and tested by Haldar et al.[80,81] In particular, in 2018 they investigated the potential antileishmanial activity of triazole-based peptides,[80] since this functional group is present in a variety of drugs for infective diseases.[82–87] Reaction of 2-nitroaniline **73** with phthalic anhydride, afforded the corresponding 1,3-isoindolinedione derivative **74**. Successive reduction of the nitro group, azide formation, and amine deprotection furnished the 2-azidoaniline **75**, which was converted into the triazole derivate **76** by click chemistry using propiolic acid.[88,89] The final amidation with the selected L-amino acid methyl ester **66a,b** (leucine,



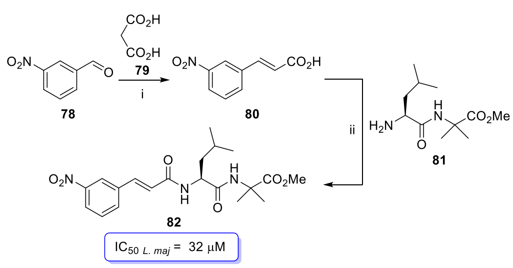
SCHEME 10 Reagents and conditions: (i) NH_4SCN, Ac_2O, 100°C, 30 min



SCHEME 11 Reagents and conditions: (i) phthalic anhydride, 215°C, 2 h, then AcOH, reflux, 30 min; (ii) Fe powder, AcOH, H_2O, acetone, reflux, 8 h; (iii) $NaNO_2$, AcOH:H_2O = 1:1, 0°C, 3 h, then NaN_3, 0°C, 30 min; (iv) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 1 h, then 1-M NaOH, rt; (v) sodium ascorbate, $CuSO_4$, propiolic acid, EtOH:H_2O = 1:1, rt, 12 h; (vi) **66a** or **66b**, DCC, HOBt, dry CH_2Cl_2, rt, 48 h

phenylalanine) in presence of DCC and HOBt led to the final triazole-based hybrid peptide **77a,b** (Scheme 11).

Regarding activity, **77a** and **77b** showed IC_{50} values of 11 μg/ml (33 μM) and 21.2 μg/ml (58 μM) on *L. major* promastigotes, respectively. The better activity of **77a** could be explained by the different lipophilicity. In fact, **77a** containing leucine moiety (which is more lipophilic than **77b**) can better pass through the *leishmania* cell membrane. Moreover, it showed 2–6-fold more potency than the standard antileishmanial drugs like sodium stibogluconate (IC_{50} > 64 μg/ml or 70 μM), ketoconazole (IC_{50} = 72 μg/ml or 135 μM), or pentastam (IC_{50} > 64 μg/ml or 70 μM).[90,91] In vitro MTT based cell viability assay indicated that this peptide even at 2 × IC_{50} concentration did not have any toxic effect on macrophage cell line J774. Morphological changes of leishmanial cells were studied upon treatment with peptide **77a** using the FESEM experiment, showing its "static" action versus *leishmania*. Indeed, cells treated with **77a**, due to inhibition of metabolic processes, endured substantial cellular stress that may not kill the parasite but stopped the normal growth in vitro and resulted in morphological abnormality (especially shorter size and rounded shape).[92]

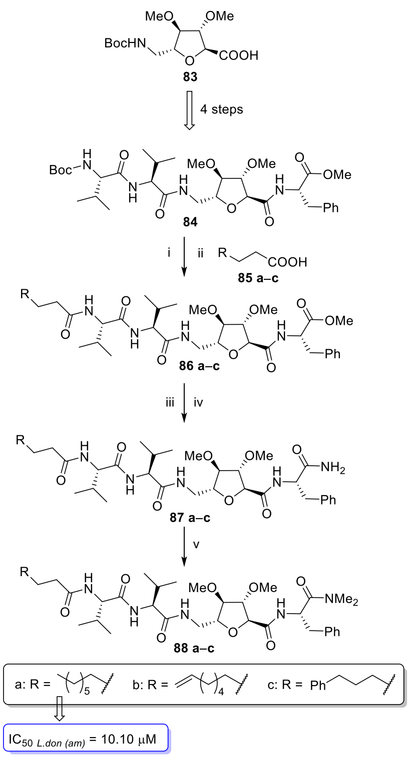


SCHEME 12 Reagents and conditions: (i) **79**, piperidine, DMF:H_2O = 20:3, 120°C, 6 h; (ii) H_2N-*L*-Leu-Aib-OMe **81**, DCC, HOBt, Et_3N, CH_2Cl_2 dry, 0°C to rt, 48 h

More recently, Haldar and coworkers reported the synthesis of the *m*-nitrocinnamic peptide **82** (Scheme 12).[81] The synthesis foresees the initial formation of the 3-(3-nitrophenyl)-acrylic acid **80** through Knoevenagel condensation reaction between 3-nitrobenzaldehyde **78** and malonic acid **79**. Solution-phase peptide synthesis between **80** and the dipeptide H_2N-L-Leu-Aib-OMe **81**, using DCC and HOBt as coupling agents, gave the final product **82**.

This peptide had exhibited considerable growth inhibition activity on *L. major* promastigotes. Its higher lipophilicity may increase the possibility of reaching this intracellular parasite. This molecule exhibited at least 2–6-fold higher potency (IC_{50} = 13 μg/ml or 32 μM) than the above mentioned standard drugs.[90,91] Moreover, in vitro MTT based cell viability assay points out that it can destroy the *Leishmania* promastigotes at a very low dose without a substantial destructive effect on the human-type macrophage cell line.

In 2017, sugar amino acid-based (SAA) linear lipopeptide analogues, having hybrid sequences of natural amino acids (AAs), unnatural (4*R*,5*S*)-4-amino-5-methylheptanoic acid (AMH), and a mannose-derived



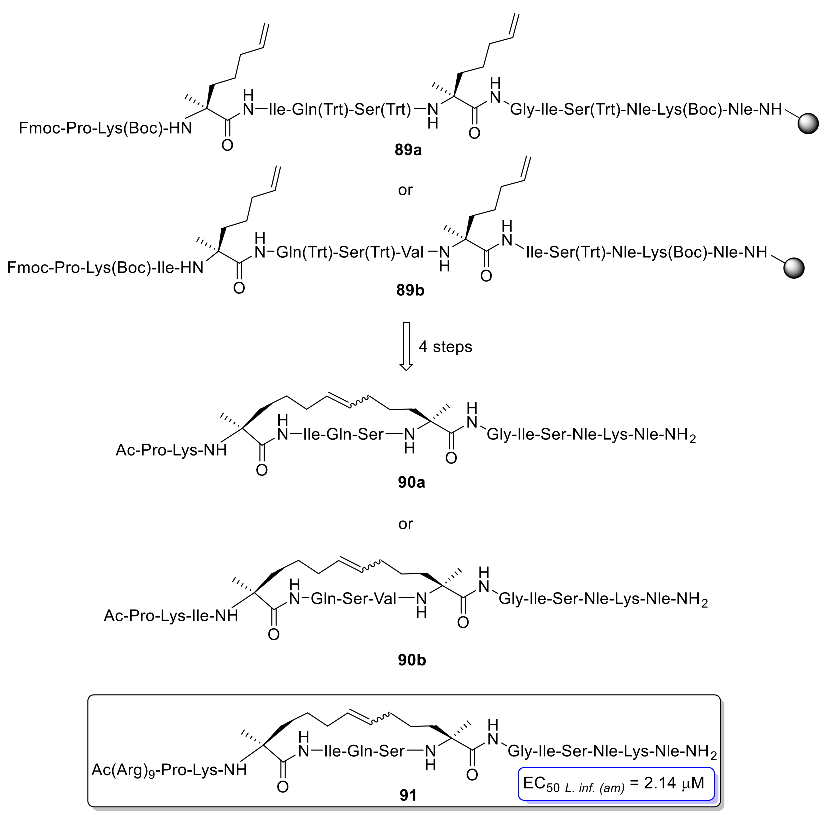
SCHEME 13 Reagents and conditions: (i) TFA, CH_2Cl_2, 0°C to rt, 30 min; (ii) **85**, HOBt, EDC hydrochloride, CH_2Cl_2, 0°C, 10 min, then DIPEA, rt, 12 h; (iii) LiOH·H_2O, THF:MeOH:H_2O = 3:1:1, 0°C to rt, 1 h; (iv) EtOCOCl, Et_3N, aq. NH_3, THF, −20°C to 0°C, 1.5 h; (v) Ag_2O, MeI, DMF, 0°C to rt, 12 h

sugar amino acid (MAA), were synthesized and examined by Chakraborty et al. to identify potential drug candidates to treat VL.[93] Besides all, MMA-permethylated analogues bearing longer hydrophobic chains at the terminal nitrogen, without AMH units, gave the better results, and only their synthesis is described here in detail (Scheme 13).

Coupling of *N*-Boc-6-amino-2,5-anhydro-3,4-di-*O*-methyl-6-deoxy-D-mannonate **83** (obtained in 11 steps from D-mannitol[94,95]) with L-phenylalanine methyl ester and, successively, with the Boc-Val-Val[96] unit, was performed by standard SPPS method using EDC and HOBt as coupling agents, obtaining the tetrapeptide **84**. Boc deprotection and coupling with various acids bearing lipophilic side chains **85a-c**, under the already described peptide coupling conditions, gave the compounds **86a-c**. Finally, compounds **88a-c** were obtained by hydrolysis of the terminal methyl ester and successive amidation, forming compounds **87a-c**, followed by *N*-permethylation.

It was observed that the permethylated SAA **88a-c** were more active against intra-macrophagic amastigotes of *L. donovani* than the unmethylated parents **87a-c**, probably due to their enhanced membrane permeability. Furthermore, NMR and SAR studies highlighted that *N*-methyl groups were essential for hampering the formation of any turn structure resulting in their increased activities. Particularly, **88a-c** showed moderate IC_{50} (10.10, 10.92 and 13.63 μM, respectively) and SI comparable to miltefosine.

Velázquez and co-workers, encouraged by the results they obtained with linear and lactam-bridged 13-residue peptides against *L. infantum*,[97] recently synthesized all-hydrocarbon stapled α-helical analogues of these peptides.[98] This series of peptides were designed to improve the *L. infantum* trypanothione reductase (Li-TryR) enzyme inhibition activity, the proteolytic stability, and the cell permeability of correspondent linear peptides which target the dimerization interface of Li-TryR. Indeed, trypanothione reductase is another well-known target for antileishmanial agents, since it maintains the cellular redox homeostasis in *leishmania*. The analogues were synthesized on Rink Amide-MBHA polystyrene resin following the standard Fmoc/^{t}Bu solid-phase orthogonal protection strategy (SPPS), essentially foreseeing cycles of Fmoc deprotections and coupling until the desired sequence of amino acids is installed. In particular, the introduction of two units of Fmoc-(*S*)-α-methyl-α-pentenylglycine at the suitable positions of the sequence during the chain elongation[99,100] allowed the synthesis of the linear precursors **89a,b**. The key step for the formation of the hydrocarbon stapled into compound



SCHEME 14 Schematic representation for the synthesis of all-hydrocarbon stapled α-helical peptides **90** and **91**

90-a,b (Scheme 14) is the ring-closing metathesis (RCM), performed on-resin. A complete conversion can be achieved using a Grubb's second-generation Ru catalyst under microwave irradiation (75°C, 1 h) with essentially no control at the stereochemistry of the double bond. Eventually, after Fmoc deprotection and N-terminus acetylation, the cleavage of the peptide from the resin, afforded the stapled hydrocarbons **90a,b**. A polyarginine (R9 CPP) carrier was linked to the N-terminal end of **90a** to facilitate the delivery of the peptides into the parasites (obtaining peptide **91**).[101–103]

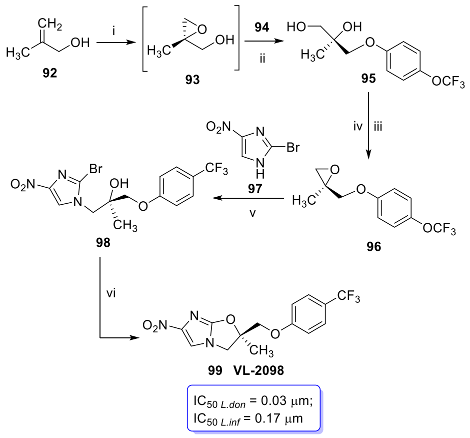
These new peptides maintained potent inhibitory activity against Li-TryR, enhancing proteolysis resistance. Interestingly, **90a,b** inhibit oxidoreductase activity in a different way, by stabilizing the TryR homodimer. These peptides were not able to cross the cell membrane, but the covalent binding of peptide **90a** with R9 CPP promoted intracellular uptake, turning this molecule into a highly active compound (**91**) against both promastigotes and amastigotes of *L. infantum* parasite.

Notably, leishmanicidal activity versus axenic amastigotes of [R9]-[stapled-peptide] conjugate **91** was comparable to miltefosine (IC_{50} = 2.14 μM vs. 2.0 μM).

4 | NITROIMADAZOXAZOLE AND -OXAZINE DERIVATIVES

One of the most promising drug candidates for the treatment of VL is the dihydroimidazoxazole VL-2098 (**99**),[104–106] In vitro biological activity highlighted the submicromolar IC_{50} values of 0.03 μM against *L. donovani*. Importantly, VL-2098 showed better activity (IC_{50} = 0.17 μM) than the corresponding (*S*)-enantiomer (IC_{50} = 0.33 μM) and was 4.5-fold more potent than racemic (IC_{50} = 0.77 μM) against *L. infantum*. In the mouse model, VL-2098 was remarkably superior to the (*S*)-enantiomer and better than racemic itself (83%, 8%, and 64% inhibition at 3.13 mg/kg, respectively), and this trend was in parallel with the microsomal stability data (0.07 μg/ml). A relative evaluation in the CDRI hamster model proved the greatest activity of the (*R*)-enantiomer (**99**) over the (*S*)-form and racemic mixture at all dose levels. Moreover, dose–response evaluations in a similar *L. infantum*-infected hamster model at LMPH also expressed an excellent in vivo efficacy of **99** (>99% inhibition in liver, spleen, and bone marrow at 25 mg/kg).[107]

Recently, Pati et al. developed a kilogram scale process for the synthesis of **99** (Scheme 15).[108] The authors, to make the process scalable, focused their attention on reducing safety hazards and facilitating separations, employing the in situ synthesis of some intermediates without their isolations.



SCHEME 15 Reagents and conditions: (i) (-)-diisopropyl-D-tartrate, Ti (O*i*-Pr)$_4$, CH_2Cl_2 dry, −30°C, 1 h, under N_2, then anhydrous TBHP, dry CH_2Cl_2, −30°C, 1 h then −5°C, 1 h, under N_2; (ii) **94**, K_2CO_3, MeOH, 60°C, 22 h; (iii) *p*-nitrobenzenesulfonyl chloride, Et_3N, CH_2Cl_2, 0°C to 30°C, 3 h; (iv) 12.5-M NaOH, CH_2Cl_2 15°C, 1 h; (v) **97**, (*i*-Pr)$_2$EtN, 115°C, 2 h; (vi) K_2CO_3, DMF, 60°C, 6 h

They began to investigate a small-scale approach (25 g). A Sharpless asymmetric epoxidation, using Ti (O*i*Pr)$_4$ together with D-(-)-diisopropyl tartrate as chiral ligand, of the β-methallyl alcohol **92**, defines the (*R*)-configuration of the stereogenic center formed leading the epoxide **93**, which, without isolation, was allowed to react with *p*-trifluoromethoxyphenol **94**, affording the diol **95**. Next, the latter was subjected to a regioselective sulfonylation reaction on the primary hydroxyl group, obtaining the corresponding nosylate, which underwent a base-catalyzed ring closure reaction to achieve the key intermediate oxirane **96** in high enantiomeric excess.

Treatment with 2-bromo-4-nitroimidazole **97**[109] in the presence of DIPEA gave **98**, followed by a further base-

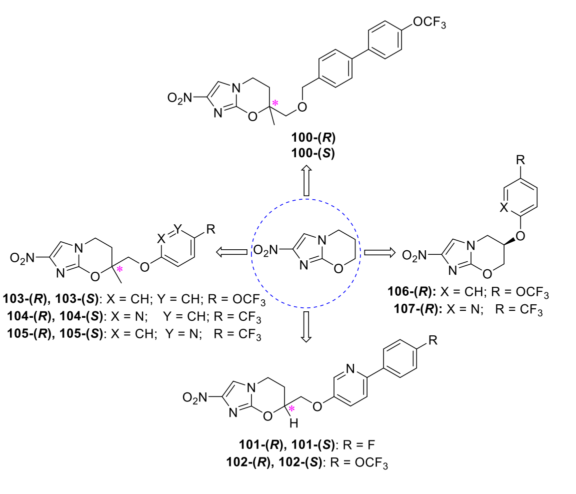
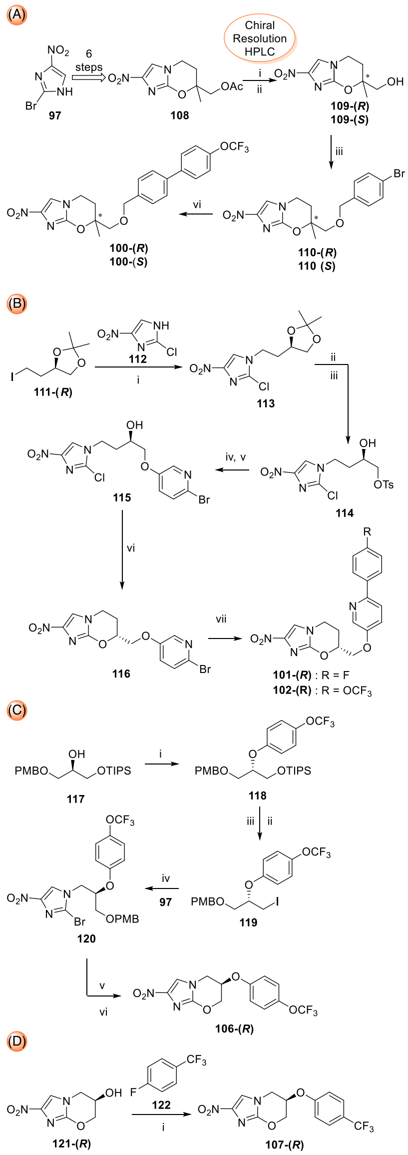


FIGURE 2 Most promising 2-nitroimidazoxazine derivatives



SCHEME 16 Reagents and conditions: (A) (i) preparative chiral HPLC (Chiral Pak IA: Amylose derivatives tris (3,5-dimethylphenylcarbamate); (ii) K_2CO_3, aq MeOH, 20°C, 4 h; (iii) 1-bromo-4-(bromomethyl)benzene, NaH, DMF, 0°C to rt, 7 h; (iv) [4-(trifluoromethoxy)phenyl] boronic acid, toluene, EtOH, 2-M Na_2CO_3, Pd (dppf)Cl_2, 90°C, 1 h, under N_2. (B) (i) **112**, K_2CO_3, DMF, 70°C, 19–72 h; (ii) 1-M HCl, MeOH, 0°C, 6 h; (iii) TsCl, pyridine, −10°C to rt, 14 h; (iv) DBU, CH_2Cl_2, 0°C, 9 h; (v) 6-bromopyridin-3-ol, K_2CO_3, methyl ethyl ketone, 80°C, 19–42 h; (vi) NaH, DMF, 0°C, 3 h; (vii) (4-fluorophenyl) boronic acid or [4-(trifluoromethoxy)phenyl] boronic acid, DMF, (toluene, EtOH), 2-M Na_2CO_3, Pd (dppf)Cl_2, 80°C, 4 h, under N_2. The (*S*)-enantiomer can be obtained starting from (*S*)-4-(2-iodoethyl)-2,2-dimethyl-1,3-dioxolane. (C) (i) 4-(trifluoromethoxy)phenol, DEAD, PPh_3, THF, 0–20°C, 60 h; (ii) TBAF, THF, 20°C, 0.5–18 h; (iii) I_2, PPh_3, imidazole, CH_2Cl_2, 20°C, 12–35 h; (iv) **97**, K_2CO_3, DMF, 90°C, 64–111 h; (v) DDQ, CH_2Cl_2, 20°C, 10–28 h, then TsOH, MeOH, 20°C, 12 h; (vi) NaH, DMF, 0–20°C, 0.25–5.5 h. (D) (i) 1-fluoro-4-(trifluoromethyl)benzene, NaH, DMF, 0–20°C, 0.25–5.5 h

catalyzed annulation, and afforded the desired compound **99**. The authors, addressing some issues due to the scale-up of the reaction (e.g., maintaining anhydrous conditions on the Sharpless epoxidation), were eventually able to produce approximately 10 kg of **99** through their synthesis.

Another recent synthetic pathway proposed by Singh and coworkers differs from the previous method essentially for the initial approach to intermediate **95** formation.[110] First, allylation of 4-trifluoromethoxy phenol with 2-methylallyl chloride, furnished the 1-[(2-methylallyl)oxy]-4-(trifluoromethoxy)benzene, which was subjected to a Sharpless asymmetric dihydroxylation using AD mix-α (containing the chiral ligand $[DHQ]_2PHAL$),[111] leading to the (*R*)-enantiomer diol key derivate **95** in high yield. Here, as already discussed, subsequent epoxidation, via mesylation, followed by successive coupling with 2-bromo-4-nitroimidazole and treatment with base, gave **99** in 36% overall yield.

Inspired by the structural analogy with **99** (VL-2098), Thompson et al. recently reported the synthesis of several substituted 2-nitroimidazoxazine and evaluated their biological activity.[112–114] They fulfilled an exceptional task, testing over a hundred racemic and enantiopure compounds against different types of *leishmania* strains. The most promising enantiopure candidates identified by the authors are reported in Figure 2.

TABLE 1 In vitro antileishmanial activities of nitroimidazoxazines

	IC_{50}[a] (μm)		
Compound	*L. don*	*L. inf*	MRC-5
(R)-**100**	0.24	1.3	>64
(S)-**100**	1.3	1.3	>64
(R)-**101**	(0.03)[b]	0.080	>64
(S)-**101**	(0.08)[b]	0.22	>64
(R)-**102**		0.11	>64
(S)-**102**		0.13	>64
(R)-**103**	0.06	0.098	>64
(S)-**103**	0.37	0.17	>64
(R)-**104**		0.29	>64
(S)-**104**		0.75	>64
(R)-**105**	0.44	1.3	>64
(S)-**105**	0.74	2.3	>64
(R)-**106**	(0.19)[b]	0.53	>64
(R)-**107**	(0.15)[b]	1.1	>64

[a] IC_{50} values of grown inhibition of leishmania stains in mouse macrophages and cytotoxicity toward human lung fibroblast (MRC-5).
[b] LMPH data.

TABLE 2 Microsomal stability and in vivo antileishmanial effect values of nitroimidazoxazines

	Microsomal Stability[a] (% remaining at 1 h)			In vivo efficacy versus *L. don*[b] (% inhibition at dose in mg/kg)					
Compound	**H**	**M**	**Ham**	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.56**
(*R*)-**100**	86	79							
(*S*)-**100**	86	59							
(*R*)-**101**	58	69	34				93		
(*S*)-**101**	63	41	5				85		
(*R*)-**102**	50	53						>99	84
(*S*)-**102**	52	46						52	57
(*R*)-**103**	63	65	1,9		>99	98	49	51	
(*S*)-**103**	50	36	1,2		66	42	56	18	
(*R*)-**104**	73	61	0,4		84				
(*S*)-**104**	70	52	1,8		38				
(*R*)-**105**	86	89	57		72				
(*S*)-**105**	89	90	55		46				
(*R*)-**106**	81	79	19	>99	>99	81	42		
(*R*)-**107**	90	92	48	>99			30		

[a]Pooled human (H), CD-1 mouse (M), or hamster (Ham) liver microsomes.
[b]Dosing was oral, 1 daily/5 days; values are the mean percentage reduction of pathogen burden in the liver.

Mainly, a chiral pool approach to introduce the desired absolute configuration at the stereogenic center was applied.

They initially focused their attention on the synthesis of methyl-*O*-diaryl substituted 2-nitroimidazoxazine (**100–102**).

Starting from the 2-bromo-4-nitroimidazole **97**, the racemic acetated compound **108**[112] was synthesized and separated in the two enantiomers using preparative chiral HPLC separation. Subjecting both **109** to a standard alkylation and a Suzuki coupling, the expected benzyl ethers (*R*)-**100**and (*S*)-**100** were eventually formed (Scheme 16 A). To obtain compounds (*R*)-**101**, (*S*)-**101**, (*R*)-**102**, and (*S*)-**102**,[112] a coupling, using the appropriate optical isomer **111**[115] [synthesis of (*R*)-enantiomer is showed in Scheme 16B] with 2-chloro4-nitroimidazole**112** gave the chiral acetal product **113**, which, after hydrolysis and tosylation, gave compound **114**. This latter was converted into the respective enantiopure epoxide. Subsequent opening by 6-bromopyridin-3-ol, ring closure and Suzuki coupling with appropriate arylboronic acids ArB $(OH)_2$ gave the enantiopure desired product (*R*)-**101** and (*R*)-**101** (Scheme 16B). More recently, they reported the synthesis of new analogues (*R*)-**103**, (*S*)-**103**, (*R*)-**104**, (*S*)-**104**, (*R*)-**105**, (*R*)-**105** using a similar synthetic approach to that proposed for the synthesis of (*R*)-**101** and (*S*)-**101**.[114] Even in this case, starting from an enantiopure protected alcohol, a coupling with 2-chloro-

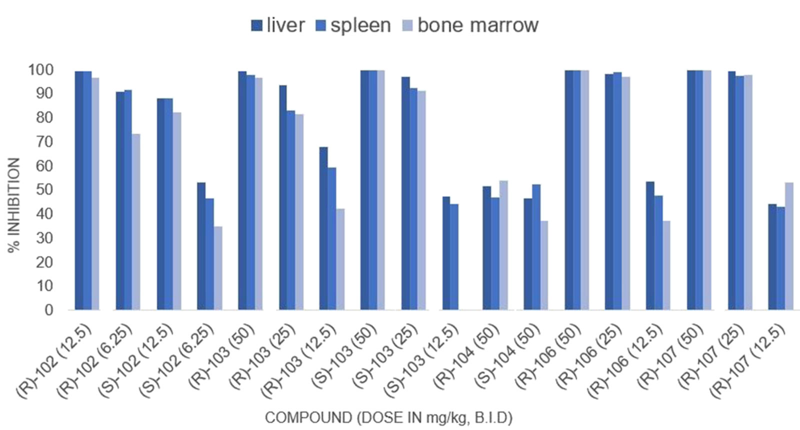


FIGURE 3 In vivo efficacy in the *L. infantum* hamster model

4-nitroimidazole, followed by the epoxidation and ring closure takes place to form the 2-nitroimidazoxazine scaffold, which was eventually functionalized at the CH_2OH position with different halo (hetero)cycles.

Moreover, they also synthesized a series of 2-nitroimidazoxazines linked to the (hetero)aryl moiety in position 6 by an etheric bond.[113] Among all these, an extensive structure–activity relationship investigation was reported for (*R*)-**106** and (*R*)-**107**, the synthesis of which is reported in Scheme 16C,D.

A Mitsunobu reaction of 4-(trifluoromethoxy)phenol with the orthogonally deprotected triol **117**[116] afforded the key intermediate **118** bearing the terminal alcohols protected by two different functional groups. The selective deprotection of these functionalities, followed by coupling with 2-bromo-4-nitroimidazole and ring closure, furnished the desired enantiomer (*R*)-**106**.

From the enantiopure chiral alcohol **121**[117] the compound (*R*)-**107** was obtained via NaH-catalyzed S_NAr displacement with 2-fluoro-5-(trifluoromethyl)pyridine.

Regarding in vitro antileishmanial activity, excellent results were achieved (in most cases with submicromolar values) as shown in Table 1. All compounds reported were non-toxic against human lung fibroblast MRC-5 cells. Confronting the activities of the couple of enantiomers, it appears that (*R*)-isomers were more potent than the relative S-enantiomer in both *leishmania* strains (*L. donovani* and *L. infantum*). In addition, microsomal stability of these series was evaluated (Table 2): an excellent percentage of remaining parent compounds (after 1 h incubation) was observed for (*R*)-**106**, (*R*)-**107**, (*R*)-**100**, (*S*)-**100**, (*R*)-**105**, and (*S*)-**105**. Successively, in vivo (mouse) efficacy against *L. donovani* at different doses was screened (Table 2): remarkable percentages of inhibition at 12.5 mg/kg were obtained with (*R*)-**106** and (*R*)-**103** (81% and 98% respectively), the latter showing a much more potent effect than the corresponding enantiomer. Lowering the dose to 6.25 mg/kg, the best results were achieved with (*R*)-**101** and (*S*)-**101** (93% and 85%). Moreover, compound (*R*)-**102** exhibited extraordinary efficacy at 1.56 mg/kg (84%), higher than the (*S*)-enantiomer (57%). The most interesting compounds were orally dosed in the early curative *L. infantum* hamster model and the results are reported in Figure 3. Best results were attained with compound (*R*)-**101**, which inhibited *L. infantum* in liver, spleen, and bone marrow at 12.5 mg/kg dose (99.5%, 99.4%, and 96.8%, respectively). Notably, also at 6.25 mg/kg, (*R*)-**101** showed exclusive inhibitory activity (91.0%, 91.6%, and 73.3%), still being more effective than the corresponding (*S*)-enantiomer. All these data clearly highlight how chirality is essential for the antileishmanial activity of this class of drugs.

5 | CONCLUSION

The syntheses of new chiral compounds with interesting antileishmanial activity reported during the last 5 years (literature update from 2017) are summarized in this review. Interestingly, from our survey, all of these active molecules presented at least one (hetero)cyclic scaffold. The most promising compounds, in terms of IC_{50}/EC_{50} against different *leishmania* strains, were divided into three chapters by chemical classification: NPs and their analogues, amino acid-containing compounds (e.g., peptides), and nitroimidazoxazines or -oxazoles. The syntheses and the antileishmanial activities are discussed in detail for each class of compounds. As might be expected, the installation of the stereogenic center(s) of the active molecule was mainly obtained through a chiral pool approach, although the asymmetric synthesis (i.e., chiral catalysis) and, in some cases, chiral resolution and kinetic resolution are also reported. Interestingly, no syntheses using a chiral auxiliary approach were reported. All the compounds presented good to excellent activities against the promastigotes and amastigotes of several *leishmania* strains and, in almost all cases, they showed less cytotoxicity against human cell lines when compared to the commercial drugs.

Moreover, it is important to notice that, at least in most of the described articles, the use of an enantiopure molecule greatly enhanced its activity toward the parasite compared to its pure enantiomer or the corresponding racemic mixture. Therefore, it appears clear that the development of new strategies for the synthesis of enantiopure active molecules against this disease is still fundamental.

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ORCID

Michele Verboni https://orcid.org/0000-0001-5648-521X
Diego Olivieri https://orcid.org/0000-0001-9411-4487
Simone Lucarini https://orcid.org/0000-0002-3667-1207

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