Review

Journal of the Brazilian Chemical Society

https://dx.doi.org/10.21577/0103-5053.20240053

J. Braz. Chem. Soc. **2024**, *35*, 10, e-20240053, 1-21
©2024 Sociedade Brasileira de Química

Amidines: The Main Discoveries in the Synthesis and Anti-Leishmanial Effects of New Chemotherapeutic Agents

Cláudio E. Rodrigues-Santos, [a] ***Maurício R. de Brito*** [a] ***and Aurea Echevarria*** [*,a]

[a]*Instituto de Química, Universidade Federal Rural do Rio de Janeiro, 23890-000 Seropédica-RJ, Brazil*

Leishmaniasis is an infectious disease caused by protozoan parasites of the *Leishmania* genus and affects more than 90 countries, especially in tropical and subtropical regions. The first treatment for these diseases involves pentavalent antimonial derivatives, which are very toxic and cause severe side effects. The other chemotherapeutic drugs used as second-line agents include different organic compound classes, such as pentamidine, which also cause severe side effects. Thus, new, safer, and efficient antileishmaniasis agents are urgently needed to control and treat these diseases. This mini review, which considered the last two decades of related research, highlighted the principal synthetic methodologies used to access amidine derivatives, focusing on more eco-friendly methods. Furthermore, the results obtained from evaluations of the anti-*Leishmania* activity and several molecular targets of the amidine derivatives are highlighted.

Keywords: leishmaniasis, trypanosomatids, amidine derivatives, chemotherapy, tropical disease

1. Introduction

Leishmaniasis is a parasitic infection that affects people in tropical and subtropical regions worldwide. Leishmaniasis is endemic in more than 90 countries; at least 350 million people are estimated to be infected, and 1.29 million new cases occur annually.[1,2] This disease is transmitted to humans through bites by infected sandflies of the Phlebotominae family.[3]

Leishmaniasis can be categorized into the following forms: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL). VL, also known as kala-azar, is the most aggressive form of infection; this disease is fatal and responsible for 20,000-40,000 deaths annually, and it is caused mainly by the species *L. donovani* and *L. infantum*. CL is the most prevalent form of disease and manifests as skin lesions, which are ulcers that leave permanent disfiguring scars; CL is also caused by several species, mainly *L. amazonensis*, *L. major*, *L. braziliensis* and *L. mexicana*. MCL affects mucosal tissues and is caused mainly by *L. vianniapanamenis*, *L. vianniabraziliensis* and *L. amazonensis*.[4-6]

*e-mail: claudioers@ufrrj.br; echevarr@ufrrj.br
Editor handled this article: Brenno A. D. Neto
It is a great honor for our research group to participate in the tribute to Prof Eliezer J. Barreiro with this review.

Leishmania is a mandatory intracellular parasitic protozoan of the Trypanosomatideae family that infects humans and mammals by infecting the sandfly of the genus Pheblotominius. According to the Centers for Disease Control and Prevention (CDC),[7] the life cycle includes two hosts. The first host is a flagellated form, named promastigote, which is inoculated in the skin through bites by an infected sandfly. Then, the second host spreads through the bloodstream, and the parasite is phagocytosed by macrophages that differentiate into the unflagellated form, termed amastigote. The amastigote subsequently breaks the macrophage membrane to infect other cells. After, if sandfly meal is used, the amastigote changes to a promastigote form, and other infections can occur when the blood is eaten.[8] Macrophages infected with amastigotes react by releasing reactive oxygen species (ROS), reactive nitrogen species (RNS), interleukins (IL-12) and interferon-γ (INF-γ). However, parasites also release proinflammatory cytokines (such as IL-12 and INF-γ) as defense mechanisms, favoring the progression of the disease and complicating treatment.[9]

The first line of treatment for these infections involves using chemotherapy (red-colored structures, Figure 1) with pentavalent antimonial derivatives, sodium stibogluconate and meglumine antimoniate, which are highly toxic and cause severe side effects and long treatment durations.[10] The second line treatments (blue-colored structures, Figure 1) include amphotericin B, pentamidine, paromomycin

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

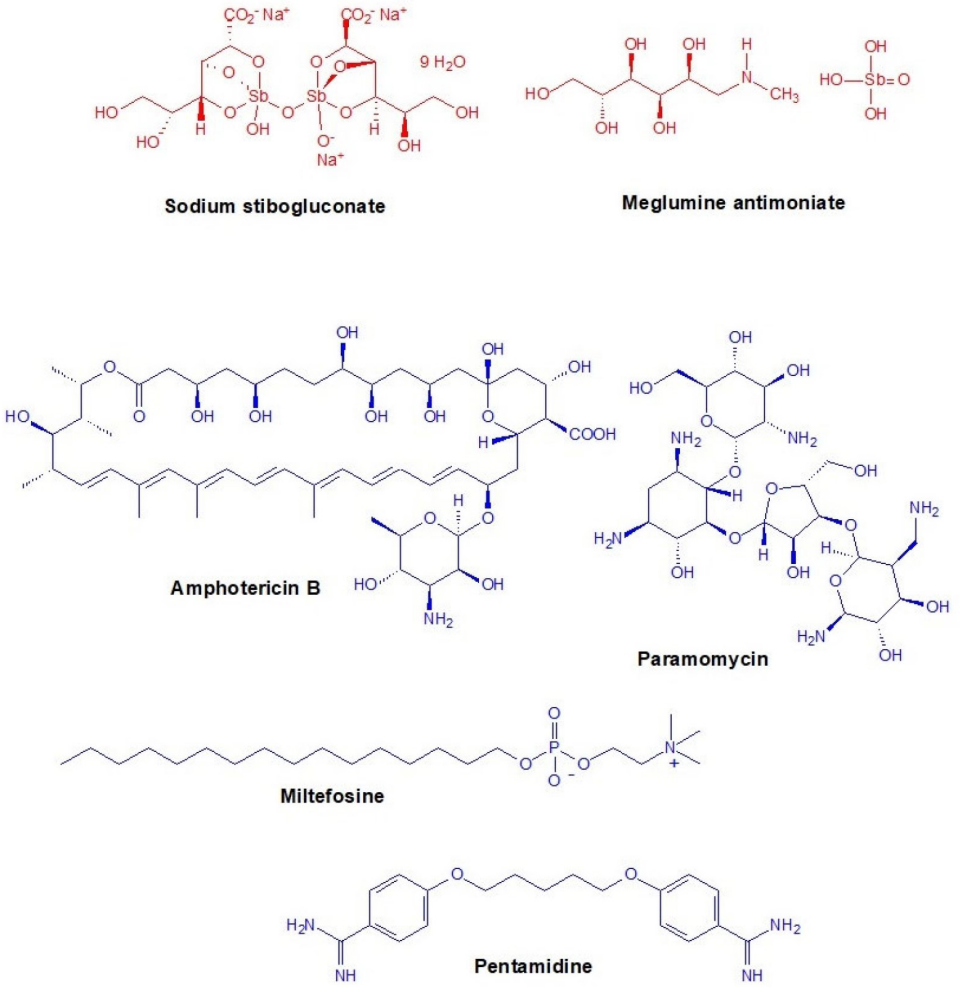


Figure 1. Chemical structures of the main drugs used in the chemotherapeutic treatment of leishmaniasis.

and miltefosine.[11] Furthermore, the parenteral or local administration of amphotericin B (which limits its use)[12] and miltefosine (which is the first oral drug) cause nephrotoxicity and haematotoxicity; however, the prolonged use of these drugs causes teratogenesis, leading to limited use.[13] Furthermore, another difficulty associated with these chemotherapeutic agents is drug resistance, which leads to inefficacy of these agents.[14]

Therefore, new drugs that are more efficient and safer are urgently needed to combat leishmaniasis. Understanding the mechanism of action of these compounds, as well as their chemotherapeutic targets, is of utmost importance. The literature reports some compounds with relevant antileishmanial activity, among which amidinic compounds stand out; notably, the aforementioned pentamidine contains an amidine moiety. In this work, we will present amidine compounds that have promising actions in combating leishmaniasis, and we will discuss the results of their known and studied chemotherapeutic targets. Initially, we will discuss the possible types of amidines that can be obtained using the main organic functional groups that generate the amidine moiety.

This research was based on the SciFinder program, considering reports from 2003 to 2023, and syntheses with yields greater than or equal to 50%; furthermore, the production of amidines from other amidines was not considered. This work describes the different types of catalysts employed in the synthesis of amidines depending on the starting group. Whenever possible, we will exemplify environmentally friendly methodologies, such as the use of alternative energy sources, ultrasound, and microwaves and the use of methodologies without the need for solvents or catalysts, and we will also present the synthesis of functionalized amidines.

2. Amidine Derivative Synthesis in the Last Two Decades

Amidines can be classified into five general types depending on the number and distribution of the

substituents on the nitrogen atoms (Scheme 1).[15] There is a large variety of possibilities for substituents on carbon or nitrogen atoms; however, in accordance with the nature of the substituents, some preparation methods may be more useful. Amidines can be synthesized from several chemical sources; however, in the last two decades, many articles have recorded the synthesis of amidines from the following functional groups: nitriles, amides, thioamides, ynamides sulfonylazides, *ortho*-ethers, imidoesters, carbodiimide, isonitrile and heterocyclics.[16,17]

2.1. Synthesis of amidines from nitriles

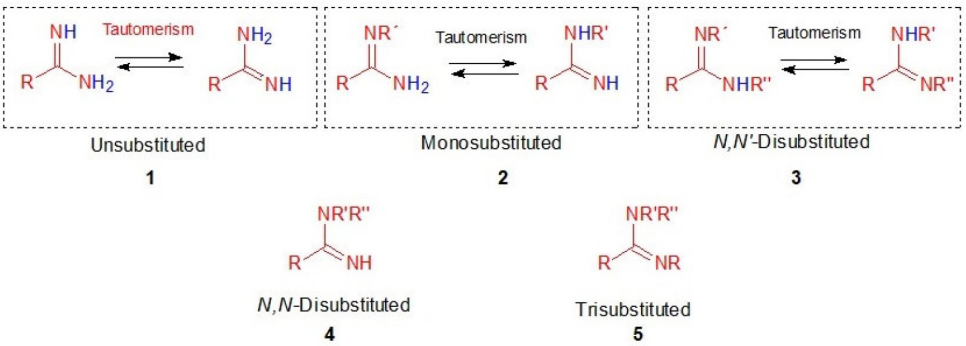
The condensation of nitriles with amines or ammonia generates amidines; however, nitriles must be activated for increased reactivity. A classic example of this activation is the Pinner reaction, which employs gaseous HCl to generate an imido ester as an intermediate; another method is attaching electron-withdrawing groups to the nitrile.[15] However, metal-based catalysts share a common underlying principle. In the literature,[18-25] the primary catalysts utilized include copper(II) triflate, palladium(II) trifluoroacetate, platinum(II) chloride, copper(II) chloride, copper(I) iodide, sulfated tungstate, and aluminium. Other metal-free catalysts, such as trimethylsilyl polyphosphate (PPSE) and trifluoromethanesulfonic acid, have also been tested.[26,27]

Chen *et al.*[18] synthesized disubstituted amidines from nitriles through a three-component reaction comprising nitrile (**6**), diazo (**8**), and sulfonamide (**7**) catalyzed by copper(II) triflate, providing excellent yields (68-94%). The results demonstrated that electron-withdrawing groups attached to the sulfonamide led to lower yields because of deactivation of the amino group (Scheme 2).

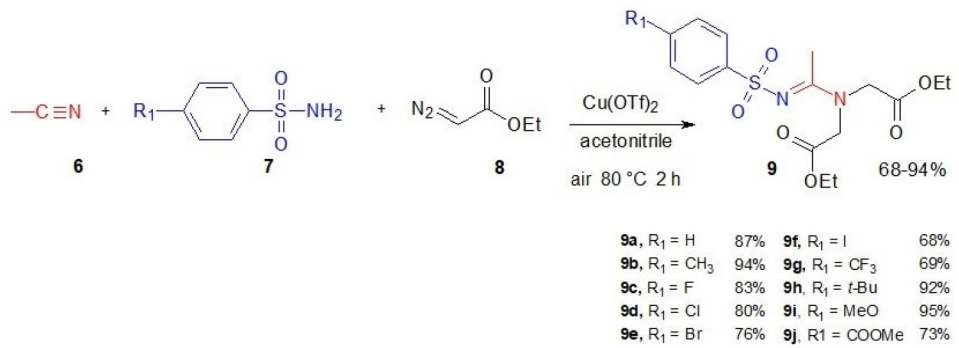
The use of microwaves often increases the reaction rate, which is an important factor in green chemistry. Several articles[28-31] have successfully used microwaves in the synthesis of amidines from nitrile (**10**). In this context, the use of DABAL-Me_3 (bis(trimethylaluminium)-1,4-diazabicyclo[2.2.2]-octane) (**12**) in conjunction with microwave irradiation furnished monosubstituted amidines (**13**) in good yields, ranging from 58 to 98%, and remarkably short reaction times (Scheme 3).[28]

The use of metallic or nonmetallic catalysts is important in the synthesis of monosubstituted or disubstituted amidines from nitriles, but cyclic amidines can be generated without the use of a catalyst.[26,32]

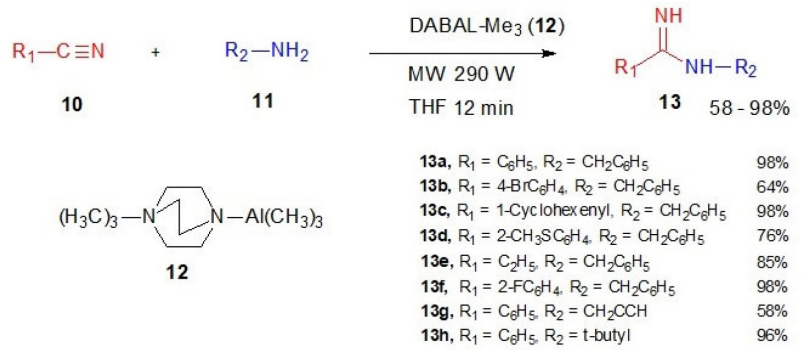
Díaz *et al.*[26] synthesized disubstituted cyclic amidines (**15**) from nitriles (**14**) without metal catalysts by employing an ethyl polyphosphate (PPE) catalyst under microwave irradiation, providing appreciable results (74-86%) and a surprisingly short reaction time of 5 min (Scheme 4).



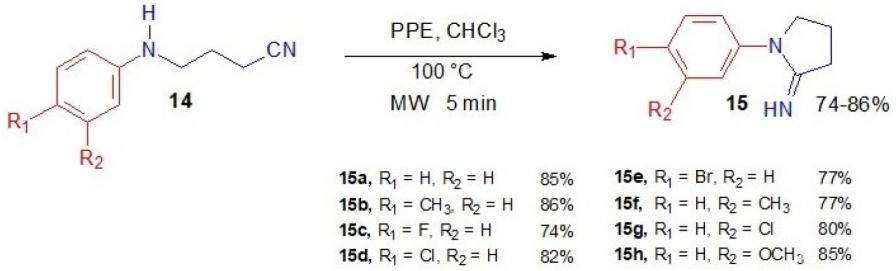
Scheme 1. Substitution of different forms of amidines.



Scheme 2. Synthesis of disubstituted amidines from nitriles catalyzed by $Cu(OTf)_2$.[18]



Scheme 3. Synthesis of amidines from nitriles under microwave irradiation catalyzed by DABAL-Me_3 (bis(trimethylaluminium)-1,4-diazabicyclo [2.2.2]-octane).[28]

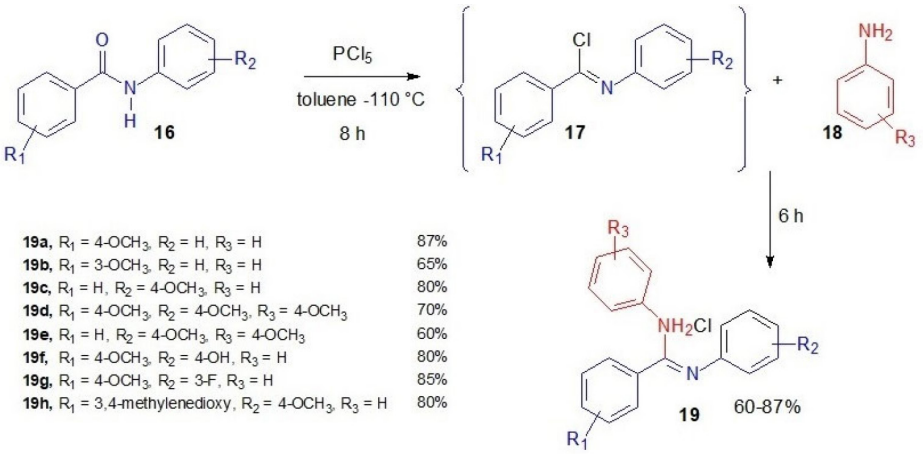


Scheme 4. Synthesis of disubstituted amidines from nitriles catalyzed by ethyl polyphosphate (PPE) under microwave irradiation.[26]

2.2. Synthesis of amidines from amides

A classic reaction for generating amidines from amides involves the formation of an imidoyl chloride intermediate, which is generated by reacting an amide with a halogenating agent, such as thionyl chloride, phosphorus oxychloride, or phosphorus pentachloride (which are dissolved in solvents such as benzene or toluene) under reflux for several hours, followed by the addition of an amine.[33,34] Among these halogenating agents, phosphorus pentachloride is the most recommended agent, and our group is proficient in using this methodology.[34] Benzanilides (**16**) can be converted to benzimidoyl chlorides (**17**) *in situ* by treatment with a halogenating reagent (PCl_5) under reflux in dry toluene for 8 h; these chlorides subsequently react with aniline (**18**) dissolved in dry toluene to furnish compound **19** in good yields (60-87%). This method is important for the synthesis of disubstituted amidines; however, this approach does not provide significant structural diversity (Scheme 5).[34]

Recently, metallic catalysts have been shown to be crucial for synthesizing amidines from amides or sulfonamides. Literature reports[35-42] describe several notable catalysts, including zinc triflate, trimethyl aluminium, magnesium oxide, titanium(II) chloride, and other metal-free catalytic agents, such as phosphorus oxychloride, ethyl polyphosphate, and *tert*-butyl hydroperoxide combined



Scheme 5. Synthesis of disubstituted amidines from amides and amines by a pentachloride halogenating reagent.[34]

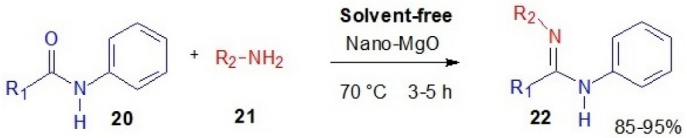
with sodium iodide and iodine with triphenylphosphine. Amidines can be generated efficiently by electrochemistry.[43] Copper oxide nanoparticles and magnesium oxide nanoparticles have been shown to be effective at catalyzing amides (**20**).[44] Das and Thakur[37] achieved high yields (85-95%) in the preparation of disubstituted amidines (**22**) using magnesium nanoparticles and solvent-free conditions. However, few examples of products were obtained through this methodology in the article (Scheme 6).

Cyclic amidines can also be synthesized from amides under microwave irradiation.[38,39,45,46] The synthesis of cyclic amidines (**24**) with rings of different sizes has been achieved through microwave irradiation from amides (**23**) catalyzed by ethyl polyphosphate or titanium(II) chloride.[38,40] Cyclic amidines of varying sizes (Scheme 7) can be obtained in high yields (42-90%) and with short reaction times (8 min).[40]

2.3. Synthesis of amidines from thioamides and ynamides

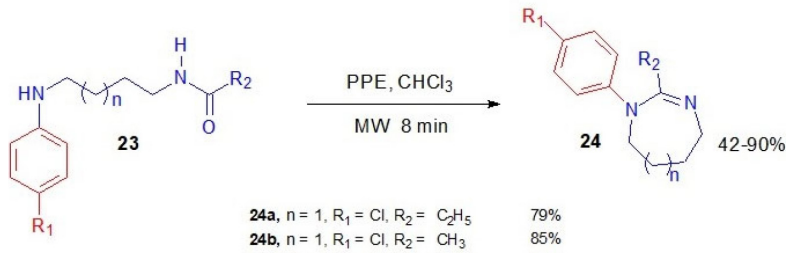
The synthesis of amidines from thioamides and ynamides is not significantly different from the synthesis of amidines from nitriles or amides. Amidine synthesis from thioamides can be catalyzed by copper(II) acetate to afford disubstituted amidines in good yields under mild reaction conditions. Additionally, silver tetrafluoroborate and caesium fluoride can be employed in this synthesis.[47-49] For certain amidine structures, synthesis can be achieved even without the presence of catalysts; however, the yields are generally low to moderate.[50] Li *et al.*[47] synthesized disubstituted amidines (**27**) from thioamide (**26**) and amine in good yields (63-91%) with a short reaction time (2.5 h) under the catalysis of palladium(II) acetate (Scheme 8).

Ynamides (**28**) are versatile groups for the synthesis



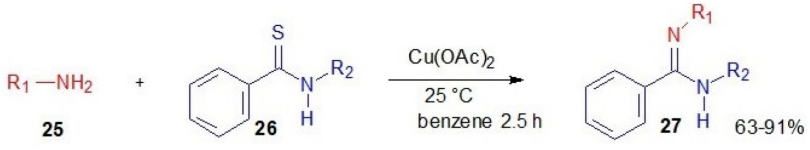
22a, $R_1 = C_6H_5$, $R_2 = C_6H_5$	94%	**22f**, $R_1 = C_6H_5$, R_2 = Cyclohexyl	85%
22b, $R_1 = C_6H_5$, $R_2 = 4\text{-}OCH_3C_6H_4$	94%	**22g**, $R_1 = CH_3$, $R_2 = C_6H_5$	92%
22c, $R_1 = C_6H_5$, $R_2 = 4\text{-}CH_3C_6H_4$	93%	**22h**, $R_1 = C_6H_{11}$, $R_2 = C_6H_5$	88%
22d, $R_1 = C_6H_5$, $R_2 = 4\text{-}NO_2C_6H_4$	90%	**22i**, $R_1 = 4\text{-}NO_2C_6H_4$, $R_2 = C_6H_5$	89%
22e, $R_1 = C_6H_5$, $R_2 = 4\text{-}ClC_6H_4$	91%	**22j**, $R_1 = 4\text{-}ClC_6H_4$, $R_2 = C_6H_5$	88%

Scheme 6. Synthesis of disubstituted amidines catalyzed by magnesium nanoparticles.[37]



24a, n = 1, R_1 = Cl, $R_2 = C_2H_5$	79%
24b, n = 1, R_1 = Cl, $R_2 = CH_3$	85%
24c, n = 1, $R_1 = CH_3$, $R_2 = CH_3$	87%
24d, n = 1, $R_1 = CH_3$, R_2 = Isopropyl	90%
24e, n = 1, R_1 = F, R_2 = Isopropyl	63%
24f, n = 2, $R_1 = CH_3$, R_2 = Isopropyl	42%

Scheme 7. Synthesis of cyclic amidines from amides catalyzed by ethyl polyphosphate PPE.[40]



27a, $R_1 = C_6H_5$, $R_2 = C_6H_5$	75%	**27e**, $R_1 = C_6H_5$, R_2 = Morpholine	91%
27b, $R_1 = C_6H_5$, $R_2 = 4\text{-}CH_3C_6H_4$	80%	**27f**, $R_1 = 4\text{-}CH_3C_6H_4$, $R_2 = C_6H_5$	76%
27c, $R_1 = C_6H_5$, $R_2 = 4\text{-}OCH_3C_6H_4$	87%	**27g**, $R_1 = 4\text{-}OCH_3C_6H_4$, $R_2 = C_6H_5$	75%
27d, $R_1 = C_6H_5$, $R_2 = 4\text{-}NO_2C_6H_4$	63%	**27h**, $R_1 = 4\text{-}NO_2C_6H_4$, $R_2 = C_6H_5$	78%

Scheme 8. Synthesis of disubstituted amidines from thioamide catalyzed by copper(II) acetate.[47]

of heterocycles and can also provide amidines. The reactions must be catalyzed to generate good yields, and the prominent catalysts in these reactions are ytterbium triflate, zinc triflate, and bis(triphenylphosphine)palladium chloride.[51-53] Chen *et al*.[52] synthesized disubstituted amidines (**30**) in reasonable yields (50-77%) and with good reaction times (Scheme 9).

2.4. Synthesis of amidines from sulfonazides

Currently, there are several articles on the synthesis of amidines from sulfonazides, providing sulfanamidines. Sulfonazides can react with alkynes, thioamides, enamines and amines.[54-57] Most reactions initially proceed by a 1,3-dipolar addition, forming a five-membered ring that subsequently cleaves, generating amidines. Additionally, sulfonazides can be employed in multicomponent reactions in the synthesis of sulfanamidines,[45-50,58] significantly contributing to the generation of complex and diverse structures. Typically, these reactions are catalyzed by metal catalysts, such as copper(I) iodide, copper(I) bromide, carbon-supported copper, iron(III) chloride, and palladium(II) acetate.[58-63] However, several metal-free catalysts can efficiently synthesize sulfonamidines, such as diethyl azodicarboxylate (DEAD), tris(pentafluorophenyl) borane and *tert*-butyl hydroperoxide.[64-66] Jian *et al*.[67] reported the synthesis of amidines through the use of sulfonazide and amines via photocatalysis.

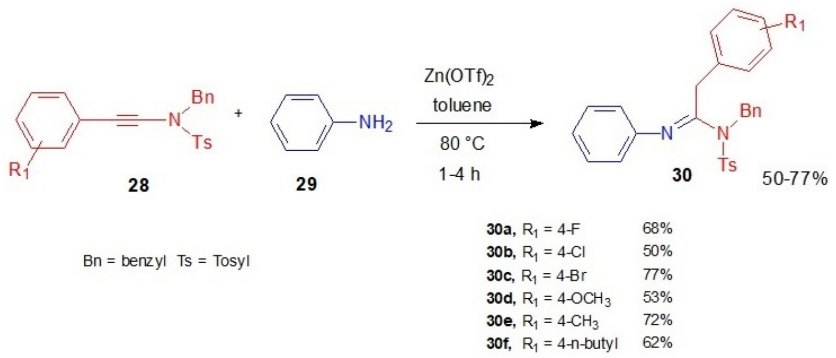
Bae *et al*.[58] obtained *N*-sulfonylamidines (**34**) via a multicomponent reaction using the following reagents: alkyne (**31**), sulfonazide (**32**), and amine (**33**). The reaction was catalyzed by copper(I) iodide, which provided very good yields ranging from 66-95% under mild conditions (Scheme 10).

In addition, sulfonamidine can be synthesized without the presence of a catalyst.[54-57,68,69] Gao *et al*.[57] synthesized a series of sulfanamidines (**37**) from sulfonazides (**35**) and enamines at room temperature without the use of catalysts, similar to Kaboudin *et al*.,[69] who also synthesized sulfonamidines from sulfonazide and amines (Scheme 11) in good yields (42-77%).

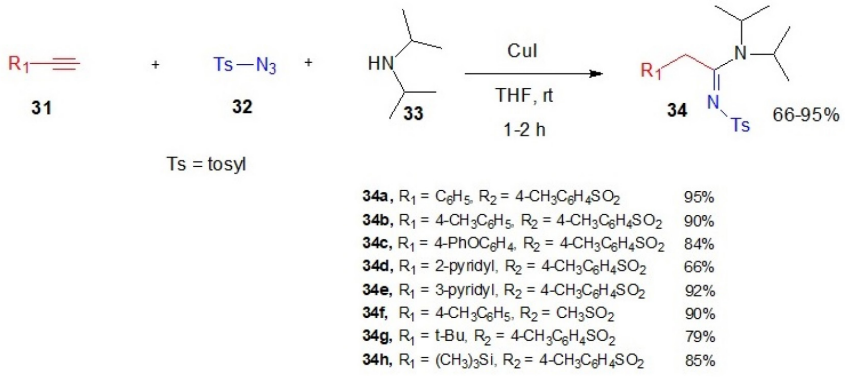
Rupakova *et al*.[54] synthesized a series of amidines (**40**) from sulfonazides (**39**) and thioamides (**39**) (Scheme 12) that exhibited significant activity against cancer cells without the use of metallic catalysts, furnishing very good results and very good yields (66-96%) without catalysts.

2.5. Synthesis of amidines from *ortho*-ethers

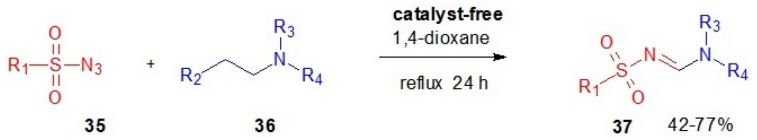
Amidines can also be synthesized by reacting amines and *ortho*-ethers. These reactions are carried out in the



Scheme 9. Synthesis of disubstituted amidines catalyzed by zinc triflate.[52]

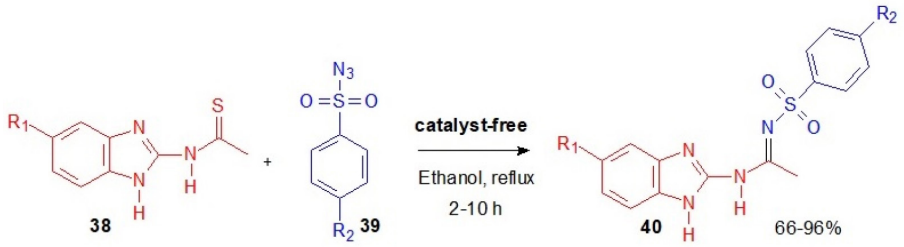


Scheme 10. Synthesis of sulfonamidines from a three-component reaction catalyzed by copper(I) iodide.[58]



37a, R_1 = 4-$CH_3C_6H_4$, R_3 = Ethyl, R_4 = Ethyl 71%
37b, R_1 = 4-ClC_6H_4, R_3 = Ethyl, R_4 = Ethyl 77%
37c, R_1 = 4-BrC_6H_4, R_3 = Ethyl, R_4 = Ethyl 68%
37d, R_1 = 4-$CH_3C_6H_4$, R_3 = Isopropyl, R_4 = Isopropyl 73%
37e, R_1 = 2,3,4-$(CH_3)_3C_6H_4$, R_3 = Isopropyl, R_4 = Isopropyl 66%
37f, R_1 = 4-$CH_3C_6H_4$, R_3 = n-propyl, R_4 = n-propyl 69%
37fg R_1 = 2,3,4-$(CH_3)_3C_6H_4$, R_3 = n-butyl, R_4 = n-butyl 46%
37h, R_1 = 2-$NO_2C_6H_4$, R_3 = n-butyl, R_4 = n-butyl 42%

Scheme 11. Catalyst-free synthesis of sulfonamidine.[69]



40a, R_1 = H, R_2 = CH_3 82%
40b, R_1 = 4$CH_3C_6H_4$, R_2 = H 66%
40c, R_1 = CH_3, R_2 = 4-FC_6H_4 67%
40d, R_1 = Ac, R_2 = CH_3 72%
40e, R_1 = Ac, R_2 = 4-$CH_3C_6H_4$ 70%
40f, R_1 = Ac, R_2 = 4-FC_6H_4 73%
40g, R_1 = H, R_2 = 4-$CH_3C_6H_4$ 66%
40h, R_1 = H, R_2 = 4-FC_6H_4 73%

Scheme 12. Synthesis of sulfonamidines from thioamides.[54]

presence of metal catalysts, such as iron(III) chloride, iron(III) oxide supported on silica, and tin chloride.[70-72] Some amidines can also be obtained by the catalytic action of cyclodextrin and acetic acid,[73,74] and there are records in the literature of the use of ionic liquids[75-77] and reactions under ultrasound and microwave irradiation.[74,78-80]

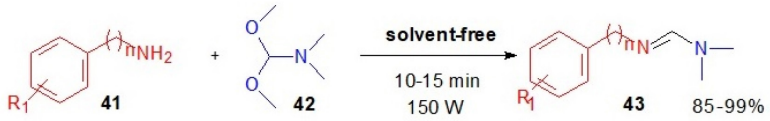
Chandna *et al.*[81] generated formamidines (**43**) in excellent yields (85-99%) from the reaction of amine (**41**) and *N*,*N*'-dimethylformamide dimethylacetal ether (**42**) (DMF-DMA) under microwave irradiation and solvent-free conditions (Scheme 13).

Dar *et al.*[80] established an excellent formamide (**46**) synthesis protocol that was solvent free and catalyst free under environmentally friendly conditions and utilized ultrasonic energy as the energy source. The yields obtained for the products were very good (64-98%), and the reaction times were extraordinary (50-90 min) compared to those of conventional methods (Scheme 14).

2.6. Synthesis of amidines from imidoesters and carbodiimides

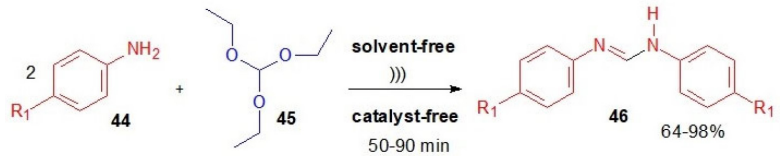
The synthesis of amidines from imidoesters (**47**) is well established and dates back to the classical Pinner reaction, in which an imidoester is generated as an intermediate.[82-85] The catalysts commonly employed for this type of reaction typically involve silicon-based compounds.[83,84] Yahyazadeh *et al.*[84] synthesized a series of formamidines (**49**) from imidoester (Scheme 15) in excellent yields (86-94%) within a short reaction time (115-195 min).

Amidines can be synthesized by reacting carbodiimides with esters. This reaction constitutes a [3+2] cycloaddition



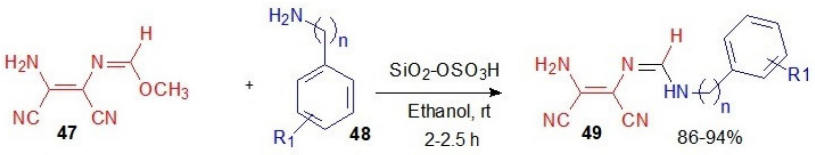
43a, R_1 = 2-Br, n = 0 85%
43b, R_1 = H, n = 0 94%
43c, R_1 = 4-NO_2, n = 0 96%
43d, R_1 = 4-F, n = 0 99%
43e, R_1 = 4-Cl, n = 0 97%
43f, R_1 = H, n = 1 94%
43g, R_1 = 4-Cl, n = 1 87%
43h, R_1 = 4-CH_3, n = 1 85%
43i, R_1 = 4-CN, n = 1 91%
43j, R_1 = H, n = 2 92%

Scheme 13. Synthesis of formamidines from DMF-DMA and amines under solvent-free microwave irradiation.[81]



46a, R_1 = H 98% **46e**, R_1 = Br 69%
46b, R_1 = CH_3 98% **46f**, R_1 = OCH_3 98%
46c, R_1 = F 81% **46g**, R_1 = NO_2 64%
46d, R_1 = I 78% **46h**, R_1 = CF_3 86%

Scheme 14. Synthesis of formamidines from amine ethers under ultrasonic irradiation without a solvent or catalyst.[80]



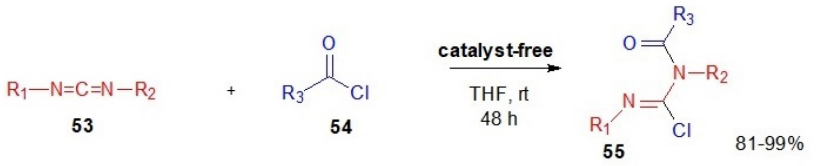
49a, R_1 = 4-OEt, n = 0 94% **49e**, R_1 = 3,4-OCH3, n = 0 94%
49b, R_1 = 4-OCH_3, n = 1 87% **49f**, R_1 = 3,4,5-OCH_3, n = 0 91%
49c, R_1 = 2-OCH_3, n = 1 86% **49g**, R_1 = 2-Cl, n = 1 91%
49d, R_1 = 4-OCH_3, n = 0 90%

Scheme 15. Synthesis of amidines from amines and imidoesters catalyzed by acidified silica.[84]

process.[86,87] These reactions can be catalyzed by tin triflate, diisobutylaluminium hydride, and iron(III) chloride.[86-88]

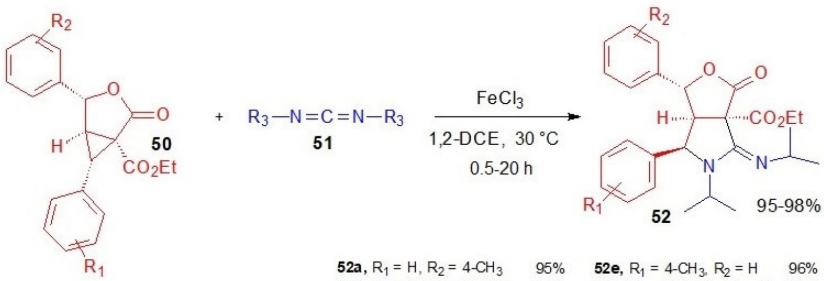
Feng *et al*.[87] synthesized cyclic amidines (**52**) by a [3+2] annulation reaction between carbodiimides (**51**) and butyrolactone (**50**) via the catalysis of iron(III) chloride (Scheme 16), for which notable yields were obtained (95-98%).

Carbodiimides (**53**) can react with esters, as mentioned before, and with acyl chloride, as indicated by Wang *et al*.[89] The researchers synthesized *N*-acyl chloroformamidines (**55**) from carbodiimide and benzoyl chloride (**54**) in very good yields (81-99%) under catalyst-free conditions (Scheme 17).



55a, R_1 = Isopropyl, R_2 = Isopropyl, R_3 = 4-$F_3CC_6H_4$ 99%
55a, R_1 = Isopropyl, R_2 = Isopropyl, R_3 = C_6H_5 99%
55a, R_1 = Isopropyl, R_2 = Isopropyl, R_3 = Cyclohexyl 98%
55d, R_1 = Cyclohexyl, R_2 = Cyclohexyl, R_3 = C_6H_5 99%
55e, R_1 = Cyclohexyl, R_2 = Cyclohexyl, R_3 = 4-$CH_3C_6H_4$ 81%
55f, R_1 = Cyclohexyl, R_2 = C_6H_5, R_3 = 4-$F_3CC_6H_4$ 99%
55g, R_1 = C_6H_5, R_2 = C_6H_5, R_3 = 4-$F_3CC_6H_4$ 89%
55h, R_1 = t-butyl, R_2 = ethyl, R_3 = 4-ClC_6H_4 93%

Scheme 17. Synthesis of amidines from carbodiimide and acyl chloride.[89]



52a, R_1 = H, R_2 = 4-CH_3 95% **52e**, R_1 = 4-CH_3, R_2 = H 96%
52b, R_1 = H, R_2 = 4-OCH_3 98% **52f**, R_1 = 3-OCH_3, R_2 = H 95%
52c, R_1 = H, R_2 = 2-OCH_3 95% **52g**, R_1 = 4-Br, R_2 = H 98%
52d, R_1 = H, R_2 = 4-Br 97% **52h**, R_1 = 4-NO_2, R_2 = H 98%

Scheme 16. Synthesis of amidines from esters and carbodiimides.[87]

2.7. Synthesis of amidines from isonitriles

The synthetic route to generate amidines from isonitriles is highly interesting because it allows for the generation of functionalized amidines, such as unsaturated amidines, in some cases. Furthermore, the reaction can be conducted by a multicomponent approach.[90-96]

Most of the literature on the synthesis of amidines from isonitriles involved a metallic catalyst. Among these, notable examples include cobalt(II) bromide, palladium(II) chloride, palladium(II) acetate, scandium(II) triflate, and rhodium(I).[90-95,97] Additionally, there are nonmetallic catalysts employed in the synthesis of amidines from amines and isonitrile, such as *p*-toluenesulfonic acid and triethylamine.[96,98]

Medda and Hulme[97] obtained trisubstituted amidines (**59**) by a multicomponent reaction involving amines (**56**), isonitriles (**57**), and aldehydes (**58**) without the need for metallic catalysts. However, acidic catalysis was needed, employing *p*-toluenesulfonic acid under microwave irradiation (Scheme 18). The results were good (39-89% yield), with notable reaction times (30 min).

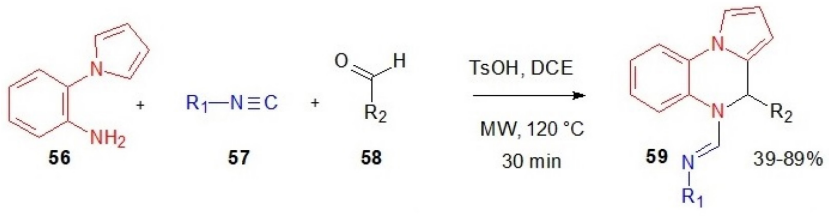
Yan *et al.*[93] obtained α,β-unsaturated amidines (**63**) by a multicomponent reaction utilizing a diazo compound (**61**), isonitrile (**60**), and sulfonamide (**62**), generating good yields (32-84%). Despite the long reaction time (16-24 h), these target compounds are considered important in terms of synthetic strategies. The products are formed by a reaction intermediate, a four-membered ring of cetenimine-imine [2+2], which subsequently opens, leading to the formation of α,β-unsaturated amidines (Scheme 19).

2.8. Synthesis of amidines from heterocyclic compounds and phosphonoacetamidines

Amidines can also be generated from heterocyclic compounds, and the literature[99-101] reports that oxadiazoles, *N*-sulfonyl triazoles, and benzotriazoles can form amidines (**66**) in good yields. Li *et al.*[100] synthesized *N*-sulfonylamidines from *N*-sulfonyl triazoles (**64**) and amines (**65**) in good yields (56-91%) and with a relatively short reaction time (4 h) without the need for catalysts (Scheme 20).

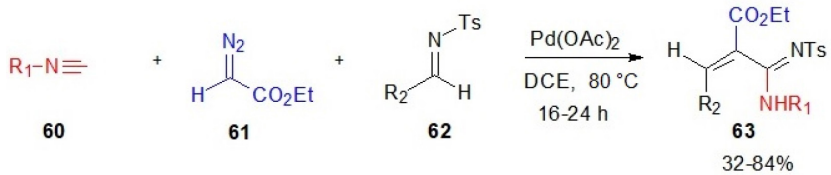
Katritzky *et al.*[101] published a comprehensive study with numerous examples of amidine (**69**) synthesis from benzotriazole (**67**) and amine (**68**) (Scheme 21) catalyzed by acetic acid for most reaction examples and, in some cases, requiring catalytic concentrations of aluminium chloride. The yields obtained were very good (76-92%), and the reaction time was notable (10 min).

As mentioned earlier, synthesizing amidines while



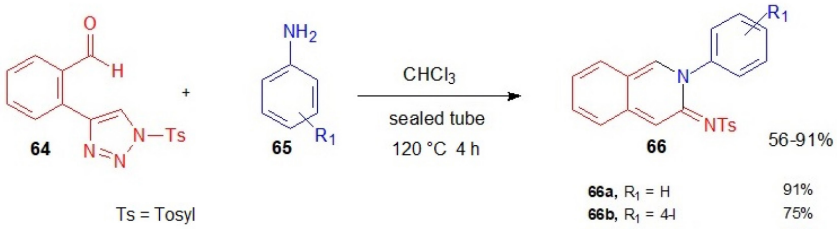
59a, R_1 = 2,5-$CH_3C_6H_3$, R_2 = 4-$NO_2C_6H_4$	62%
59b, R_1 = 2,5-$CH_3C_6H_3$, R_2 = C_6H_5	64%
59c, R_1 = 2,5-$CH_3C_6H_3$, R_2 = $C_2H_4C_6H_5$	67%
59d, R_1 = 2,5-$CH_3C_6H_3$, R_2 = $C_2H_4SCH_3$	49%
59e, R_1 = (2,2,3,3-tetramethyl)butyl, R_2 = 3-FC_6H_4	89%
59f, R_1 = t-butyl, R_2 = 3OCH3C_6H_4	62%
59g, R_1 = 2-CH3-5Cl-C_6H_3, R_2 = C_6H_5	64%
59h, R_1 = n-butyl, R_2 = C_6H_5	39%

Scheme 18. Synthesis of amidines by a multicomponent reaction under microwave irradiation.[98]



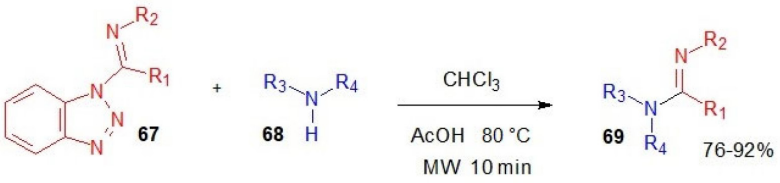
63a, R_1 = t-butyl, R_2 = C_6H_5	85%	**63e**, R_1 = 2,5-$CH_3C_6H_3$, R_2 = C_6H_5	32%
63b, R_1 = t-butyl, R_2 = 4-$F_3CC_6H_4$	86%	**63f**, R_1 = t-butyl, R_2 = 3-ClC_6H_4	73%
63c, R_1 = t-butyl, R_2 = 4-$CH_3C_6H_4$	74%	**63g**, R_1 = t-butyl, R_2 = 4-FC_6H_4	79%
63d, R_1 = t-butyl, R_2 = 4-CNC_6H_4	82%	**63h**, R_1 = t-butyl, R_2 = 4-BrC_6H_4	65%

Scheme 19. Synthesis of α,β-unsaturated amidines by a $Pd(OAc)_2$-catalyzed multicomponent reaction.[93]



66a,	R_1 = H	91%
66b,	R_1 = 4-I	75%
66c,	R_1 = 4-Br	70%
66d,	R_1 = 4-Cl	66%
66e,	R_1 = 4-F	86%
66f,	R_1 = 4-OCH_3	86%
66g,	R_1 = 4-C_2H_5	84%
66h,	R_1 = 4-CH_3	93%
66i,	R_1 = 4-CH_2CH_2OH	91%
66j,	R_1 = 4-$CH_2CH_2N_3$	56%

Scheme 20. Synthesis of *N*-sulfonylamidine from triazole.[100]



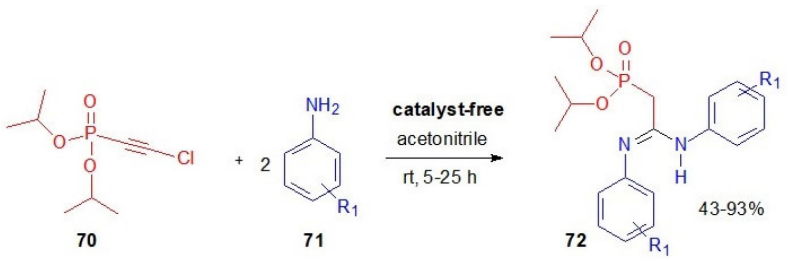
69a,	R_1 = CH_3, R_2 = C_6H_5, R_3, R_4 = $(CH_2)_2O(CH_2)_2$	76%
69b,	R_1 = CH_3, R_2 = C_6H_5, R_3 = C_2H_5, R_4 = C_2H_5	88%
69c,	R_1 = C_6H_5, R_2 = C_6H_5, R_3 = 4-$CH_3C_6H_4$, R_4 = H	89%
69d,	R_1 = 2-furyl, R_2 = 4-$CH_3C_6H_4$, R_3 = 4-$CH_3C_6H_4$, R_4 = H	90%
69e,	R_1 = 2-furyl, R_2 = 4-$CH_3C_6H_4$, R_3, R_4 = $(CH_2)_2O(CH_2)_2$	89%
69f,	R_1 = CH_3, R_2 = 4-$CH_3C_6H_4$, R_3, R_4 = $(CH_2)_2O(CH_2)_2$	92%

Scheme 21. Synthesis of amidines from benzotriazole and amines catalyzed by acetic acid under microwave irradiation.[101]

retaining other chemical functionalities is not a trivial task. Erkhitueva *et al*.[102] developed an efficient approach for the synthesis of *N*-aryl-C-phosphonoacetamidines (**72**) from di-isopropyl(chloroethynyl)phosphonate (**70**) and amine (Scheme 22), obtaining good yields (43-93%) without the need for catalysts. Amidines can also be synthesized from other methodologies, but there are few articles or examples of reactions in the last two decades compared to the functional groups discussed in this work.[103-106]

3. Amidine Derivatives with Antileishmanial Activity and Their Potential Targets

Based on the literature, we will show that amidinic derivatives achieved more promising results against various types of leishmaniasis caused by different species, such as *L. amazonensis*, *L. major*, *L. braziliensis*, *L. donovani*, *L. tropica* and *L. infantum*; in addition, we will describe the various types of essays carried out, such as *in vitro* studies on different evolutionary stages of the



72a,	R_1 = H	87%	**72f,**	R_1 = 4-Br	78%
72b,	R_1 = 4-CH_3	88%	**72g,**	R_1 = 4-I	72%
72c,	R_1 = 4-OCH_3	93%	**72h,**	R_1 = 4-CF_3	81%
72d,	R_1 = 4-F	81%	**72i,**	R_1 = 4-NO_2	43%
72e,	R_1 = 4-Cl	82%	**72j,**	R_1 = 4-Ac	65%

Scheme 22. Synthesis of *N*-aryl-C-phosphonoacetamidines from di-isopropyl(chloroethynyl)phosphonate and amine.[102]

parasite, promastigote and amastigote, with the parasite (amastigote) infecting inside cells, macrophages, and even results conducted *in vivo*. Furthermore, the results of the structure-activity relationship (SAR) regarding the anti-*Leishmania* activity and the chemotherapeutic targets of the compounds are interesting. Initially, we will describe the results obtained from studies on chemotherapeutic targets and recent studies on the first amidinic compound that became a drug, pentamidine (Figure 1), and subsequently, the results of studies of other amidinic derivatives.

3.1. Pentamidine

Basselin *et al.*[107] demonstrated through experimental studies that pentamidine interferes with the synthesis of polyamines, inhibiting the use of S-adenosyl-L-methionine by inhibiting enzymes such as ornithine decarboxylase and spermidine synthetase; thus, the synthesis of molecules important for the maintenance of life of the parasite is prevented. Theoretical studies by Montanari *et al.*[108] indicated that both pentamidines and their analogues can bind to deoxyribonucleic acid (DNA) in the minor groove, which is a region rich in adenine-timina (A-T). The experimental results obtained by Yang *et al.*[109] also demonstrated that pentamidine binds to the minor grooves of double-stranded DNA helices.

Systemic administration of pentamidine induces significant side effects, notably nephrotoxicity. Recent studies[110] have focused on the utilization of pentamidine within innovative nanoadministration systems, with the goal of repositioning this drug. The bis-cationic form of pentamidine has been employed as a pharmaceutical delivery vehicle. Among various applications, a study by Banerjee *et al.*[111] highlighted the encapsulation of pentamidine isethionate within sugar-grafted liposomes, which were evaluated against leishmaniasis in infected hamsters. Several sugars were assessed; mannose-grafted liposomes containing pentamidine isethionate exhibited an 85.1% reduction in parasite load in the spleen, whereas sugar-free liposomes achieved only a 46.6% reduction in parasite load in golden hamsters infected with Indian kala-azar (leishmaniasis) patients; similar studies were conducted by Román-Álamo *et al.*[112]

3.2. Amidine derivatives

Stephens *et al.*[113] evaluated several amidinic derivatives named 2,5-bis-[4-(2-pyridylimino)-phenyl]-furan dihydrochloride (**72**, Figure 2) against *L. donovani* in the amastigote form and found excellent results in the range of 0.10-1.14 μmol L^{-1}. However, when the pyridyl group was replaced with a phenyl group, the activity decreased approximately 100-fold (half-maximal inhibitory concentration (IC_{50}) > 100 μmol L^{-1}). These same compounds showed affinity for DNA binding and exhibited cellular toxicity in the range of 4.2-143 μmol L^{-1} (IC_{50}).

De Souza *et al.*[114] evaluated the effects of furamidine compounds against *L. amazonensis* and obtained promising results, with an IC_{50} of 32 μM (**73**, R = H, Figure 2) and an IC_{50} of 3.7 μM (**73**, R = Ph, Figure 2). The data indicate that the phenyl effect is important for the activity, suggesting greater p-stack interaction with the bioreceptor and/or increased lipophilicity. These same compounds also showed significant activities against *Trypanosoma cruzi* and even against cancer cells. However, both molecules damage organelles containing DNA, similar to pentamidine.[115] Some studies[116] have shown the leishmanicidal action of berenil (Figure 2), but its efficacy is much lower than that of pentamidine.

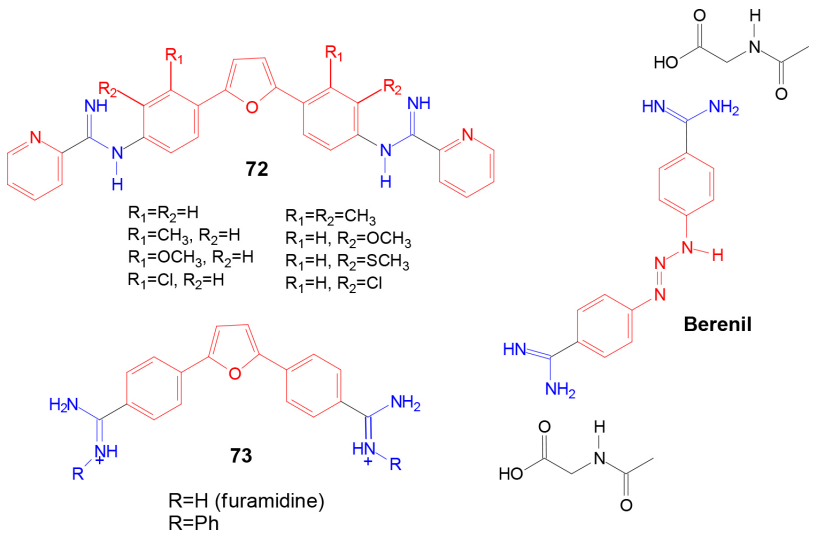


Figure 2. Chemical structures of amidines **72**, berenil and furamidins **73**.

Huang *et al.*[117] investigated the antiplasmodial and anti-*L. donovani* activities of a series of 52 pentamidine analogues in which the links between two phenyl amidine moieties were highly variable. The IC_{50} values ranged from 0.290 to over 97.8 μmol L^{-1} for 44 compounds assayed at a maximum concentration of 100 μmol L^{-1}. The most active compound, **74** (Figure 3, IC_{50} = 0.290 μmol L^{-1}), did not cause cytotoxicity in Vero cells up to 19.6 μmol L^{-1} and was 68-fold more toxic to *L. donovani* than to Vero cells.

Rosypal *et al.*[118] evaluated a series of 35 dicationic aromatic reversed diamidines against *L. infantum* promastigotes isolated from North American foxhound. Among the assayed compounds, **75** (Figure 3) showed the best activity, with an IC_{50} of 0.0042 μmol L^{-1}. In contrast, IC_{50} of 14.2 μmol L^{-1} was observed for pentamidine, which was used as a positive control, and the cytotoxic indices obtained using L-6 rat myoblast cells were 9.2 and 0.3 for compound **75** and pentamidine, respectively.

The effects of diamidine azaterphenyl and its analogues on the axenic amastigotes of *L. donovani* were investigated. Among the eighteen compounds evaluated, nine obtained IC_{50} values lower than 0.10 μmol L^{-1}. In this study, the authors revealed that the anti-*Leishmania* activity of diamidines depends on the position of the nitrogen atom in the ring relative to the amidine group, and this is correlated with DNA affinity. Among the most active compounds, **76** and **77** (Figure 3; IC_{50} = 0.063 and 0.084 μmol L^{-1}, respectively) possess nitrogen atoms at the *ortho* position on the ring neighboring the amidine group; however, when two nitrogen atoms are introduced, the inhibitory effect on the axenic amastigotes decreases. Unfortunately, **76** and **77** were not effective in assays of infected macrophages.[119]

Eighteen pentamidine analogues were evaluated *in vitro* against *L. major* and *L. tropica*. These species are causative agents of cutaneous leishmaniasis in the Old World. Among the compounds assayed, compounds **78** and **79** were most active (Figure 4), with IC_{50} values of 0.0007 μmol L^{-1} for both compounds towards *L. major* (promastigotes) and 0.002 and 0.0065 μmol L^{-1} for *L. tropica* (promastigotes) and 0.25 and 0.29 μmol L^{-1} for *L. donovani* (amastigotes), respectively.[120]

Bakunova *et al.*[121] reported the synthesis of cationic 2-phenylbenzofurans and their effect on protozoa, including the axenic amastigotes of *L. donovani*. Among the forty-nine compounds assayed against *L. donovani*, five showed better results than pentamidine, with IC_{50} values less than 2 μmol L^{-1}, and compound **80** was the most active compound (IC_{50} = 0.99 μmol L^{-1}). In this study,[122] the authors observed, in general, that substituting the amidine group led to a decrease in activity. The same group of authors reported the synthesis of eighteen pyridyl-pentamidine analogues with antiprotozoal activity, including *L. donovani*. Compound **81** (Figure 4) was the most active compound, with an IC_{50} less than 1 μmol L^{-1}, and was nearly four times more potent than pentamidine.

Arylimidamides were evaluated for their ability to treat *L. amazonensis*, *L. major* intracellular amastigotes, and *L. donovani* intracellular and axenic amastigotes. These compounds showed exceptional activity with an $IC_{50} \leq 0.12$ μmol L^{-1} and did not exhibit mutagenicity in the Ames assay. The most active compound, **82** (Figure 5), was assayed *in vivo* by two efficient models in *L. donovani*-infected mice and hamsters, in which 71 and 89% of liver parasitemia was inhibited, respectively. However, after *in vivo* treatment, chlorydrate (**82**) in a murine model had adverse effects on histopathology in tissue samples.[123,124] Continuing the studies with arylimidamide, Pandharkar and co-workers[125] assayed *L. donovani* axenic

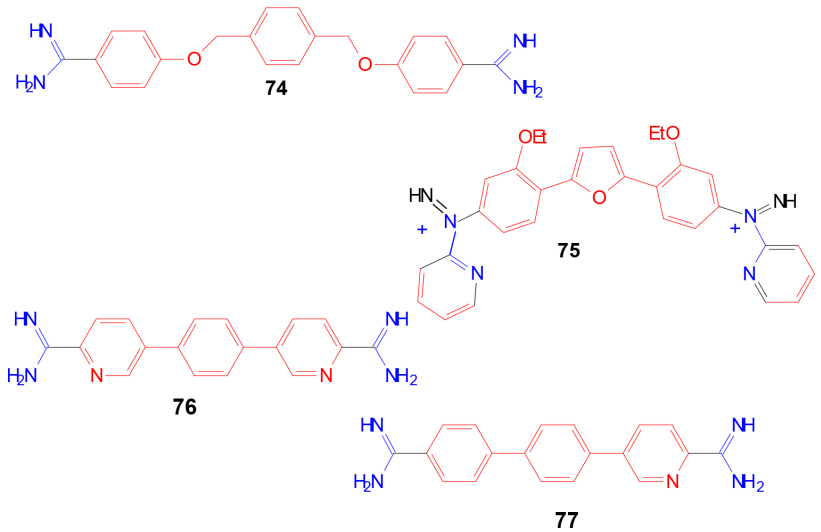


Figure 3. Chemical structures of dicationic furamidine and di-amidines with anti-*Leishmania* activity.

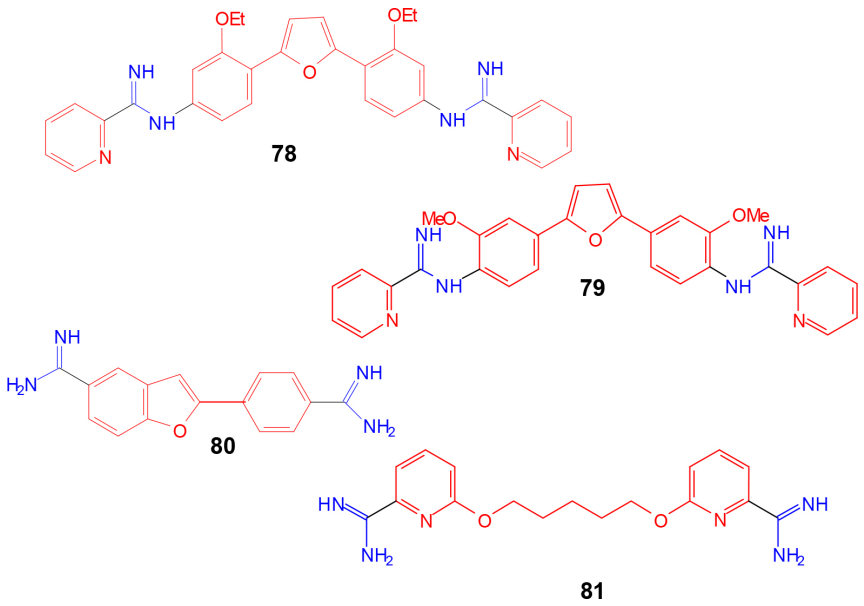


Figure 4. Chemical structures of reversed furamidine derivatives and pentamidine analogues with anti-*Leishmania* activity.

amastigotes, which are resistant to this compound and are twice as sensitive to miltefosine. Furthermore, the authors prepared eighteen arylimidamides containing pyridylimidamide terminal groups, six of which had nanomolar IC_{50} values against intracellular amastigotes of *L. donovani* and *L. amazonensis*, and compound **83** (Figure 5) reduced *L. donovani* liver parasitemia by 46% after an oral dose of 100 mg kg^{-1}.

A series of new imidinoximes were prepared and investigated for their anti-*L. donovani* activity. Among all

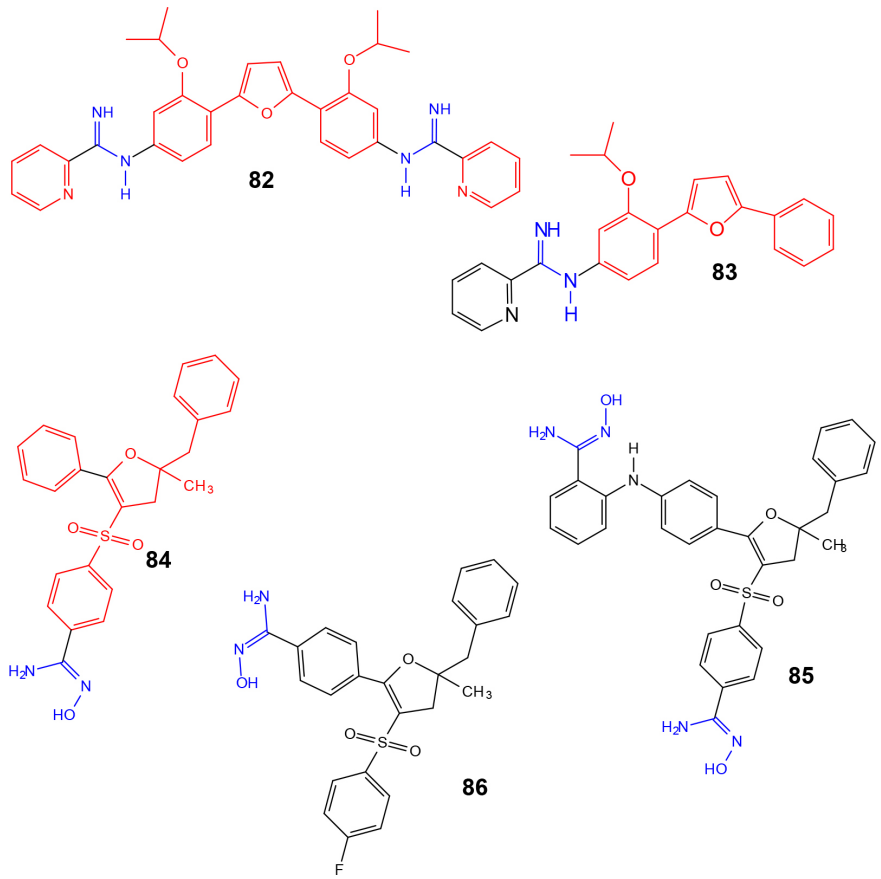


Figure 5. Chemical structures of arylimidamides and amidinoximes with anti-*Leishmania* activity.

the compounds, only two compounds, **84** and **85** (Figure 5), generated IC_{50} values less than 10 μmol L^{-1} (8.3 and 8.8 μmol L^{-1}, respectively) against promastigotes and were cytotoxic to human THP-1 cells; these compounds exhibited a better selectivity index than that of pentamidine.[126] Then, the authors prepared thirteen new imidinoximes, and the anti-*L. donovani* promastigotes obtained IC_{50} values in the range of 5.21-7.89 μmol L^{-1}, the most active compound **86** (Figure 5), with a selectivity index 40 times greater than that of pentamidine.[127]

As previously mentioned, imidinoximes exhibit significant activity against *L. donovani*, and studies conducted by Clement and Struwe[128] have increased knowledge on the biological mechanisms underlying the activity of this class of compounds by investigating *N*-hydroxylated pentamidine derivatives. The research group observed low *in vitro* antiprotozoal activity and significantly decreased DNA binding. However, when the monohydroxylated and bishydroxylated pentamidine derivatives were assayed *in vivo*, the results were consistent with the *in vivo* reduction of the amidoxime group to amidine (Figure 6). Amidoximes can be used as prodrugs with greater flexibility than other agents, and OH groups can also be used to esterate different carboxylic acids. Prodrugs based on amidoximes exhibit interesting pharmacokinetic and pharmacological properties.[128]

A series of 5-(5-nitrofuran-2-y1)-1,3,4-thiadiazoles

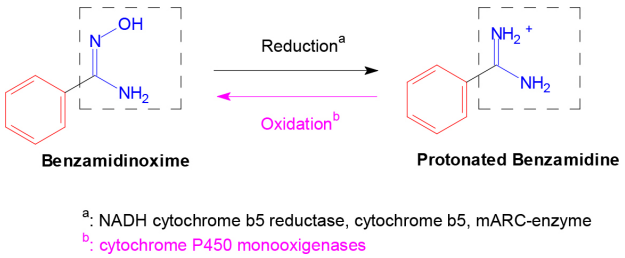


Figure 6. Reduction of benzamidinoxime to benzamidine and oxidation of benzamidoxime (adapted from Clement and Struwe).[128]

with piperazinyl-linked benzamidine substituents were synthesized as scaffolds and analogues and evaluated against promastigotes and amastigotes of *L. major*. The compounds containing *n*-propyl, *n*-butyl and benzyl side chains in benzamidine exhibit very good activity, highlighting compound **87** (Figure 7), with an *n*-propyl moiety, which obtained an IC_{50} of 0.08 μmol L^{-1}, after 72 h of treatment in promastigote form; furthermore, the compound exhibited very low toxicity in macrophages.[129]

As mentioned earlier, Yang *et al.*[109] also investigated some diamidines using *L. donovani* promastigotes. The investigations included compound **88** and other mono- and di-amidines. The most active compound, **89** (IC_{50} = 3.20 μmol L^{-1}), exhibited strong DNA binding properties (Figure 7). All diamidines assayed dose-dependently affected kDNA but not nuclear DNA replication.

Hybrids of arylimidamides containing the catechol moiety were prepared and evaluated against promastigotes of *L. major* and *L. infantum* and against axenic amastigotes of *L. major*. When the terminal phenyl group was replaced with a catechol moiety, the antiparasitic effect improved 10-fold. The most active compound, **90** (Figure 8), exhibited IC_{50} values of 0.29 and 0.32 μmol L^{-1} against *L. major* in the promastigote and axenic amastigote developmental stages, respectively, and 0.36 μmol L^{-1} against *L. infantum* in the promastigote stage.[130]

Nué-Martinez *et al.*[131] reported the synthesis and SAR study of three series of compounds that target AT-rich mitochondrial DNA (kDNA), focusing on their antiprotozoal activity. Among these series, the bis-arylimidamides included some compounds with antiparasitic effects against *L. donovani*, *T. brucei* and *T. cruzi*, with IC_{50} values < 1 μmol L^{-1}. The two most active compounds, **91** and **92** (Figure 8), exhibited IC_{50} values of 0.26 μmol L^{-1} and 0.33 and 0.65 μmol L^{-1} against the amastigote form, respectively, and 0.65 μmol L^{-1} and

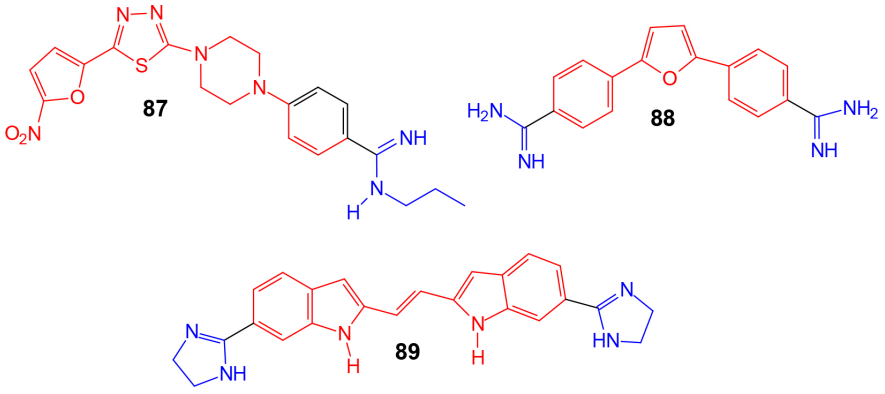


Figure 7. Chemical structures of 5-(5-nitrofuran-2-y1)-1,3,4-thiadiazoles with piperazinyl-linked benzamidine, arylimidamide, and mono- and diamidine derivatives with anti-*Leishmania* activity.

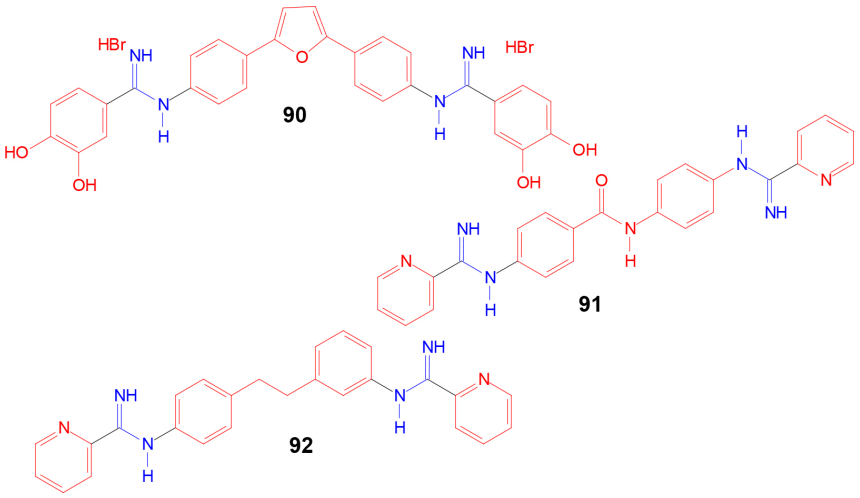


Figure 8. Chemical structures of reversed diamidine derivatives with anti-*Leishmania* activity.

1.11 and 0.65 μmol L^{-1} against the promastigote form, respectively. In general, the compounds in the series of bis-arylimidamides strongly bound to the DNA minor groove, and the pK_a values obtained were correlated with the DNA binding affinities.

A series of 2'-arylsubstituted-1*H*,1'*H*-[2,5']-bis-benzimidazolyl-5-carboxamidines were synthesized, and the effects of these compounds on several parasites and fungal species, including *L. donovani*, were investigated; however, these compounds were not active against *Leishmania* parasites, for example, compound **93** (Figure 9).[132]

Boechat *et al.*[133] prepared thirty-four compounds, including fifteen containing an amidine group, for example, compound **94** (Figure 9), which targets the enzyme arginase of *L. amazonensis*. Unfortunately, after promastigote assays, only three compounds showed IC_{50} values in the range of 30-70 μmol L^{-1} and no correlation was detected between anti-*L. amazonensis* activity and arginase interaction by molecular docking.

3.3. *N*-Phenyl-*N*'-R_1,R_2-phenyl-*p*-methoxy-benzamidine derivatives

Interesting results were obtained for the series of *N*-phenyl-*N*'-R_1,R_2-phenyl-*p*-methoxy-benzamidine compounds against *L. amazonensis in vitro* in the promastigote form, especially for compound **95** (Figure 9), which has an IC_{50} of 14.00 μM. Temporal *et al.*[134] evaluated the cytotoxicity of compound **95** and pentamidine (positive control) *in vitro*, in macrophages infected with

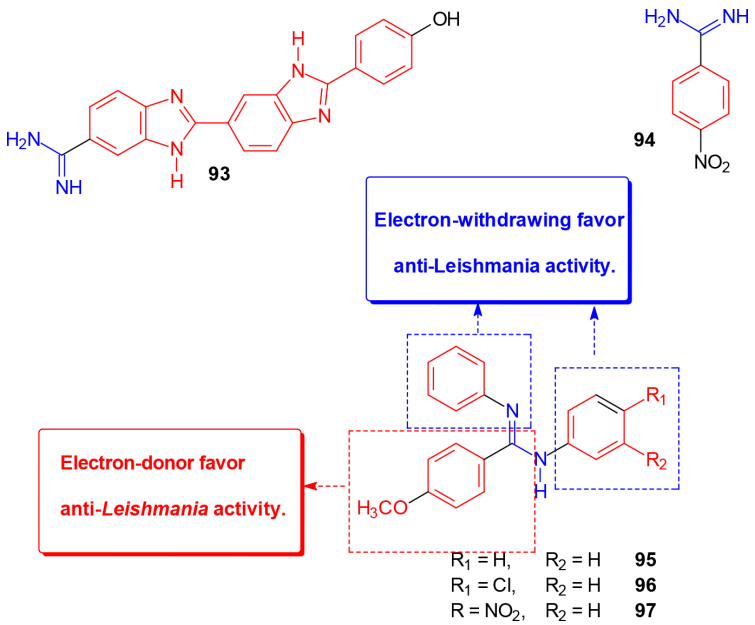


Figure 9. Chemical structures of 2'-4-hidroxyaryl-1*H*,1'*H*-[2,5']-bisbenzimidazolyl-5-carboxamidines (**93**), nitrobenzamidine (**94**) and *N*-phenyl-*N*'-R_1,R_2-phenyl-4-methoxt-bezamidine (**95**, **96** and **97**).

L. amazonensis, and *in vivo*, in BALB/c mice also infected with *L. amazonensis.* Surprisingly, the compound decreased the percentage of parasites *in vitro* without damaging the host cell, and *in vivo*, the compound prevented infection in the animals. Notably, the reference drug pentamidine was not effective in either experiment.

Recently, studies have been performed to determine the mechanism of action. In this context, Genestra *et al.*[135] evaluated the effects of compound **95** and pentamidine isethionate (as a reference drug) on nitric oxide radical ($NO^{\bullet}$) production by promastigotes and axenic amastigotes of *L. amazonensis*. The results revealed 23.53% $NO^{\bullet}$ inhibition in promastigotes and 52.94% inhibition in axenic amastigotes when compared to 3.78 and 25.9%, respectively, for pentamidine. Based on these results, the action of the parasite's NOS enzyme is important and does not affect activity of the host. Figure 10 shows the inhibition of $NO^{\bullet}$ production by *L. amazonensis* axenic amastigotes and promastigotes after treatment with compound **95** and pentamidine.[136] Genestra *et al.*[135] subsequently showed that *L. amazonensis* axenic amastigotes produce more $NO^{\bullet}$ than that generated by promastigotes.

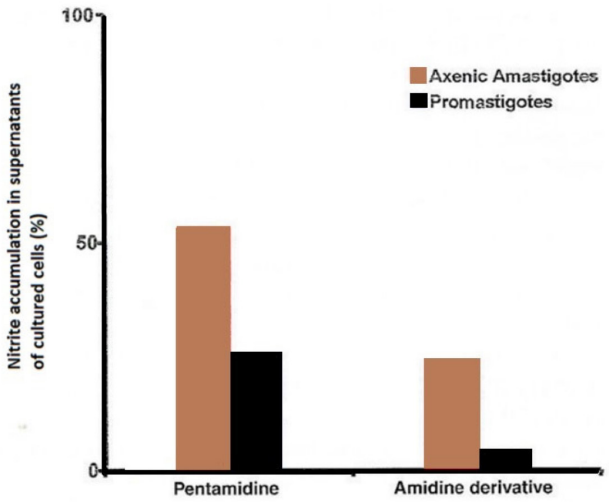


Figure 10. Inhibition of nitric oxide ($NO^{\bullet}$) production by *L. amazonensis* axenic amastigotes and promastigotes. Nitrite accumulation in the supernatants of cultured cells was used as an indicator of $NO^{\bullet}$ production and was determined by the Griess reaction.[135]

Another chemotherapeutic target studied was the enzyme trypanothione reductase (TR). The TR in the soluble fraction of *L. amazonensis* and its expression were significantly different between infected axenic amastigotes and lesion-containing promastigotes. The results of the experiments showed that compound **95** and pentamidine inhibited the TR enzyme; however, only pentamidine inhibited glutathione reductase, suggesting its connection to toxic effects. These results indicated the importance of the TR enzyme in the survival of intracellular parasites and indicated that TR is a possible target for anti-trypanosomatid drugs.[137]

The effect of compound **95** on the $NO^{\bullet}$ production of parasite-macrophage interactions after 24 h of infection was evaluated. Interestingly, compound **95** destroyed intracellular parasites without affecting host cells, and these data corroborate the results of Temporal *et al.*[134] Pretreatment with pentamidine under the same conditions did not help prevent infection.[138]

Rodrigues-Santos *et al.*[34] synthesized two new series of 23 *N*,*N*'-diphenyl-*p*-X-benzamidine based on good results of 4-methoxy-phenyl-*N*,*N*'-diphenyl-benzamidine (**95**) on *L. amazonensis*. A series of compounds were evaluated against *L. amazonensis*, *L. chagasis* and *L. braziliensis*, and the most active amidines were **96** and **97** (Figure 9), with IC_{50} values of 12.60 and 13.00 μmol L^{-1} against *L. amazonensis*, respectively. The SAR and QSAR studies indicated that the best results were achieved when electron-donor groups were linked to the ring attached to the amidine carbon atom and when electron-withdrawing moieties were linked to the rings attached to the nitrogen atom. Furthermore, the QSAR data indicated the importance of hydrophobicity for anti-*Leishmania* activity in these series. This work was revisited by Kapil *et al.*[139] and included in a review focusing on small-molecule strategies targeting leishmaniasis chemotherapy.

Petiz *et al.*[140] investigated the toxicity of compound **95** against *L. amazonensis* in isolated rat liver mitochondria at the same concentration used for the anti-*Leishmania* effect. The sites of inhibition in the respiratory chain were complex I and between segment ubiquinone and complex III. This effect was confirmed by the dose-dependent increase in oxygen consumption during stage 4 respiration induced by oligomycin. Furthermore, mitochondrial swelling was inhibited by compound **95**, suggesting that this amidine affects mitochondrial membrane permeability and fluidity. These results indicated that compound **95** has a slight effect on energy-like functions in isolated mitochondria at the medium lethal dose (LD_{50}).

4. Conclusions

Based on the bibliographic survey of this work, the synthesis of unsubstituted or monosubstituted amidines can be primarily achieved from nitriles in good reaction times, requiring a catalyst. However, the synthesis of disubstituted and trisubstituted amidines typically requires longer reaction times and the addition of other functionalities, such as amides, thioamides, sulfonylazides, *ortho*-ethers, isonitriles, imidoesters, heterocyclics, carbodiimides,

and ynamides, necessitating expensive catalysts. There are few examples of functionalized amidines synthesized in the literature, and this method remains a synthetic challenge. However, in the past two decades, there have been significant advancements in the use of eco-friendly methods, such as microwave irradiation, ultrasound, and solvent-free and catalyst-free methods; however, these methods are still insufficient, especially for the synthesis of disubstituted or trisubstituted amidines.

Regarding the results obtained for the antileishmanial activity of the amidine derivatives, derivatives containing the furan group and, especially, neutral or di-cationic bis-amidines are notable; thus, compounds of these scaffolds appear to be promising for leishmaniasis treatment. Concerning molecular targets in intracellular parasites, there have been few advances, and most of the tests have been carried out *in vitro*; therefore, much research is still needed; however, the results suggest that DNA intercalation contributes to the activity and side effects of most bis-amidines because the two amidinic groups are diametrically opposed. These data highlight the future need to evaluate new bis-amidines with both amidic groups on the same side of the molecule. Such structural characteristics would preserve the lipophilicity of the molecule and should influence DNA binding. Furthermore, it is necessary to evaluate new amidoximes to combat leishmaniasis. This group represents a useful strategy for enhancing the bioavailability of amidines produced from amidoximes in biological assays. The bioavailability of amidines could also be improved using liposomes and nanoparticles of different compositions. These two strategies for enhancing bioavailability could be applied not only to bis-amidines but also to monoamidinic compounds, as previously performed with the *N*,*N*-phenyl-*N*'-R_1,R_2-phenyl-*p*-methoxy-benzamidine series, which has been shown to be extremely promising.

Acknowledgments

The authors would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant No. 30864/2019-2), Coordenação de Aperfeiçoamento de Nível Superior (CAPES, financial code 001), and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for fellowships and financial support.

Cláudio E. Rodrigues-Santos *received his bachelor's and teaching degree in Chemistry in 2002, and he obtained his MSc (2002) and PhD (2008) in Science from the Rural University of Rio de Janeiro. Currently, he is an Associate Professor in the Department of Organic Chemistry of the Rural University of Rio de Janeiro. His research interests are focused on the synthesis of pyrazolo[3,4-b]pyridin-6-ones, 4-aryl-3,4-dihydrocoumarins, amidines, the development of green protocols, microwave and ultrasound utilization, and the planning of bioactive molecules. Recently, he has been working to develop new teaching methodologies.*

Maurício R. de Brito *is an MSc Student at UFRRJ Chemistry Post Degree Program (Seropédica, Rio de Janeiro State, Brazil) and opted for the emphasis on materials chemistry. He also holds a bachelor's degree in Chemical Engineering (UFRRJ) and acts as a temporary researcher in the UFRRJ Chemistry Institute's photochemistry and photocatalysis group. His major interests are organic synthesis, theoretical chemistry, and photochemistry.*

Aurea Echevarria *is a Professor of Organic Chemistry at the Universidade Federal Rural do Rio de Janeiro (UFRRJ, Brazil). She is graduated and Master in Chemistry at Universidade Estadual de Campinas (UNICAMP, Brazil), and obtained a doctorate degree in Organic Chemistry at Universidade de São Paulo (USP, Brazil) working on physical organic chemistry involving mesoionic compounds (1986). She subsequently completed a postdoctorate at the Instituto de Química Medica (CSIC, Madrid). In fact, she has developed projects in medicinal chemistry involving cancer and neglected diseases, as well as new organic materials.*

References

1. Drugs for Neglected Diseases Initiative (DNDi); *Visceral leishmaniasis*, https://www.dndi.org/diseases-projects/leishmaniasis, accessed in March 2024.
2. World Health Organization (WHO); *Leishmaniasis*, https://who.int/news-room/fact-sheets/detail/leishmaniasis, accessed in March 2024.
3. Güran, M. In *Vectors and Vector-Borne Zoonotic Diseases*; Savić, S., ed.; IntechOpen Limited: London, UK, 2019, ch. 5. [Crossref]
4. McCall, L.-I.; Zhang, W.-W.; Matlashewski, G.; *PLoS Pathog.* **2013**, *9*, e1003053. [Crossref]
5. Samy, A. M.; Doha, S. A.; Kenawy, M. A.; *Mem. Inst. Oswaldo Cruz* **2014**, *109*, 299. [Crossref]

6. Bekhit, A. A.; El-Agroudy, E.; Helmy, A.; Ibrahim, T. M.; Shavndi, A.; Bekhit, A. El-D. A.; *Eur. J. Med. Chem.* **2018**, *160*, 229. [Crossref]
7. D'Alessandro, S.; Parapini, S.; Corbett, Y.; Frigerio, R.; Delbue, S.; Modenese, A.; Gramiccia, M.; Ferrante, P.; Taramelli, D.; Basilico, *N.; Pathogens* **2021**, *10*, 1380. [Crossref]
8. Inbar, E.; Hughitt, V. K.; Dillon, L. A. L.; Ghosh, K.; El-Sayed, N. M.; Sacks, D. L.; *mBio* **2017**, *8*, e00029-17. [Crossref]
9. Roma, E. H.; Macedo, J. P.; Goes, G. R.; Gonçalves, J. L.; de Castro, W.; Cisalpino, D.; Vieira, L. Q.; *Parasites Vectors* **2016**, *9*, 193. [Crossref]
10. McGwire, B. S.; Chang, K.-P.; Engman, D. M.; *Infect. Immun.* **2003**, *71*, 1008. [Crossref]
11. Andrade-Neto, V. V.; Cunha-Junior, E. F.; Faioes, V. S.; Martins, T. P.; Silva, R. L.; Leon, L. L.; Torres-Santos, E. C.; *Front. Biosci.-Landmark* **2018**, *23*, 967. [Crossref]
12. Reithinger, R.; Dujardin, J.-C.; Louzir, H.; Pirmez, C.; Alexander, B.; Brooker, S.; *Lancet Infect. Dis.* **2007**, *7*, 581. [Crossref]
13. Pal, R.; Teli, G.; Akhtar, Md J.; Matada, G. S. P.; *Eur. J. Med. Chem.* **2023**, *258*, 115609. [Crossref]
14. Soni, M.; Pratap, J. V.; *Phatogens* **2022**, *11*, 950. [Crossref]
15. Shriner, R. L.; Neumann, F. W.; *Chem. Rev.* **1944**, *35*, 351. [Crossref]
16. Gautier, J. A.; Miocque, M.; Farnoux, C. C.; *The Chemistry of Amidines and Imidates*, vol. 1; Patai, S., ed.; John Wiley & Sons: New York, 1975.
17. dos Santos, M. S.; Bernardino, A. M. R.; de Souza, M. C.; *Quim. Nova* **2006**, *29*, 1301. [Crossref]
18. Chen, J.; Long, W.; Zhao, Y.; Li, H.; Zheng, Y.; Lian, P.; Wan, X.; *Chin. J. Chem.* **2018**, *36*, 857. [Crossref]
19. Abe, T.; Takeda, H.; Miwa, Y.; Yamada, K.; Yanada, R.; Ishikura, M.; *Helv. Chim. Acta* **2010**, *93*, 233. [Crossref]
20. Rydfjord, J.; Skillinghaug, B.; Brandt, P.; Odell, L. R.; Larhed, M.; *Org. Lett.* **2017**, *19*, 4066. [Crossref]
21. Dunsford, J. J.; Camp, J. E.; *Tetrahedron Lett.* **2013**, *54*, 4522. [Crossref]
22. Abreu, P. A.; Castro, H. C.; Paes-de-Carvalho, R.; Rodrigues, C. R.; Giongo, V.; Paixão, I. C. N. P.; Santana, M. V.; Ferreira, J. M.; Caversan, O. M.; Leão, R. A. C.; Marins, L. M. S.; Henriques, A. M.; Farias, F. M. C.; Albuquerque, M. G.; Pinheiro, S.; *Chem. Biol. Drug Des.* **2013**, *81*, 185. [Crossref]
23. Yavari, I.; Nematpour, M.; Sodagar, E.; *Synlett* **2013**, *24*, 161. [Crossref]
24. Veer, S. D.; Katkar, K. V.; Akamanchi, K. G.; *Tetrahedron Lett.* **2016**, *57*, 4039. [Crossref]
25. Lee, D. S.; Amara, Z.; Poliakoff, M.; Harman, T.; Reid, G.; Rhodes, B.; Brough, S.; Mclnally, T.; Woodward, S.; *Org. Process Res. Dev.* **2015**, *19*, 831. [Crossref]
26. Díaz, J. E.; Mollo, M. C.; Orelli, L. R.; *Beilstein J. Org. Chem.* **2016**, *12*, 2026. [Crossref]
27. Murali, A.; Sen, S. K.; Baskaran, S.; *Synthesis* **2011**, *11*, 1771. [Crossref]
28. Saikia, U. P.; Borah, G.; Pahari, P.; *Eur. J. Org. Chem.* **2018**, *2018*, 1211. [Crossref]
29. Rydford, J.; Svensson, F.; Trejos, A.; Sjöberg, P. J. R.; Sköld, C.; Sävmarker, J.; Odell, L. R.; Larhed, M.; *Chem. - Eur. J.* **2013**, *19*, 13803. [Crossref]
30. Sävmarker, J.; Rydford, J.; Gising, J.; Odell, L. R.; Larhed, M.; *Org. Lett.* **2012**, *14*, 2394. [Crossref]
31. Sondhi, S. M.; Rani, R.; Roy, P.; Agrawal,S.K.; Saxena, A. K.; *Eur. J. Med. Chem.* **2010**, *45*, 902. [Crossref]
32. Tkachuk, V. A.; Omelchenko, I. V.; Hordiyenko, O. V.; *Synlett* **2017**, *28*, 851. [Crossref]
33. Calderón-Díaz, A.; Arras, J.; Miller, E. T.; Bhuvanesh, N.; McMillen, C. D.; Stollenz, M.; *Eur. J. Org. Chem.* **2020**, *2020*, 3243. [Crossref]
34. Rodrigues-Santos, C. E.; Leon, L. L.; Bortoluzzi, A. J.; Canto-Cavalheiro, M. M.; Machado, G. C.; Echevarria, A.; *Eur. J. Med. Chem.* **2013**, *67*, 166. [Crossref]
35. Chen, J.; Long, W.; Fang, S.; Yang, Y.; Wan, X.; *Chem. Commun.* **2017**, *53*, 13256. [Crossref]
36. Velavan, A.; Sumathi, S.; Balasubramanian, K. K.; *Eur. J. Org. Chem.* **2014**, *2014*, 5806. [Crossref]
37. Das, V. K.; Thakur, A. J.; *Tetrahedron Lett.* **2013**, *54*, 4164. [Crossref]
38. Hellal, M.; Bihel, F.; Mongeot, A.; Bourguignon, J.-J.; *Org. Biomol. Chem.* **2006**, *4*, 3142. [Crossref]
39. Xuan-Wu, Y.; Wusiman, A.; *Phosphorus, Sulfur Silicon Relat. Elem.* **2020**, *195*, 115. [Crossref]
40. Díaz, J. E.; Bisceglia, J. Á.; Mollo, Ma. C.; Orelli, L. R.; *Tetrahedron Lett.* **2011**, *52*, 1895. [Crossref]
41. Chen, S.; Xu, Y.; Wan, X.; *Org. Lett.* **2011**, *13*, 6152. [Crossref]
42. Phakhodee, W.; Wangngae, S.; Wiriya, N.; Pattarawarapan, M.; *Tetrahedron Lett.* **2016**, *57*, 5351. [Crossref]
43. Mahanty, K.; Halder, A.; De Sarkar, S.; *Adv. Synth. Catal.* **2023**, *365*, 96. [Crossref]
44. Taj, M. B.; Tirmizi, S. A.; Raheel, A.; Alelwani, W.; Hajjar, D.; Makki, A. A.; Ali, U.; Darroudi, M.; Ali, H. B. M.; *Russ. J. Gen. Chem.* **2018**, *88*, 2425. [Crossref]
45. Taj, M. B.; Raheel, A.; Alelwani, W.; Babteen, N.; Kattan, S.; Alnajeebi, A.; Sharif, M.; Ahmad, R. H.; Abbas; Hazeeq, A.; Tirmizi, S. A.; Ali, H. B. M.; *Russ. J. Org. Chem.* **2019**, *55*, 1047. [Crossref]
46. Mateu, N.; Ciordia, M.; Delgado, O.; Sánchez-Roselló, M.; Trabanco, A. A.; Gool, M. V.; Tresadern, G.; Pérez-Benito, L.; Fustero, S.; *Chem. - Eur. J*. **2015**, *21*, 11719. [Crossref]
47. Li, J.-S.; Xue, Y.; Li, P.-Y.; Li, Z.-W.; Lu, C.-H.; Liu, W.-D.; Pang, H.-L.; Liu, D.-H.; Lin, M.-S.; Luo, B.-B.; Jiang, W.; *Res. Chem. Intermed.* **2013**, *41*, 2235. [Crossref]
48. Okano, A.; James, R. C.; Pierce, J. G.; Xie, J.; Boger, D. L.; *J. Am. Chem. Soc.* **2012**, *134*, 8790. [Crossref]

49. Biswas, K.; Greaney, M. F.; *Org. Lett.* **2011**, *13*, 4946. [Crossref]
50. Scherbakow, S.; Namyslo, J. C.; Gjikaj, M.; Schimidt, A.; *Synlett* **2009**, 1964. [Crossref]
51. Zeng, X.; Gu, Q.; Dai, W.; Xie, Y.; Liu, X.; Wu, G.; *Synthesis* **2021**, *53*, 2889. [Crossref]
52. Chen, Z.; Nie, X.-D.; Sun, J.-T.; Yang, A.-M.; Wei, B.-G.; *Org. Biomol. Chem.* **2021**, *19*, 2492. [Crossref]
53. DeKorver, K. A.; Johnson, W. L.; Zhang, Y.; Hsung, R. P.; Dai, H.; Deng, J.; Lohse, A. G.; Zhang, Y.-S.; *J. Org. Chem.* **2011**, *76*, 5092. [Crossref]
54. Rupakova, N. A.; Bakulev, V. A.; Knippschild, U.; García-Reyes, B.; Eltsov, O. S.; Slesarev, G. P.; Beliaev, N.; Slupukhin, P. A.; Witt, L.; Peifer, C.; Beryozkina, T. V.; *Arkivoc* **2017**, *iii*, 225. [Crossref]
55. Dianova, L.; Berseneva, V.; Beryozkina, T.; Efimov, I.; Kosterina, M.; Eltsov, O.; Dehaen, W.; Bakulev, V.; *Eur. J. Org. Chem.* **2015**, *2015*, 6917. [Crossref]
56. Gui, J.; Xie, H.; Jiang, H.; Zeng, W.; *Org. Lett.* **2019**, *21*, 2804. [Crossref]
57. Gao, T.; Zhao, M.; Meng, X.; Li, C.; Chen, B.; *Synlett* **2011**, *9*, 1281. [Crossref]
58. Bae, I.; Han, H.; Chang, S.; *J. Am. Chem. Soc.* **2005**, *127*, 2038. [Crossref]
59. Kim, J.; Lee, S. Y.; Lee, J.; Do, Y.; Chang, S.; *J. Org. Chem.* **2008**, *73*, 9454. [Crossref]
60. Yao, B.; Shen, C.; Liang, Z.; Zhang, Y.; *J. Org. Chem.* **2014**, *79*, 936. [Crossref]
61. Wang, J.; Lu, P.; Wang, Y.; *Org. Chem. Front.* **2015**, *2*, 1346. [Crossref]
62. Ghorai, S.; Lee, D.; *Org. Lett.* **2021**, *23*, 697. [Crossref]
63. Chow, S. Y.; Odell, L. R.; *J. Org. Chem.* **2017**, *82*, 2515. [Crossref]
64. Xu, X.; Li, X.; Ma, L.; Ye, N.; Weng, B.; *J. Am. Chem. Soc.* **2008**, *130*, 14048. [Crossref]
65. Cao, V. D.; Mun, S. H.; Kim, S. H.; Kim, G. U.; Kim, H. G.; Joung, S.; *Org. Lett.* **2020**, *22*, 515. [Crossref]
66. Rouzi, A.; Hudabaierdi, R.; Wusiman, A.; *Tetrahedron* **2018**, *74*, 2475. [Crossref]
67. Jian, Y.; Chen, M.; Yang, C.; Xia, W.; *Synthesis* **2019**, *51*, 4425. [Crossref]
68. Wang, S.; Wang, Z.; Zheng, X.; *Chem. Commun.* **2009**, 7372. [Crossref]
69. Kaboudin, B.; Torabi, S.; Kazemi, F.; Aoyama, H.; *RSC Adv.* **2020**, *10*, 26701. [Crossref]
70. Chakraborty, P.; Roy, S. C.; *Green Sustainable Chem.* **2013**, *3*, 26. [Crossref]
71. Ziyadi, H.; Baghali, M.; Heydari, A.; *Heliyon* **2021**, *7*, e07165. [Crossref]
72. Azizi, N.; Gholibeglo, E.; Babapour, M.; Ghafuri, H.; Bolourtchian, S. M.; *C. R. Chim.* **2012**, *15*, 768. [Crossref]
73. Patil, D. R.; Dalal, D. S.; *Chin. Chem. Lett.* **2012**, *23*, 1125. [Crossref]
74. Cibian, M.; Langis-Barsetti, S.; Hanan, G. S.; *Synlett* **2011**, 405. [Crossref]
75. Lakeh, E. H.; Shariyati, A. Y.; Langarudi, M. S. N.; Tajik, H.; Shirini, F.; *ChemistrySelect* **2020**, *5*, 7488. [Crossref]
76. Mazloumi, M.; Shirini, F.; Goli-Jolodar, O.; Seddighi, M.; *New J. Chem.* **2018**, *42*, 5742. [Crossref]
77. Nasresfahani, Z.; Kassaee, M. Z.; Eidi, E.; *Appl. Organomet. Chem.* **2017**, *31*, e3800. [Crossref]
78. Siutkina, A. I.; Kalinina, S.; Liu, R.; Heitman, L. H.; Junker, A.; Daniliuc, C. G.; Kalinin, D. V.; *ACS Omega* **2023**, *8*, 14097. [Crossref]
79. Cao, H.; Bie, F.-s.; Liu, X.-j.; Han, Y.; Ma, J.; Shi, Y.-j.; Yan, P.; Sun, C.-y.; Wang, H.-m.; *Tetrahedron* **2020**, *76*, 131205. [Crossref]
80. Dar, B. A.; Armad, S. N.; Wagay, M. A.; Hussain, A.; Ahmad, N.; Bhat, K. A.; Khuroo, M. A.; Sharma, M.; Singh, B.; *Tetrahedron Lett.* **2013**, *54*, 4880. [Crossref]
81. Chandna, N.; Chandak, N.; Kumar, P.; Kapoor, J. K.; Sharma, P. K.; *Green Chem.* **2013**, *15*, 2294. [Crossref]
82. Clodt, J. I.; Wigbers, C.; Riermann, R.; Fröhlich, R.; Würthwein, E.-U.; *Eur. J. Org. Chem.* **2011**, *2011*, 3197. [Crossref]
83. Cigbers, C.; Prigge, J.; Mu, Z.; Fröhlich, R.; Chi, L.; Würthwein, E.-U.; *Eur. J. Org. Chem.* **2011**, *2011*, 861. [Crossref]
84. Yahyazadeh, A.; Nea, M. H.; Majnooni, S.; *Eur. Chem. Bull.* **2013**, *2*, 855. [Link] accessed in April 2024
85. Debnath, P.; Baeten, M.; Lefèvre, N.; Van Daele, S.; Maes, B. U. W.; *Adv. Synth. Catal.* **2015**, *357*, 197. [Crossref]
86. Goldberg, A. F. G.; O'Connor, N. R.; Craig II, R. A.; Stoltz, B. M.; *Org. Lett.* **2012**, *14*, 5314. [Crossref]
87. Feng, M.; Yang, P.; Yang, G.; Chen, W.; Chai, Z.; *J. Org. Chem.* **2018**, *83*, 174. [Crossref]
88. Zhao, Y.; Ma, X.; Yan, B.; Ni, C.; He, X.; Peng, Y.; Yang, Z.; *J. Organomet. Chem.* **2021**, *946-947*, 121879. [Crossref]
89. Wang, Y.; Chi, Y.; Zhao, F.; Zhang, W.-X.; Xi, Z.; *Synthesis* **2013**, *45*, 347. [Crossref]
90. Gu, Z.-Y.; Han, H.; Li, Z.-Y.; Ji, S.-J.; Xia, J.-B.; *Org. Chem. Front.* **2021**, *8*, 1544. [Crossref]
91. Li, M.; Fang, S.; Zheng, J.; Jiang, H.; Wu, W.; *Org. Lett.* **2019**, *21*, 8439. [Crossref]
92. Dai, Q.; Jiang, Y.; Yu, J.-T.; Cheng, J.; *Chem. Commun.* **2015**, *51*, 16645. [Crossref]
93. Yan, X.; Liao, J.; Lu, Y.; Liu, J.; Zeng, Y.; Cai, Q.; *Org. Lett.* **2013**, *15*, 2478. [Crossref]
94. Keung, W.; Bakir, F.; Patron, A. P.; Rogers, D.; Priest, C. D.; Darmohusodo, V.; *Tetrahedron Lett.* **2004**, *45*, 733. [Crossref]
95. Tetala, K. K. R.; Whitby, R. J.; Light, M. E.; Hurtshouse, M. B.; *Tetrahedron Lett.* **2004**, *45*, 6991. [Crossref]
96. Ai, H.-J.; Cao, C.-X.; Qi, X.; Peng, J.-B.; Ying, J.; Zheng, F.; Wu, X.-F.; *Tetrahedron Lett.* **2017**, *58*, 3751. [Crossref]

97. Medda, F.; Hulme, C.; *Tetrahedron Lett.* **2014**, *55*, 3328. [Crossref]
98. Zhang, Z.; Huang, B.; Qiao, G.; Zhu, L.; Xiao, F.; Chen, F.; Fu, B.; Zhang, Z.; *Angew. Chem., Int. Ed.* **2017**, *56*, 4320. [Crossref]
99. Sendzik, M.; Hui, H. C.; *Tetrahedron Lett.* **2003**, *44*, 8697. [Crossref]
100. Li, G.; Zhao, M.; Xie, J.; Yao, Y.; Mou, L.; Zhang, X.; Guo, X.; Sun, W.; Wang, Z.; Xu, J.; Xue, J.; Hu, T.; Zhang, M.; Li, M.; Hong, L.; *Chem. Sci.* **2020**, *11*, 3586. [Crossref]
101. Katritzky, A. R.; Cai, C.; Singh, S. K.; *J. Org. Chem.* **2006**, *71*, 3375. [Crossref]
102. Erkhitueva, E. B.; Panikorobskii, T. L.; Svintsitskaya, N. I.; Trifonov, R. E.; Dogadina, A. V.; *Synlett* **2018**, *29*, 933. [Crossref]
103. Huang, B.; Yang, C.; Zhou, J.; Xia, W.; *Chem. Commun.* **2020**, *56*, 5010. [Crossref]
104. Carvalho, M. H. R.; Ribeiro, J. P. R. S.; De Castro, P. P.; Passos, S, T. A.; Neto, B. A. D.; Dos Santos, H. F.; Amarante, G. W.; *J. Org. Chem.* **2022**, *87*, 11007. [Crossref]
105. Kubíčková, A.; Markos, A.; Voltrová, S.; Marková, A.; Filgas, J.; Klepetářová, B.; Slavíček, P.; Beier, P.; *Org. Chem. Front.* **2023**, *10*, 3201. [Crossref]
106. Vogel, J. A.; Miller, K. F.; Shin, E.; Krussman, J. M.; Melvin, P. R.; *RSC Adv.* **2023**, *13*, 30129. [Crossref]
107. Basselin, M.; Badet-Denisot, M.-A.; Lawrence, F.; Robert-Gero, M.; *Exp. Parasitol.* **1997**, *85*, 274. [Crossref]
108. Montanari, C. A.; Trent, C. O.; Jenkins, T. C.; *J. Braz. Chem. Soc.* **1998**, *9*, 175. [Crossref]
109. Yang, G.; Choi, G.; No, J. H.; *Antimicrob. Agents Chemother.* **2016**, *60*, 6828. [Crossref]
110. Andreana, I.; Bincoletto, V.; Milla, P.; Dosio, F.; Stella, B.; Arpicco, S.; *Drug Delivery Transl. Res.* **2022**, *12*, 1911. [Crossref]
111. Banerjee, G.; Nandi, G.; Mahato, S. B.; Pakrashi, A.; Basu, M. K.; *J. Antimicob. Chemother.* **1996**, *38*, 145. [Crossref]
112. Román-Álamo, L.; Allaw, M.; Avalos-Padilla, Y.; Manca, M. L.; Manconi, M.; Fulgheri, F.; Fernández-Lajo, J.; Rivas, L.; Vázquez, J. A.; Peris, J. E.; Roca-Geronès, X.; Poonlaphdecha, S.; Alcover, M. M.; Fisa, R.; Riera, C.; Fernández-Busquets, X.; *Pharmaceutics* **2023**, *15*, 1163. [Crossref]
113. Stephens, C. E.; Brun, R.; Salem, M. M.; Werbovetz, K. A.; Tanious, F.; Wilson, W. D.; Boykin, D. W.; *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2065. [Crossref]
114. de Souza, E. M.; Lansiaux, A.; Bailly, C.; Wilson, W. D.; Hu, Q.; Boykin, D. W.; Batista, M. M.; Araújo-Jorge, T. C.; Soeiro, M. N. C.; *Biochem. Pharmacol.* **2004**, *68*, 593. [Crossref]
115. Lansiaux, A.; Tanious, F.; Mishal, Z.; Dassonneville, L.; Kumar, A.; Stephens, C. E.; Hu, K.; Wilson, W. D.; Boykin, D. W.; Bailly, C.; *Cancer Res.* **2002**, *62*, 7219. [Link]
116. Soares-Bezerra, R. J.; Leon, L.; Genestra, M.; *Braz. J. Pharm. Sci.* **2004**, *40*, 139. [Link] accessed in April 2024
117. Huang, T. L.; Eyde, J. J. V.; Mayence, A.; Donkor, I. O.; Khan, S. I.; Tekwani, B. L.; *J. Pharm. Pharmacol.* **2006**, *58*, 1033. [Crossref]
118. Rosypal, A. C.; Hall, J. E.; Bakunova, S.; Patrick, D. A.; Bakunov, S.; Stephens, C. E.; Kumar, A.; Boykin, D. W.; Tidwell, R. R.; *Vet. Parasitol.* **2007**, *145*, 207. [Crossref]
119. Hu, L.; Arafa, R. K.; Ismail, M. A.; Wenzler, T.; Brun, R.; Munde, M.; Wilson, W. D.; Nzmiro, S.; Samyesudhas, S.; Werbovetz, K. A.; Boykin, D. W.; *Bioorg. Med. Chem. Lett.* **2008**, *18*, 247. [Crossref]
120. Rosypal, A. C.; Werbovetz, K. A.; Salem, M.; Stephens, C. E.; Kumar, A.; Boykin, D. W.; Hall, J. E.; Tidwell, R. R.; *J. Parasitol.* **2008**, *94*, 743. [Crossref]
121. Bakunova, S. M.; Bakunov, S. A.; Wenzler, T.; Barszcz, T.; Werbovetz, K. A.; Brun, R.; Tidwell, R. R.; *J. Med. Chem.* **2009**, *52*, 4657. [Crossref]
122. Bakunov, S. A.; Bakunova, S. M.; Wenzler, T.; Barszcz, T.; Werbovetz, K. A.; Brun, R.; Tidwell, R. R.; *J. Med. Chem.* **2008**, *51*, 6927. [Crossref]
123. Wang, M. Z.; Zhu, X.; Srivastava, A.; Liu, Q.; Sweat, J. M.; Pandharkar, T.; Stephens, C. E.; Riccio, E.; Parman, T.; Munde, M.; Mamdal, S.; Madhubala, R.; Tidwell, R. R.; Wilson, W. D.; Boykin, D. W.; Hall, J. E.; Kyle, D. E.; Werbovetz, K. A.; *Antimicrob. Agents Chemother.* **2010**, *54*, 2507. [Crossref]
124. Zhu, X.; Liu, Q.; Yang, S.; Parman, T.; Green, C. E.; Mirsalis, J. C.; Soeiro, M. N. C.; Souza, E. M.; Silva, C. F.; Batista, D. G. J.; Stephens, C. E.; Banerjee, M.; Farahat, A. A.; Munde, M.; Wilson, W. D.; Boykin, D. W.; Wang, M. Z.; Werbovetz, K. A.; *Antimicrob. Agents Chemother.* **2012**, *56*, 3690. [Crossref]
125. Zhu, X.; Farahat, A. A.; Mattamana, M.; Joice, A.; Pandhakar, T.; Holt, E.; Banerjee, M.; Gragg, J. L.; Hu, L.; Kumar, A.; Yang, S.; Wang, M. Z.; Boykin, D. W.; Werbovetz, K. A.; *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2551. [Crossref]
126. Bouhlel, A.; Curti, C.; Dumètre, A.; Laget, M.; Crozet, M. D.; Azas, N.; Vanelle, P.; *Bioorg. Med. Chem.* **2010**, *18*, 7310. [Crossref]
127. Paloque, L.; Bouhlel, A.; Curti, C.; Dumètre, A.; Verhaeghe, P.; Azas, N.; Vanelle, P.; *Eur. J. Med. Chem.* **2011**, *46*, 2984. [Crossref]
128. Clement, B.; Struwe, M. A.; *Molecules* **2023**, *28*, 4713. [Crossref]
129. Tahghighi, A.; Marznaki, F. R.; Kobarfard, F.; Dastmalchi, S.; Mojarrd, J. S.; Razmi, S.; Ardestani, S. K.; Emami, S.; Shafiee, A.; Foroumadi, A.; *Eur. J. Med. Chem.* **2011**, *46*, 2602. [Crossref]
130. Rezaei, F.; Saghaie, L.; Sabet, R.; Fassihi, A.; Hatam, G.; *Chem. Biodiversity* **2018**, *15*, e1800228. [Crossref]
131. Nué-Martinez, J. J.; Cisneros, D.; Moreno-Blazquez, M. V.; Fonseca-Berzal, C.; Manzano, J. I.; Kraeutler, D.; Ungogo, M. A.; Aloraini, M. A.; Elati, H. A. A.; Ibáñez-Escribano,

A.; Lagartera, L.; Herraiz, T.; Gamarro, F.; de Koning, H. P.; Gómez-Barrio, A.; Dardonville, C.; *J. Med. Chem.* **2023**, *66*, 13452. [Crossref]

132. Alp, M.; Göker, H.; Brun, R.; Yildiz, S.; *Eur. J. Med. Chem.* **2009**, *44*, 2002. [Crossref]
133. Boechat, N.; Lages, A. S.; Santos-Filho, O. A.; Genestra, M.; Bastos, M. M.; Kover, W. B.; *J. Microbiol. Antimicrob.* **2013**, *5*, 72. [Crossref]
134. Temporal, R. M.; Cysne-Finkelsteina, L.; Echevarria, A.; Souza, M. A. S.; Sertã, M.; Silva-Gonçalves, A. J.; Pirmez, C.; Leon, L. L.; *Arzneimittelforschung* **2002**, *52*, 489. [Crossref]
135. Genestra, M.; Guedes-Silva, D.; Souza, W. J. S.; Cysne-Finkelstein, L.; Soares-Bezerra, R. J.; Monteiro, F. P.; Leon, L. L.; *Arch. Med. Res.* **2006**, *37*, 328. [Crossref]
136. Genestra, M.; Echevarria, A.; Cysne-Finkelstein, L.; Vignólio-Alves, L.; Leon, L. L.; *Nitric Oxide* **2003**, *8*, 1. [Crossref]
137. Castro-Pinto, D. B.; Echevarria, A.; Genestra, M. S.; Cysne-Finkelstein, L.; Leon, L. L.; *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 57. [Crossref]
138. Temporal, R. M.; Cysne-Finkelstein, L.; Echevarria, A.; Silva-Gonçalves, A. J.; Leon, L. L.; Genestra, M. S.; *J. Enzym Inhib. Med. Chem.* **2005**, *20*, 13. [Crossref]
139. Kapil, S.; Singh, P. K.; Silakari, O.; *Eur. J. Med. Chem.* **2018**, *157*, 339. [Crossref]
140. Petiz, L. L.; Pires, A. R. A.; Echevarria, A.; Rodrigues-Santos, C. E.; Rocha, M. E. M.; Acco, A.; Cadena, S. M. S. C.; *Chem.-Biol. Interact.* **2018**, *279*, 34. [Crossref]

Submitted: February 1, 2024
Published online: April 12, 2024