Similarity-based profiling of hydrazone-containing scaffolds active against leishmania amastigotes

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Abstract

This review explores the potential of hydrazone-containing scaffolds as antileishmanial agents, particularly targeting Leishmania amastigotes. Through a strategy focusing on the 3D electroshape properties of compounds, molecular alignment techniques were applied to analyze and compare structurally similar hydrazone derivatives. Rather than relying solely on traditional functional group analysis, this review adopts a shape-based profiling approach to uncover key structural features linked to antileishmanial activity. The review systematically compiles data on hydrazone-based compounds from various studies, with particular emphasis on those exhibiting significant activity against intracellular amastigotes. Structural comparisons were performed using molecular overlays, allowing the identification of promising compounds and their potential mechanisms of action. The selected hydrazone derivatives demonstrated noteworthy antileishmanial activity *in vitro*, with a focus on those active against the human phase of the parasite. By integrating both computational and experimental approaches, the review provides insights that may guide the optimization of hydrazone scaffolds for enhanced efficacy, bioavailability, and safety. Despite these promising findings, further investigation into the precise molecular targets of these compounds is necessary, as the exact mechanisms of action remain to be fully understood. Overall, this review highlights the potential of hydrazone-containing compounds as a valuable foundation for the development of new therapeutic agents against leishmaniasis, using a novel shape-based molecular alignment strategy to support drug discovery efforts.

1. Background and introduction

Leishmaniasis is a parasitic disease caused by protozoa from the genus *Leishmania*, transmitted by female *Phlebotomine sandflies*. It manifests in four primary clinical forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), visceral leishmaniasis (VL or kala azar), and post-kala azar dermal leishmaniasis (PKDL). In mammalian hosts, Leishmania proliferates as intracellular amastigotes within phagocytic cells, particularly

macrophages. The sandflies ingest these amastigotes while feeding, which then transform into motile promastigotes in the insect gut, completing the infection cycle.[1]

According to the World Health Organization (WHO), more than one billion people live at risk of infection in endemic areas for leishmaniasis, with approximately 30 000 new cases of VL and more than one million new cases of CL occurring annually (www.who.int/health-topics/leishmaniasis). Regions with the highest disease burden include India, Bangladesh, Nepal,[2] East Africa, and Brazil.[3] HIV coinfection further complicates treatment, increasing parasite burden, worsening prognosis, and elevating relapse rates.[4]

Existing therapies for VL exhibit variable efficacy and significant toxicity. Of the available treatments, only miltefosine is administered orally, while others—such as liposomal amphotericin B and paromomycin—require intravenous or intramuscular administration, posing logistical challenges in many endemic regions.[5] There is an urgent need for simple, affordable oral therapies that are both safe and effective across different populations.[6] In recent years, new drug combinations involving liposomal amphotericin B, paromomycin, and miltefosine have been introduced, offering improved safety and tolerability. However, these therapies remain costly, difficult to administer, and poorly stable in the high temperatures common to endemic areas.[7] Furthermore, a disparity in efficacy persists across regions: while treatment needs are somewhat met in South Asia, drug efficacy and tolerability remain problematic in East Africa and Latin America.[8]

The ideal treatment for VL would consist of a short-course oral therapy that maintains efficacy, improves tolerability, and prevents resistance. Research efforts are focused on developing combination regimens with distinct mechanisms of action to counter the emergence of drug resistance. The DNDi has outlined Target Product Profiles (TPPs) emphasizing the importance of therapies with high efficacy within a 10-day treatment window, effective against resistant strains, and suitable for use across various regions.[9]

A central focus of this review is on hydrazone-based compounds (Fig 1), a class of molecules recognized for their ease of synthesis and broad biological activities. These compounds include aminoguanidine hydrazones, thiosemicarbazones, N-acylhydrazones, and semicarbazones, which are often produced through simple condensation reactions. Despite their popularity in medicinal chemistry, only a limited number of these scaffolds have advanced to clinical applications. Their structural flexibility makes them excellent candidates for optimization in antileishmanial drug discovery.

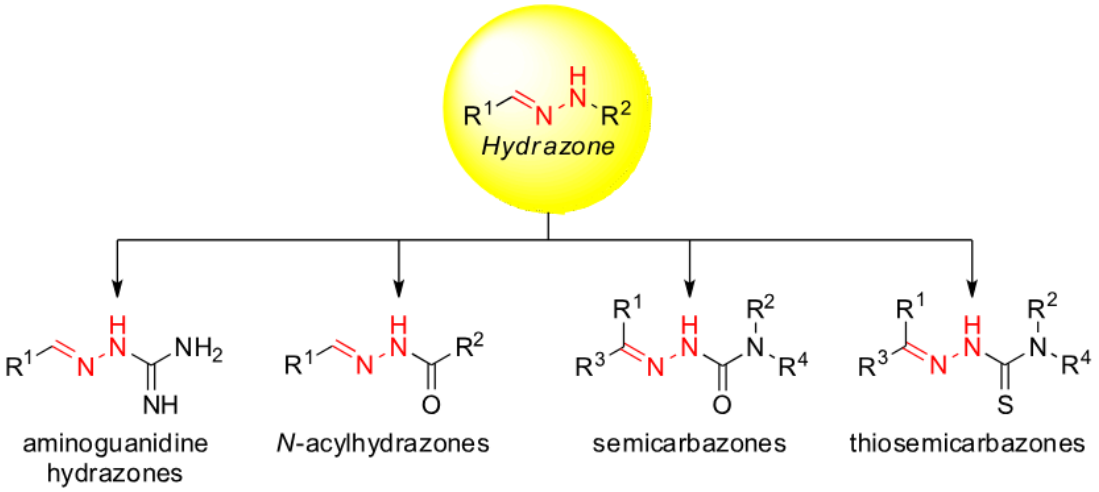

Fig. 1. Representation hydrazone-based compounds.

Rather than focusing solely on molecular functional groups, this review adopts an unconventional approach by prioritizing the 3D electroshape similarities among compounds from the literature. Using molecular alignment techniques, including shape-based overlays, the review compares structurally similar hydrazone-containing scaffolds tested on amastigotes. This approach aims to uncover key insights into their potential mechanisms of action and identify promising candidates with similar properties. Furthermore, the review explores well-studied related compounds, not necessarily tested against Leishmania, to generate insights that may reinforce hypotheses about possible molecular targets.

This comparative reviewing approach, integrating shape-based alignments and electroshape analysis, offers a fresh perspective on literature reviewing. By focusing not only on molecular similarity but also on local and global shape alignments, the review aims to provide researchers with new hypotheses for experimental testing. These hypotheses can be explored through in vitro assays or *in silico* modeling to investigate drug-target interactions and refine compound designs. The insights gained from this analysis may support the development of related compounds with improved efficacy, bioavailability, and enhanced potential as antileishmanial leads.

2. Review strategy

This review followed a systematic strategy to compile compounds from the literature. First, the keywords “Leishmania,” “amastigotes,” and either “synthesis” or “natural” were used to search for relevant studies, starting from the earliest indexed paper on Scopus in 1904. The most active compounds tested on models mimicking the human phase of the parasite—including axenic amastigotes, macrophage amastigotes, and *in vivo* tests—were selected. When compounds were not close analogues, more than one representative from each study was included. Compounds were drawn based on their indicated names or directly using Marvin Sketch,[10] and their 3D structures were optimized using the PM7[11] semi-empirical method in Mopac.[12]

Structural comparisons of all compounds within each series were performed using LS-align to generate molecular overlays. Proper inputs were created for the Gephi software,[13] where nodes represented the reviewed compounds and edges indicated LS-align[14] similarity scores. Low-similarity edges were filtered to remove unrelated nodes from the analysis. To identify direct analogues of the compounds discussed in the article, similarities were measured using the Tanimoto equation implemented in PubChem[15] dictionary-based binary fingerprint. These tools allowed correlating the selected compounds with annotated records in the database, facilitating the identification of potential biological targets. Additionally, this approach provided common names and relevant literature for each compound, aiding in the verification of their identities and supporting assumptions regarding their biological activities.

3. Hydrazone-Containing compounds and related scaffolds

If this review were focused on scaffolds containing a specific functional group tested against *Leishmania* amastigotes, it would encompass a wide range of molecules, potentially including all compounds from various studies. However, the emphasis on 3D electroshape similarities introduces numerous possible alignments and significantly raises the computational cost of analysis. Such an extensive comparison could undermine the clarity and precision intended for this review. To ensure coherence, only one representative compound from each study was selected when others were close analogues; when structural differences were more pronounced, multiple compounds were considered. Priority was given to the most potent compound tested against amastigotes, whether axenic, intramacrophagic, or ideally *in vivo*, ensuring a focused and meaningful analysis of the most effective molecules.

The selected compounds were grouped based on their structural similarities and visualized using Gephi[13] to reveal connections in molecular shapes (Fig. 2). This strategy helped organize the review and illuminated meaningful patterns among structurally related compounds. By clustering these molecules, the analysis offered the potential to form hypotheses about their molecular targets, providing further insights into the possible mechanisms of action. These visualized clusters also enabled commentary on the findings reported by the original authors, offering a more comprehensive understanding of the relationships between the compounds and their biological activities. This approach enhanced the clarity of the review by illustrating connections that may not have been immediately evident from the individual studies.

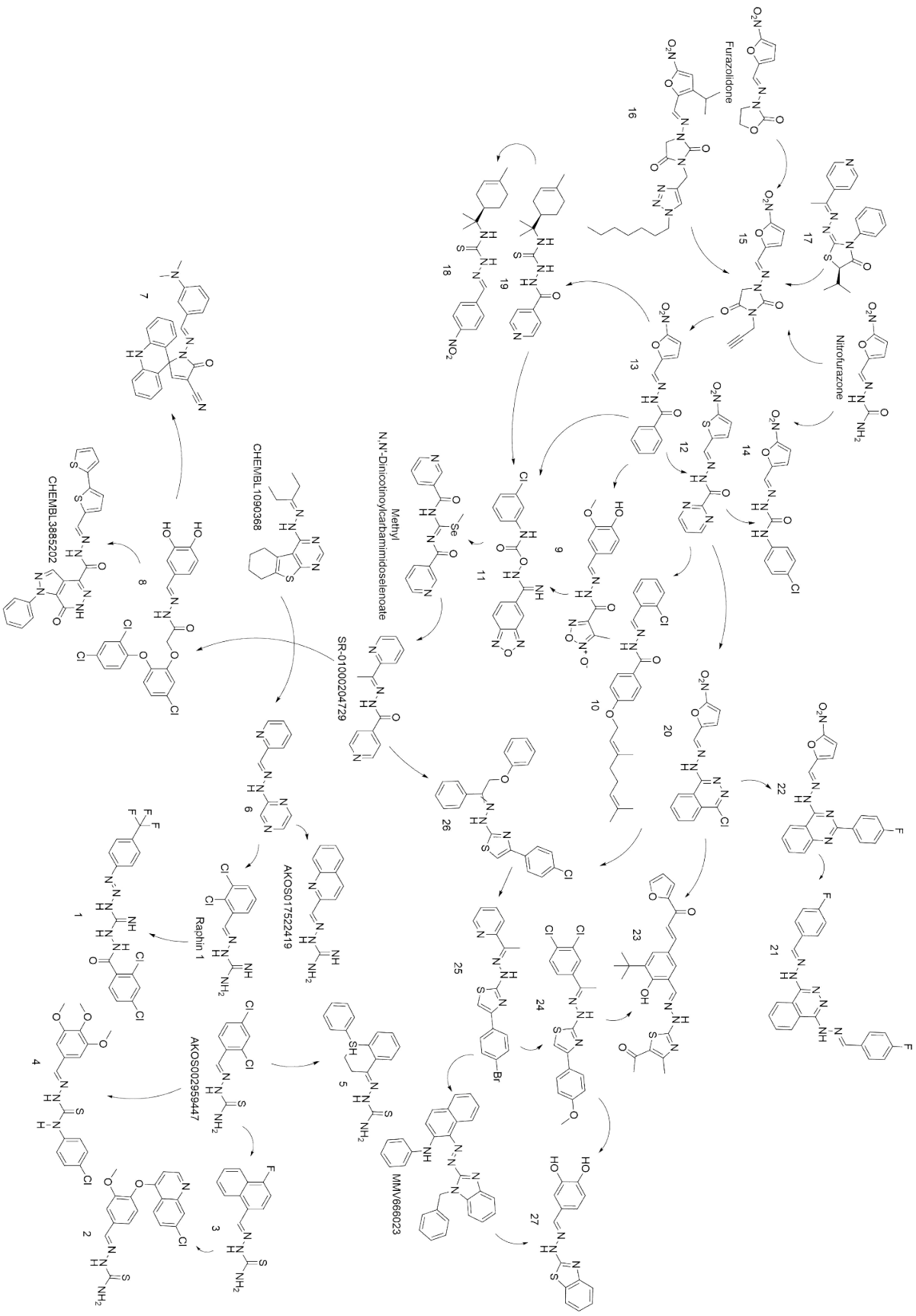

Fig. 2. Graph presenting the structural relationship network of representative compounds from various studies, each selected for their activity against amastigote forms of *Leishmania*. The compounds are

displayed as nodes, with connecting edges indicating structural similarity based on three-dimensional features.

Compounds **Raphin 1** (**1**) and **AKOS017522419** (**2**) (Fig. 3) both were designed and evaluated for their activity against *Leishmania chagasi*.[16] **Raphin 1** is a well-known inhibitor of the regulatory subunit PPP1R15B (R15B) of protein phosphatase, with a dissociation constant (K_d) of 33 nM. It has previously demonstrated efficacy in a mouse model of Huntington's disease.[17] However, *Leishmania* lacks a direct counterpart to PPP1R15B, making it difficult to establish a direct correlation between **Raphin 1**'s mechanism of action in *Leishmania* and its known targets in other organisms.

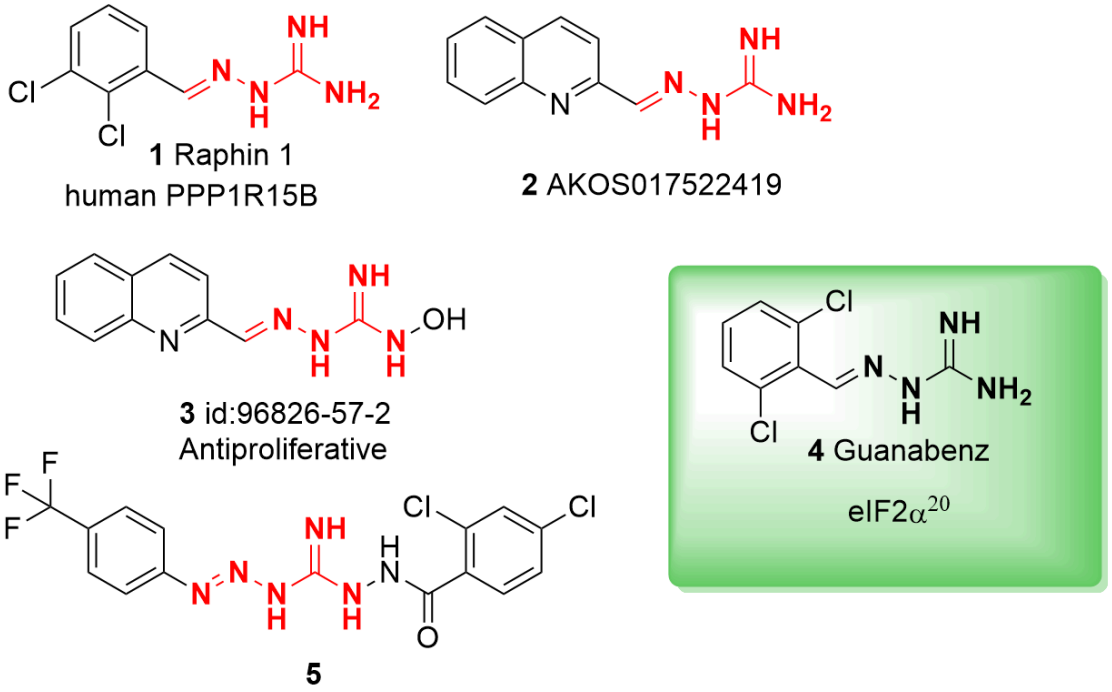

Fig 3. Compounds **Raphin 1** (**1**)**, AKOS017522419** (**2**) and (**5**) and related compounds.

The compound **AKOS017522419**[16] is commercially available and structurally related to **96826-57-2** (**3**),[18] which has known antiproliferative properties. These compounds share a connection to **guanabenz** (**4**) with notable antiparasitic activity. **Guanabenz**, an alpha-2 adrenoceptor agonist, also showed efficacy against *Plasmodium falciparum*[19] and *Toxoplasma gondii* by inhibiting eIF2α phosphorylation.[20] Molecular docking performed by the authors suggested that **AKOS017522419** may interact with Leishmania trypanothione reductase,[16] although this interaction has yet to be confirmed. It is plausible that, like **guanabenz,**[21] **Raphin 1** and **AKOS017522419** may affect *Leishmania* eIF2α biochemical processes.[20] However, further investigation is needed to verify this mechanism.

The unique carbamoyl-N-aryl-imine-urea framework **5** (Fig. 3), was evaluated for its antileishmanial activity against the amastigote forms of *Leishmania amazonensis* and *Leishmania braziliensis*. *In vivo* tests were also conducted using a murine model of cutaneous leishmaniasis to assess the compound's efficacy.[22] The *in vitro* assays demonstrated significant leishmanicidal activity, particularly against *L. amazonensis* amastigotes. In the murine model, Compound **5** showed promising results by reducing both

parasite burden and lesion size, suggesting that it interferes with critical biological processes within the parasite. However, no similar compounds were identified, and a specific mechanism of action was not reported, leaving the molecular target and the precise mode of interference with parasite biology to be further investigated.

The compound **AKOS002959447** (**6**) (Fig. 4) produced by the same authors[16] was also found to be active against *Mycobacterium tuberculosis* in High-throughput tests performed by the VanderVen Lab, College of Veterinary Medicine, Cornell University (Bioassay: 1259343). Its closely related compound, **Benzalthiosemicarbazone** (**7**) (Fig 4), has demonstrated activity against *Plasmodium falciparum* dihydrofolate reductase,[23] suggesting that **AKOS002959447** might share the same target.

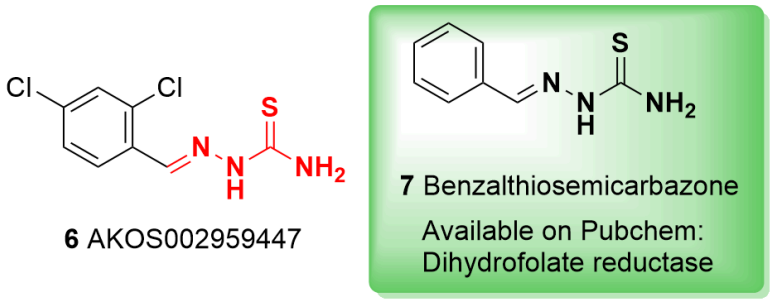

Fig 4. **AKOS002959447** and related compound **Benzalthiosemicarbazone**.

Compound **8** (Fig. 5), a thiosemicarbazone chimerized with chloroquinoline, was tested against *Leishmania donovani* with a focus on its potential synergistic effects when combined with standard antileishmanial drugs miltefosine and amphotericin B.[24] The study demonstrated that **8** exhibited significant synergy with these drugs, particularly against the amastigote form. A key finding was its ability to disrupt mitochondrial membrane potential, leading to parasite cell death, a mechanism further amplified when used in combination. The compound's design, incorporating both chloroquinoline and thiosemicarbazone moieties, mirrors that of **CHEMBL5286667** (**9**), a structurally similar molecule with activity against *Mycobacterium tuberculosis*. **9** showed low micromolar efficacy against the *M. tuberculosis* H37Rv strain and inhibited DNA gyrase.[25] This mechanism could also apply to **8** in *Leishmania*, as topoisomerase dysfunction is linked to mitochondrial activity regulation.[26]

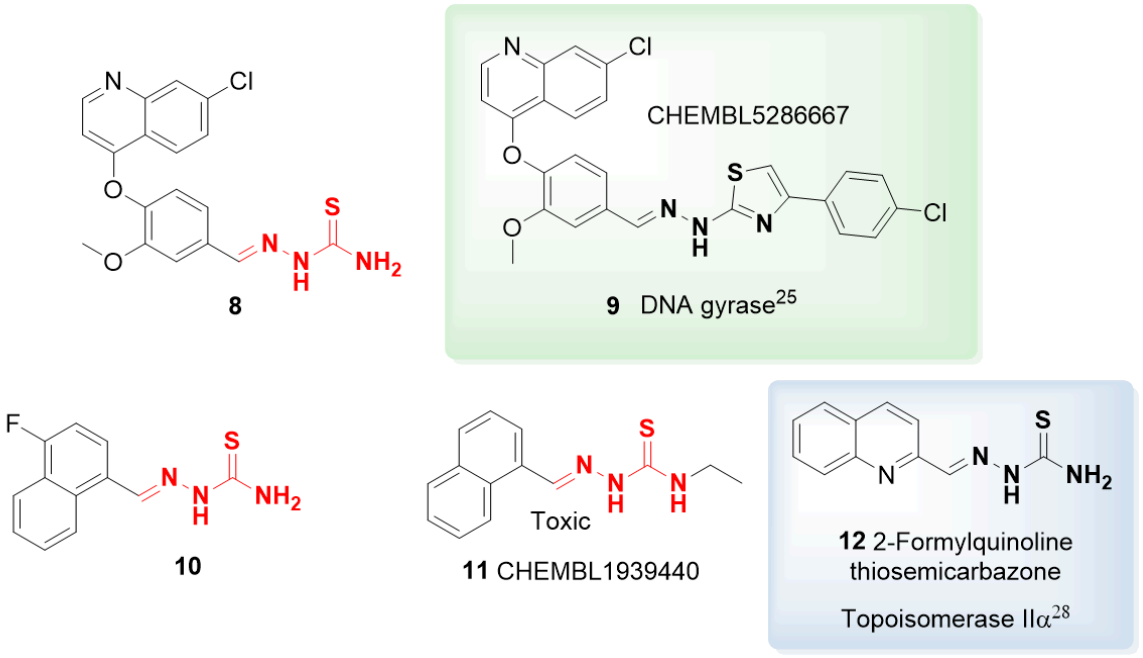

Fig 5. Compound **8** to **12**.

In another study, among 32 synthesized compounds, **10** (Fig. 5) exhibited EC_{50} values below 10 μM, with a selectivity index (SI) greater than 250, against *Leishmania donovani* amastigotes.[27] **10** is closely related to the highly toxic molecule **CHEMBL1939440** (**11**), which has shown acute oral toxicity and significant toxicity to aquatic life (European Chemicals Agency). The similarity to **2-Formylquinoline thiosemicarbazone** (**12**) is also notable, as this molecule has been found to be active in DNA biochemical processes, particularly through inhibition of Topoisomerase IIα catalytic activity.[28] These findings suggest that compounds **8** and **10** may also act on essential targets involved in the maintenance of mitotic chromosomal structure, and **10** may be toxic.

In the study, **13** (Fig. 5) demonstrated notable antifungal and antiparasitic activities, particularly against *Leishmania amazonensis*.[29] **13** was more effective against promastigotes than intracellular amastigotes, likely due to the biological barriers it must overcome to reach intracellular parasites. A related analogue **CHEMBL2007612** (**14**) (Fig. 5) has shown activity against tyrosyl-DNA phosphodiesterase 1 (TDP1) (bioassay: 686978), which plays a role in mitochondrial base excision repair, an essential process for repairing oxidative damage in mitochondrial DNA. As human and *Leishmania* TDP1 share reasonable sequence identity, this enzyme could be a promising target for leishmanial treatment strategies.[30]

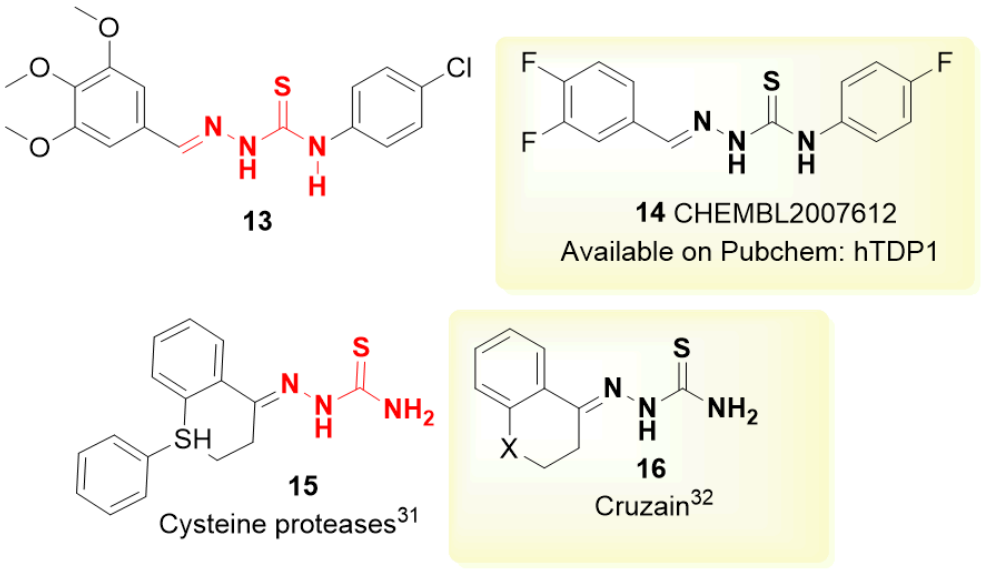

Fig 6. Compounds **13** to **16**.

Closely related hydrazone derivatives were investigated for their potential to enhance the antileishmanial activity of thiochroman-4-ones. The study evaluated the *in vitro* activity of **15** against *Leishmania panamensis* amastigotes, with a focus on identifying potent compounds with low cytotoxicity.[31] The paper suggests that cysteine proteases, such as cathepsin L, may play a role in the mechanism of action. Similar thiosemicarbazone derivatives (**16**) were tested against *T. cruzi* **cruzain**, aligning with the cysteine protease inhibition hypothesis.[32]

A series of related pyrazyl and pyridylhydrazone derivatives were investigated for their efficacy against *Leishmania amazonensis* and *Leishmania braziliensis* amastigotes.[33] These compounds are structurally similar 1-[(phenylmethylidene)amino]guanidine,[16] with the group being replaced by the 2-pyridylhydrazone moiety, maintaining similar pharmacophoric properties. **17** (Fig. 7) was found to induce reactive oxygen species (ROS) accumulation and mitochondrial membrane, leading to the disruption of energy production and apoptosis-like cell death in the parasite, without toxicity to macrophages.[33] A closely related compound, **CHEBI:120813** (**18**) (bioassay: 651718), has a confirmed target in *Leishmania*'s biochemical machinery—methionine sulfoxide reductase A (MsrA), an enzyme crucial for protecting the parasite against oxidative stress and supporting growth in macrophages.[34] This aligns with authors findings reinforcing the role of ROS and mitochondrial dysfunction in the compound's mechanism of action.

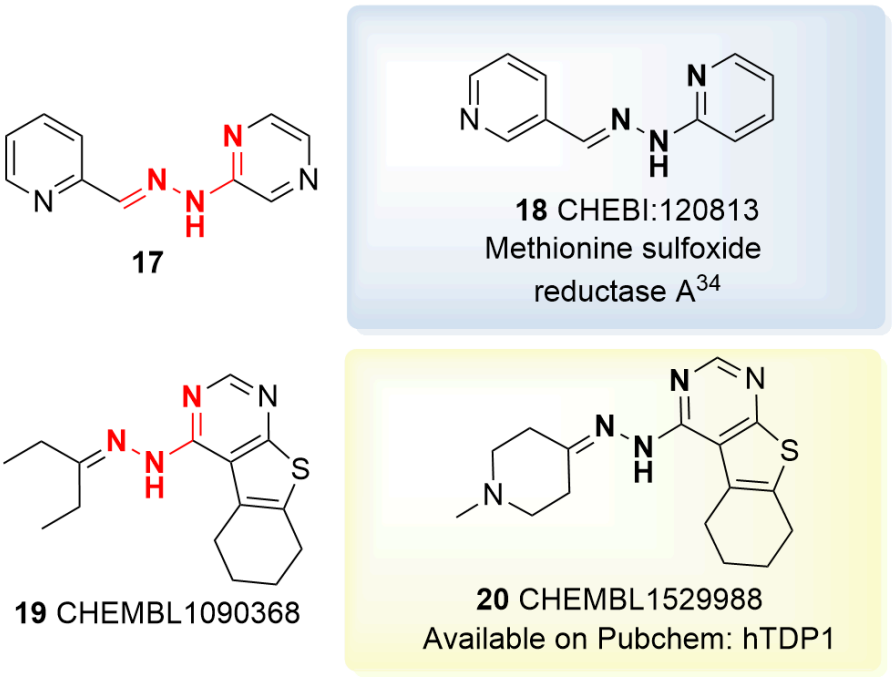

Fig 7. Compound **6** and **CHEMBL1090368** and related compounds.

Similar tetrahydrobenzothienopyrimidine compounds, including **CHEMBL1090368** (**19**), were evaluated for *Leishmania amazonensis*[35] using BALB/c mice (Fig. 7). However, the study did not provide detailed insights into its specific leishmanicidal mechanism of action. Interestingly, **CHEMBL1529988** (**20**) (Fig. 7), a closely related compound, was found to inhibit tyrosyl-DNA phosphodiesterase 1 (TDP1), a target also implicated in the action of **14** (Fig. 5). TDP1 may be a potential shared molecular target for these compounds.

Compounds **21**,[36] **22**,[37] and **CHEMBL3885202** (**23**)[38] (Fig. 8) are unique, as no structurally similar scaffolds have been tested to draw definitive conclusions about their biological targets in *Leishmania*. The authors hypothesized several biological targets for the spiro-acridine derivative **21**, including trypanothione reductase (TryR), Leishmania donovani topoisomerase I (LdTopoI), and CYP51; however, no confirmatory tests were conducted. The precise mechanism of action for compound **22** remains unconfirmed. Additionally, **23** has been reported to induce increased ROS production, cell shrinkage, phosphatidylserine exposure, and DNA fragmentation—hallmark indicators of apoptosis-like cell death in the parasite.

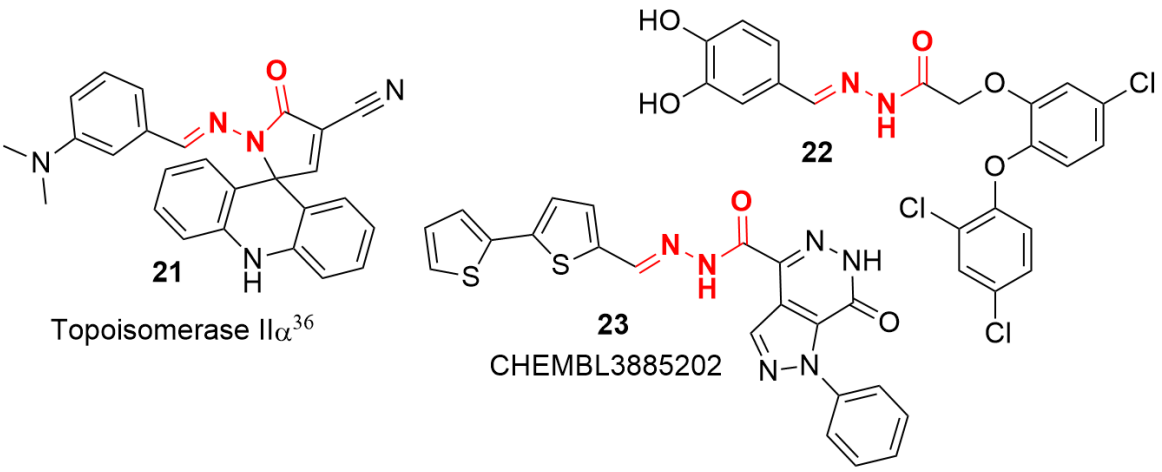

Fig. 8. Compounds **21**, **22** and **23.**

The cytotoxicity and antileishmanial activity of isoniazid-derived hydrazones and 2-pyrazineformamide thiosemicarbazones against *Leishmania braziliensis*, identifying **SR-01000204729** (**24**) (Fig. 9) as having a favorable balance between cytotoxicity and antileishmanial activity.[39] **PCIH** (**25**) (Fig. 9), which is nearly identical to **24**, is a tridentate chelator used in managing iron-overload diseases.[40] This iron interaction may be relevant in explaining **24**'s antileishmanial effect. Iron plays a critical role in host-pathogen interactions, as intracellular pathogens like *Leishmania* rely on host Fe for survival, growth, and virulence. *Leishmania* disrupts iron sequestration into ferritin by cleaving Fe-chaperones such as poly(rC)-binding proteins,[41] thereby promoting intracellular growth. It can be hypothesized that **24** may interfere with these processes, potentially inhibiting the parasite's ability to utilize host iron. However, further studies are required to confirm this proposed mechanism, though it is supported by existing literature.

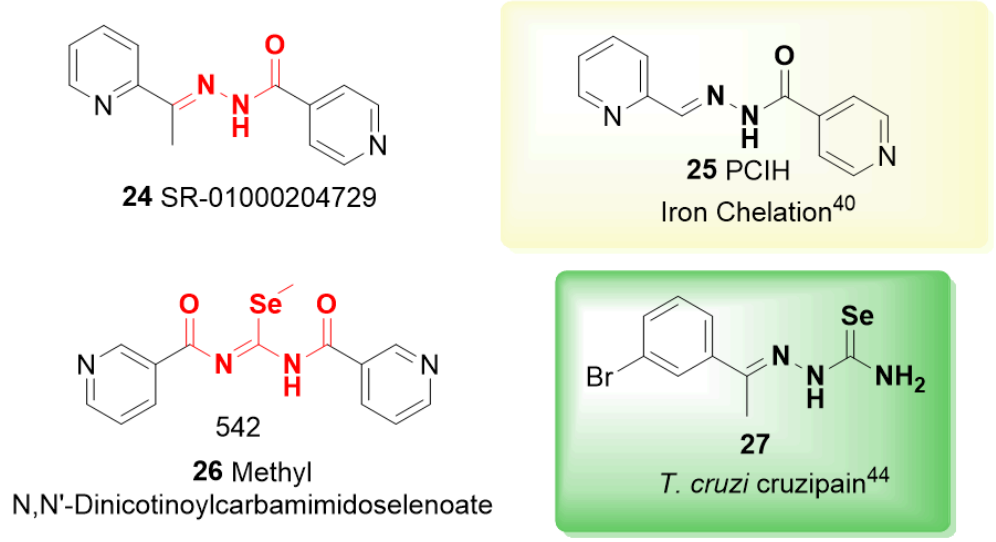

Fig. 9. Compounds **24** to **27**.

Methyl N,N'-dinicotinoylcarbamimidoselenoate (**26**) (Fig. 9), a related imidoselenocarbamate, was investigated for its antileishmanial potential against *Leishmania infantum*.[42] **26** demonstrated moderate antileishmanial activity with low toxicity to host cells, making it a promising candidate for further development. Selenosemicarbazones and similar

compounds have been extensively reviewed[43] highlighting their predominant exploration for antichagasic activity. For instance, a selenosemicarbazone **27** showed high potency against cruzipain, a crucial cysteine protease in *Trypanosoma cruzi*.[44] Given the structural similarity, cysteine peptidase A (CPA, XP_001465113.1) in *L. infantum* may serve as a closely related target, suggesting that selenosemicarbazones could potentially be their inhibitors.

A series of hybrid furoxanyl N-acylhydrazone derivatives as potential drug candidates a*Leishmania amazonensis*. Among them, **28** (Fig. 10) demonstrated superior selectivity - 50-fold higher than Amphotericin B with greater potency.[45] Its structure is closely related to oxadiazole **CHEMBL3196729** (**29**) (Fig. 10), which is active against *Leishmania mexicana* pyruvate kinase (Bioassay: 1721) , further supporting the potential of compound **28** to exhibit similar properties.

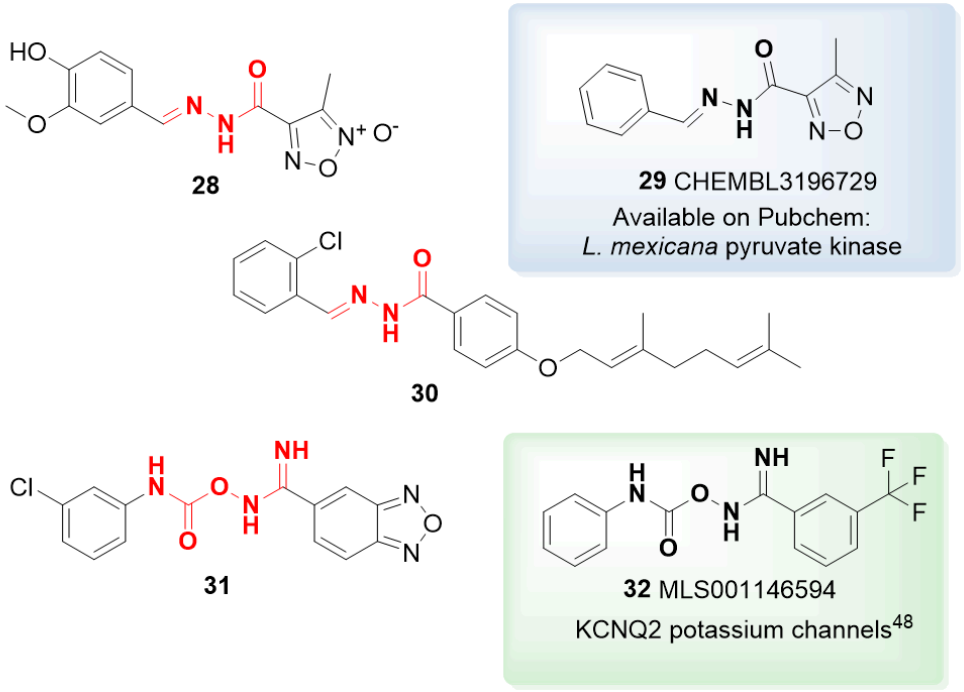

Fig. 10. Compound **28** to **32**.

The unique compound **30** (Fig. 10) was extensively evaluated for its antiprotozoal efficacy, particularly through *in vitro* assays against various parasitic protozoans, including *Trypanosoma brucei brucei*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Leishmania infantum*.[46] The compound demonstrated significant activity against *L. infantum*, positioning it as a promising candidate for antileishmanial therapy. Its structural features, especially the presence of a chloro functional group, were noted as potential contributors to its broad-spectrum activity, likely by disrupting essential metabolic or signaling pathways required for parasite survival. Notably, no closely related compounds were found in the PubChem database, suggesting the compound's novelty and potential for further exploration.

An interesting related scaffold, carboxyimidamide-substituted benzo[c][1,2,5]oxadiazoles, demonstrated promising activity, with **31** (Fig. 10) proved to be active against *Leishmania donovani*.[47] A structurally related carboxyimidamide **MLS001146594** (**32**) (Fig 10) is well known for its action on KCNQ2 potassium channels, which are also present in *L. donovani*

(CAJ1987321.1). These putative potassium channels may be involved in several cellular pathways,[48] suggesting a potential mechanism of action for **31** (Fig. 10).

Compound **32** (Fig. 11), derived from a series of 2-pyrimidinyl hydrazones, was evaluated for its activity against *Leishmania amazonensis*, with a focus on mitochondrial dysfunction and ROS production as part of its mechanism of action.[49] The study indicated that the mitochondrial membrane is a critical target, as the compound induces mitochondrial depolarization and disrupts the membrane potential. Additionally, ROS production increased following treatment, further contributing to parasite death. A closely related compound, **CHEMBL1974036** (**33**) (Fig. 11), was extensively tested (Bioassay: 624296), although no conclusive information on its mechanism of action was reported.

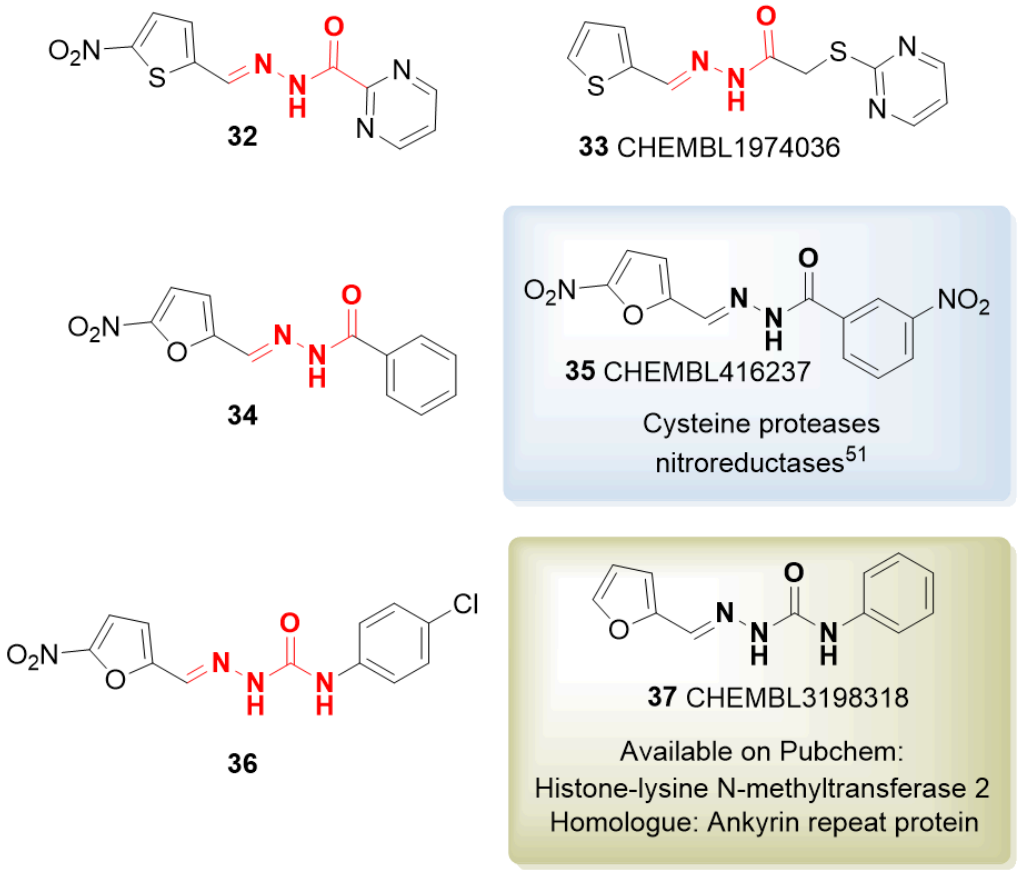

Fig. 11. Compounds **12**, **13** and **14** and analogues.

Compound **34** (Fig. 11) has an established scaffold with known antiparasitic properties against *Leishmania* promastigotes and amastigotes, continuing a long tradition of research into related structures.[50] **CHEMBL416237** (**35**), for instance, was found to have antimalarial and antichagasic activity by inhibiting cysteine proteases.[51] **34** showed IC_{50} values lower than those of the reference drugs pentamidine and amphotericin B. However, unlike **35**, the study proposed that the primary mechanism of action was the reduction of the nitro group by unspecific nitroreductases, without specifically implicating cysteine protease targets.

A related semicarbazone derivative **36** (Fig. 11) was tested *in vitro* and *in vivo* against *Leishmania amazonensis* and *Leishmania braziliensis* amastigotes. It exhibited potent leishmanicidal activity, significantly reducing lesion size in BALB/c mice with intraperitoneal

administration.[52] The authors suggested that the mechanism of action involves mitochondrial dysfunction, induction of apoptosis through caspase-like activity, and autophagy. The compound was reported to cause mitochondrial membrane depolarization and activate apoptosis-like pathways in Leishmania. A conjectural molecular target may involve a mechanism similar to that of **CHEMBL3198318** (**37**) (Fig. 11), which was tested on human euchromatic histone-lysine N-methyltransferase 2 (Bioassay: 504332) and has a counterpart in Leishmania, the **ankyrin repeat protein** (XP_003723231.1).

Nitrofurazone (**38**) (Fig. 12) is an older compound known for its good activity against amastigotes.[53] **38** likely exerts its antileishmanial activity through a mechanism similar to its action in bacteria, undergoing enzymatic reduction, potentially mediated by a leishmanial reductase. This reduction process converts nitrofurazone into reactive intermediates which can covalently bind to proteins and possibly nucleic acids, leading to damage to essential cellular macromolecules. The reduction process relies on the presence of NADPH or NADH to facilitate nitrofurazone's activation.[54] Inspired by nitrofurazone, compounds **39** and **41** (Fig 12) were evaluated for their in vitro antileishmanial activity against *Leishmania donovani* and *Leishmania major*, targeting both promastigote and amastigote forms.[55] Compounds **39** and **41** exhibited significantly better activity against *Leishmania donovani* amastigotes compared to the parent drug, **nitrofurazone**, demonstrating success in optimizing antileishmanial drug design. These findings highlight their potential as promising candidates for further development in the treatment of leishmaniasis.[55]

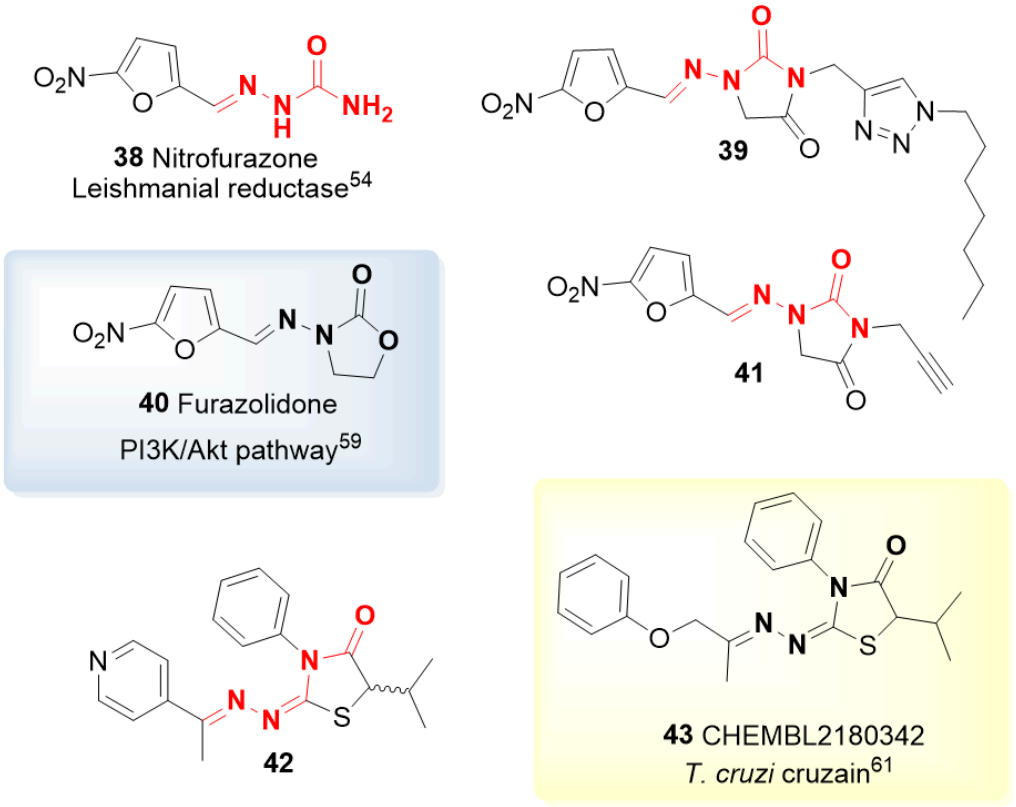

Fig. 12. Compounds **38** to **43**.

Furazolidone (**40**) (Fig. 12), an classical antibacterial drug,[56] was evaluated for antileishmanial activity against several *Leishmania* species, focusing particularly on

Leishmania chagasi. **40** displayed potent activity against *L. chagasi* intracellular amastigotes, although it exhibited cytotoxicity at higher concentrations. The drug induced mitochondrial swelling, vacuolization, and nuclear damage, leading to the loss of intracellular organelles and parasite death.[57] This activity is attributed its ability to induce apoptosis through a reactive oxygen species (ROS)-dependent mitochondrial signaling pathway and suppression of the PI3K/Akt pathway.[58] While this mechanism can result in undesirable cytotoxic effects, the development of safer analogues could improve its therapeutic profile, making it a promising lead for new antiparasitic drug development. **40** was identified as loosely electroshape-related to compound **42**[59] that displayed promising activity against *L. amazonensis* amastigotes. Compound **42** shared structural similarity with **CHEMBL2180342** (**43**), which exhibited significant activity against *Trypanosoma cruzi* through cruzain inhibition.[60] This similarity suggests that the inhibition of cysteine proteases in *L. amazonensis* may similarly contribute to the observed activity.

Compound **44** (Fig. 13), a 4-nitrobenzaldehyde thiosemicarbazone derived from S-limonene, against *Leishmania amazonensis*. It exhibited greater toxicity toward the parasite than toward mammalian J774A1 macrophages, inducing significant ultrastructural changes, including mitochondrial swelling, disorganization of the inner mitochondrial membrane, accumulation of lipid bodies, and cytoplasmic vacuolization.[61] The same research group also investigated a related limonene-acylthiosemicarbazide hybrid, compound **45** (Fig. 13), which demonstrated the most potent antiproliferative activity and a higher selectivity index for intracellular amastigotes than **44**. **45** primarily targeted the Golgi complex of the parasite, causing structural disorganization and vesiculation in the flagellar pocket. These ultrastructural changes suggest that the compound may interfere with lipid biosynthesis and secretion pathways.[62] Compound **CHEMBL3235021** (**46**) (Fig. 13), a structurally related thiosemicarbazone, exhibited excellent anticancer properties, making it one of the most relevant analogue in the literature.[63] Numerous antiproliferative properties are attributed to the thiosemicarbazone class and are listed on PubChem. Uncovering the precise mechanism of action of these derivatives could pave the way for the development of potent antiparasitic drugs as well.

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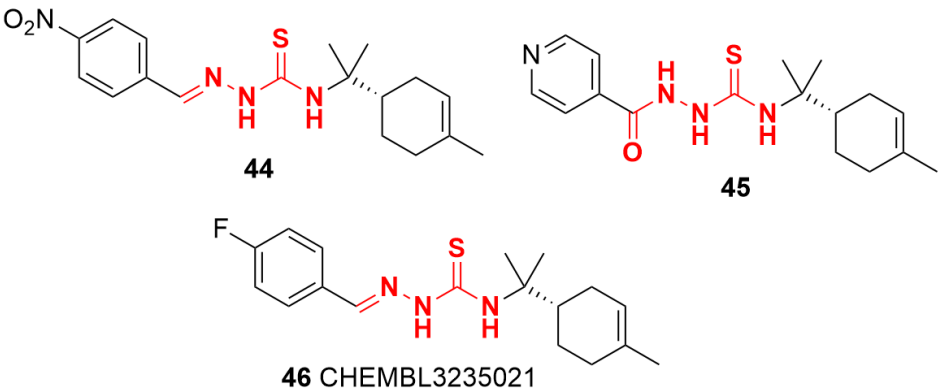

Fig 13. Compound **44**, **45** and related **CHEMBL3235021** (**46**).

Compound **47** (Fig. 14), a phthalazine derivative and closed-ring N'-(furan-2-ylmethylideneamino) benzenecarboximidamide, was tested *in vitro* for its antileishmanial activity against *Leishmania braziliensis*.[64] This compound, a closed-ring structure analogue similar to compounds **17** (Fig. 11)[33] and **46**[35] (Fig. 14), is thought to exert

its effects through oxidative stress and mitochondrial dysfunction. It induces oxidative stress in the parasite, leading to impaired mitochondrial dehydrogenase activity. Although superoxide dismutase (SOD) was initially proposed as a target, molecular docking studies revealed weak interactions with this enzyme, suggesting that the compound may act through alternative mechanisms.[64]

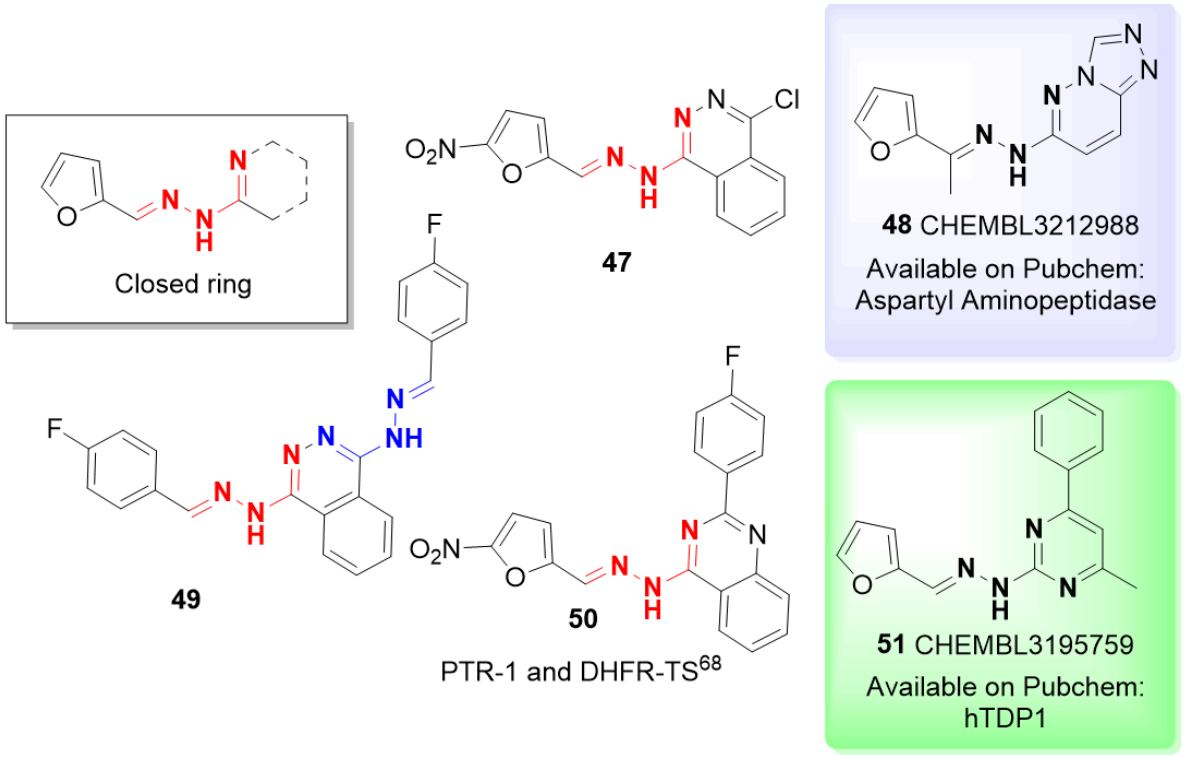

Fig. 14. Closed ring aminoguanidine-hydrazones analogues, **47** to **51**.

Compound **49** (Fig. 14), a 1,4-bis(substituted benzalhydrazino)phthalazine derivative, was tested against *Leishmania braziliensis* and *Leishmania mexicana*.It was evaluated using murine macrophages, and molecular docking studies were conducted to investigate potential interactions with superoxide dismutase (SOD).[65] **49** showed promising results, exhibiting better antileishmanial activity against *L. braziliensis* compared to the reference drug **Glucantime**. However, enzymatic inhibition assays for SOD demonstrated poor inhibitory activity for the active compounds, confirming that SOD inhibition is not the mechanism of action. **CHEMBL3212988** (**48**) (Fig. 14), a structurally similar compound to **47** and **49**, is known to inhibit *Plasmodium falciparum* M18 Aspartyl Aminopeptidase (PFM18AAP). However, a homologous target in *Leishmania* (XP_001566576.1) shows low sequence identity, making it a less likely candidate for activity in *Leishmania*, and thus a distant possibility.[66]

Compound **50** (Fig. 14) was proposed to target folate pathways in *Leishmania*, with mechanistic assays showing interactions with pteridine reductase 1 (PTR1) and dihydrofolate reductase-thymidylate synthase (DHFR-TS) in *Leishmania infantum*.[67] A closely related analogue, **CHEMBL3195759** (**51**) (Fig. 14), demonstrated similar activity, particularly against TDP1 in human cells.[68] TDP1 repeatedly emerges as a promising target for the revised compounds, being a promising one due to its critical role in repairing oxidative damage in mitochondrial DNA.[69]

Benzylidene-hydrazineyl-thiazole **52** (Fig. 15) can be considered part of the same closed-ring analogue, now incorporating a bioisosteric thiazole ring. This compound induced reactive oxygen species (ROS) and nitric oxide (NO) production, which led to apoptosis-like death in *Leishmania* amastigotes. The compounds triggered the externalization of phosphatidylserine, a hallmark of apoptosis, in treated parasites. *In vivo*, compound **52** demonstrated a 73% reduction in parasite load in the spleens of infected hamsters.[70] It shares remarkable structural similarities with the antifilarial chalcone-thiazole derivative **CHEMBL3260651** (**53**), which exhibited promising activity against *Brugia malayi*, demonstrating 100% embryostatic effects and moderate microfilaricidal activity in *in vivo* models.[71] However, the precise mechanism of action for **53** remains unknown. Further research is needed to clarify the exact mechanisms by which these compounds exert their antiparasitic activities.

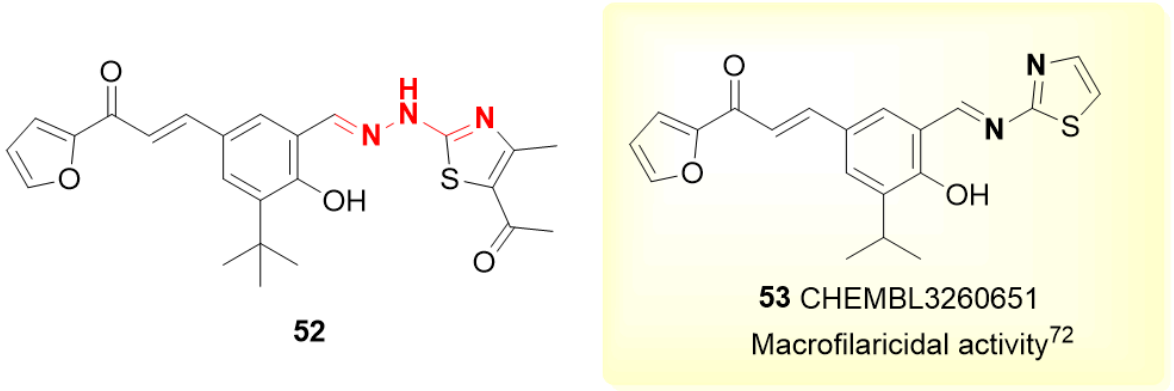

Fig. 15. Compound **52** and analogue **CHEMBL3260651** (**53**).

The structurally related compounds **54**[72] and **55**[73] (Fig. 16) demonstrated potent activity against both promastigotes and amastigotes of *Leishmania infantum*. These compounds caused significant morphological changes in the parasites, including mitochondrial swelling, cellular disorganization, and direct structural damage, nearly eliminating macrophage infection at the highest tested concentration (1 mg/mL). **56** (Fig. 16), a branched analogue of **54** and **55**, also showed strong antileishmanial effects against *L. major* promastigotes and amastigotes, with potency approximately six times greater than the standard drug **Glucantime**.[74] These compounds share structural similarities with **CHEMBL3194563** (**57**) (Fig. 16), which has been tested across multiple biological assays, showing activity against malarial parasites, *T. cruzi* replication. TDP1 emerged again as a possible target for these compounds.[68]

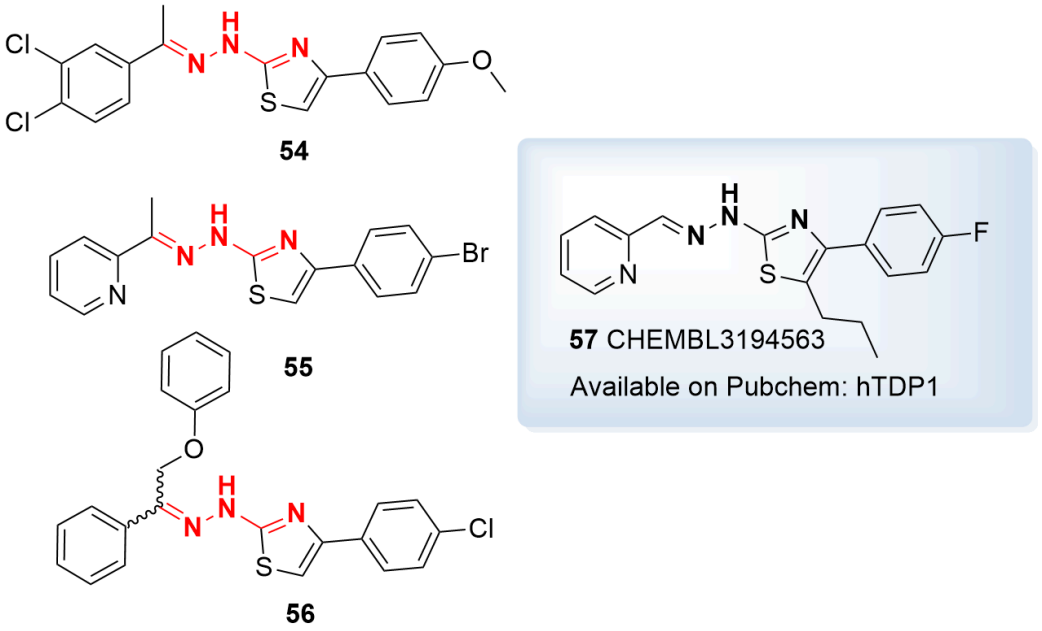

Fig 16. Compounds **54**, **55** and **56** and related **CHEMBL3194563** (**57**).

In a study investigating the antileishmanial activity of compounds from the Medicines for Malaria Venture (MMV) Malaria Box collection against intracellular *Leishmania major* amastigotes, compound **MMV666023** (**58**) emerged as active in an intracellular assay using luciferase-expressing parasites to measure proliferation.[75] As with typical screening protocols, no specific molecular targets were investigated at this stage, leaving the mechanisms of action for **58** unexamined. However, in another study, **58** was evaluated for its potential to inhibit *Plasmodium falciparum* deoxyhypusine hydroxylase (DOHH), an enzyme critical for the biosynthesis of hypusine, a modification essential for parasite survival.[76] *Leishmania donovani* deoxyhypusine synthase (DHS), identified as an essential enzyme for the parasite survival.[77] This enzyme catalyzes the first step in the post-translational modification of eukaryotic initiation factor 5A (eIF5A). DHS34, specifically, plays a vital role in *L. donovani* survival, and due to its structural differences from human DHS, it presents a potential drug target. Given **58**'s activity and the essential role of DHS34, it is plausible that it may bind to this target, providing a direction for further investigation.

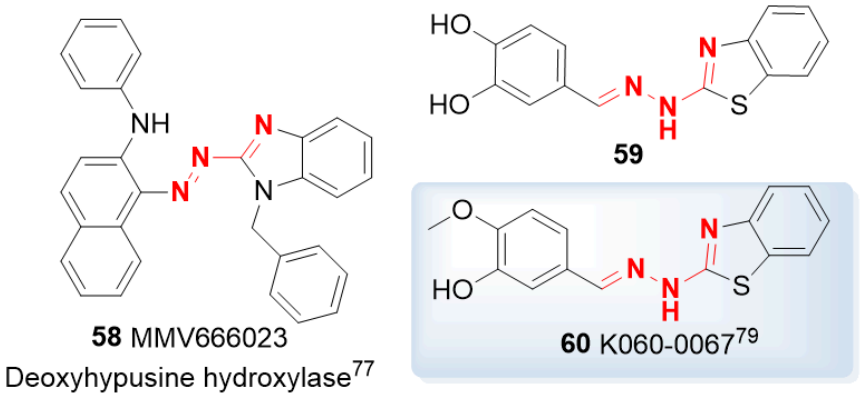

Fig 17. **MMV666023** (**58**) and **59** and **K060-0067** (**60**).

Benzothiazole derivative **59** (Fig. 17) demonstrated significant efficacy against both the promastigote and intracellular amastigote forms of *Leishmania amazonensis*, surpassing the reference drug miltefosine in performance.[78] Further investigation into its mechanism of action revealed that **59** induced mitochondrial dysfunction, leading to the depolarization of the mitochondrial membrane potential without triggering ROS production. Additionally, **59**'s analogue, **K060-0067** (**60**), was included in a chemogenomic screening of 188 synthetic compounds, aimed at identifying bioactive substances.[79] Although **60** exhibited extensive bioactivities and appeared to interact with specific genetic pathways in yeast models, particularly through gene deletion assays that mapped sensitivities and resistances, its exact molecular targets and mechanism of action were not fully defined, highlighting the need for further studies.

4. Overview of Relevant Targets Reported for Parent Compound and Close Analogues

Compounds **13**[29]**, 19**[35] (Fig. 7); **50**[68] (Fig. 14); **54**[72]**, 55**[73] and **66**[74] (Fig. 16) have closely related compounds known to inhibit human TDP1. In *Leishmania donovani*, TDP1 plays a crucial role in repairing DNA damage caused by topoisomerase I inhibitors, such as **camptothecin**, by removing covalent topoisomerase I-DNA complexes, which would otherwise result in DNA fragmentation and cell death.[68] Due to its key role in DNA repair, LdTDP1 is an attractive target for the development of new inhibitors designed to disrupt the DNA repair mechanism in *Leishmania*.[30,68] This hypothesis may be explored for the design of potent, selective inhibitors targeting Leishmania TDP1.

Compounds **8**[24] (Fig. 5); **17**[33] (Fig. 7); **23**[38] (Fig. 8); **32**[49], **36**[52] (Fig. 11); **38**[55], (Fig. 12) , **40**[58] (Fig. 12); **44**[61] (Fig. 13); **47**[64] (Fig. 14); **52**[70] (Fig. 15); **54**[72]**, 55**[73] (Fig. 16) and **59**[78] (Fig. 17) have been demonstrated by their respective authors to disrupt key mitochondrial features, potentially involving (ROS)-dependent mitochondrial signaling pathways, such as those mediated by methionine sulfoxide reductase A[34]. Some of these disruptions may be linked to DNA biochemical processes, particularly through the inhibition of topoisomerase activity[28] DNA gyrase[25], ankyrin repeat protein (XP_003723231.1) or TDP1 inhibition.[68] Proving these assumptions may lead to the discovery of new potent mitochondrial disruptor compounds.

Cysteine proteases play a pivotal role in both *Leishmania* and *Trypanosoma cruzi*, making them valuable targets for drug discovery despite structural differences between the cathepsin-like proteases in *Leishmania* and cruzain in *T. cruzi*. Compound **42**[59] (Fig. 12) is active against *Leishmania* and closely resembles **43**[60], which has demonstrated significant activity against *T. cruzi* through cruzain inhibition. Selenium-containing compounds, such as **26**[42], have shown antileishmanial potential, while selenosemicarbazones (like **27**[44]) are recognized for their antichagasic activity as cruzain inhibitors. Related thioflavanone **15**[31] is thought to bind to cathepsin L in *Leishmania*. Although binding between these proteases across species may not be directly relatable, it may aid in designing inhibitors with cross-species properties, warranting further exploration and validation.

In conclusion, while many of the reviewed compounds exhibited significant antileishmanial activity, particularly against intracellular amastigotes, most mechanisms of action remain to

be fully elucidated. Future work should focus on identifying precise molecular targets, especially those involved in mitochondrial functions, oxidative stress pathways, and cysteine protease inhibition. This understanding will be crucial in advancing these promising compounds from in vitro efficacy to viable therapeutic candidates.

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