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Original article

Synthesis and anti-protozoal activity of novel dihydropyrrolo[3,4-d][1,2,3]triazoles

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ABSTRACT

1,2,4-Oxadiazole and 1,2,3-triazole containing heterocyclic compounds continue to gain interest in synthesis of chemical entities and exhibit various biological activities as anti-protozoal and anti-cancer agents. By using the principle of bioisosterism, a series of novel oxadiazolyl pyrrolo triazole diones; namely, (3a*S*,6a*R*)-1-((3-(4-substituted phenyl)-1,2,4-oxadiazol-5-yl)methyl)-5-phenyl-1,6a-dihydropyrrolo[3,4-d][1,2,3] triazole-4,6(3a*H*,5*H*)-diones (**5a**–**k**) was designed and synthesized by the 1,3-dipolar cycloaddition reaction of novel 5-azidomethyl 3-aryl substituted 1,2,4-oxadiazoles (**4a**–**k**) with *N*-phenyl maleimide. The structures of all the cycloadducts were elucidated by means of spectroscopic methods and physical characteristics. The *in vitro* anti-protozoal and cytotoxic activities of these novel heterocyclic compounds were investigated.

1. Introduction

Vector-borne infectious parasitic diseases, caused by single-cell protozoans and transmitted to human by the bite of arthropod vectors constitute a significant proportion of the global infectious disease burden. Malaria, the most significant parasitic disease worldwide transmitted to human by mosquito bites is due to several *Plasmodium* species. The World Health Organization (WHO) reports around 300 million new cases, and around one million deaths due to *falciparum* malaria each year [1]. Human African trypanosomiasis, or sleeping sickness is ranked ninth out of 25 human infectious and parasitic diseases in Africa [2]. The acute form of the disease in East Africa is established by *Trypanosoma brucei rhodesiense* and transmitted to human by an infected tsetse fly, which can be fatal within months or even weeks [3]. Chagas disease, caused by the flagellate protozoan *Trypanosoma cruzi* and transmitted to human by triatomine bugs, is endemic in Latin America. An estimated 10 million people are infected worldwide and more than 25 million people are at risk of the illness. It is estimated that in 2008 Chagas disease killed more than 10,000 people [4]. Leishmaniasis is caused by parasitic protozoa of the genus *Leishmania*. The human infections occur via the bite of phlebotomine sandflies, which breed in forest areas, caves, or the burrows of small rodents. Of the four main types of the disease, visceral leishmaniasis (kala azar), caused principally by *Leishmania donovani*, *Leishmania infantum*, and *Leishmania chagasi*, is the most life-threatening form and if left untreated, can have a fatality rate as high as 100% within two years [5]. There is currently no long-term effective vaccine for the prophylaxis of these neglected diseases and the arthropod vectors have developed resistance to the existing insecticides. Moreover, the available antiparasitic drugs are far from ideal and hampered by safety, resistance, stability, efficacy and cost issues. The urgent need for effective anti-protozoal drugs has stimulated the search for new compounds, of both natural and synthetic origin, with potential clinical utility.

1,2,4-Oxadiazole derivatives are important heterocyclic compounds that have been used as heterocyclic amide and ester bioisoster in the synthesis of peptide building blocks and in the formation of dipeptidomimetics. This class of compounds also receive research attention due to their various bioactivities, such as tyrosine kinase inhibition, muscarinic agonism, histamine H3 antagonism, anti-inflammatory, antitumoral and monoamine

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oxidase inhibition [6–9]. Azides are also very important precursors for the preparation and synthesis of heterocyclic aromatic compounds which can find various applications both in industry, medicine and pharmacy. For example, azidothymidine (zidovudine), an azidonucleoside, are used in treatment of AIDS and azapride, azidamphenicol, azidomorphine are utilized as pharmaceuticals (Scheme 1) [10–17]. Triazoles which can be derived from azides find increasing attraction in chemistry, biology, and medicine due to their stable metabolism, high selectivity and less adverse reaction. They were widely used as antifungal, anticonvulsant agents and especially popular in designing anti-cancer agents (Scheme 1), e.g. compound (**a**) as aromatase inhibitor in estrogen-dependent breast cancer; compound (**b**) as epithelia proliferation inhibitor and compound (**c**) as Oophoroma cell suppressant [18–22].

The oxadiazole heterocycle is well known for its antiparasitic properties for some time [23]. Due to their chemical stability and ability to act as an amide bioisoster, the oxadiazole structure is emerging as a valuable scaffold for anti-protozoal drug design [23,24]. For example some 3-(4-substituted-aryl)-1,2,4-oxadiazoles were found to be as potent as the reference drug, benznidazole against *T. cruzi* [24]. In mice, the 3-aryl-5-thiocyanatomethyl-1,2,4-oxadiazoles decrease parasitemia caused by *L. donovani* through both i.v. and oral routes [25]. Dicationic triazoles show anti-protozoal activities against *T. rhodesiense* and *Plasmodium falciparum* as potent as the reference drugs artemisinin and melarsoprol, *in vitro* and *in vivo* [26]. Also triazoles and pyrroles of synthetic or natural origin have been reported to have promising anti-protozoal activities [27–29]. Taking account of the above considerations, plus our continuous interest in 1,3-dipolar cycloaddition chemistry [30–32] and discovery of novel anti-protozoal agents, we have focused on the synthesis of the various 5-azidomethyl-3-aryl substituted-1,2,4-oxadiazol compounds starting from 5-chloromethyl 1,2,4-oxadiazoles. These azides were then converted to target molecules (**5a**–**k**) by 1,3-dipolar cycloaddition of *N*-phenylmaleimide. Because the novel cycloadducts obtained in this study (**5a**–**k**) carry all these 1,2,4-oxadiazole, triazole and pyrrole substructures, we expected the overall structures to show anti-protozoal activities. Hence, their growth inhibitory activity was assessed against a small panel of parasites, i.e. *T. b. rhodesiense*, *T. cruzi*, *L. donovani* and *P. falciparum*. Selective toxicity of all compounds was determined against L6 cells, a primary cell line derived from rat skeletal myoblasts.

2. Results and discussion

2.1. Chemistry

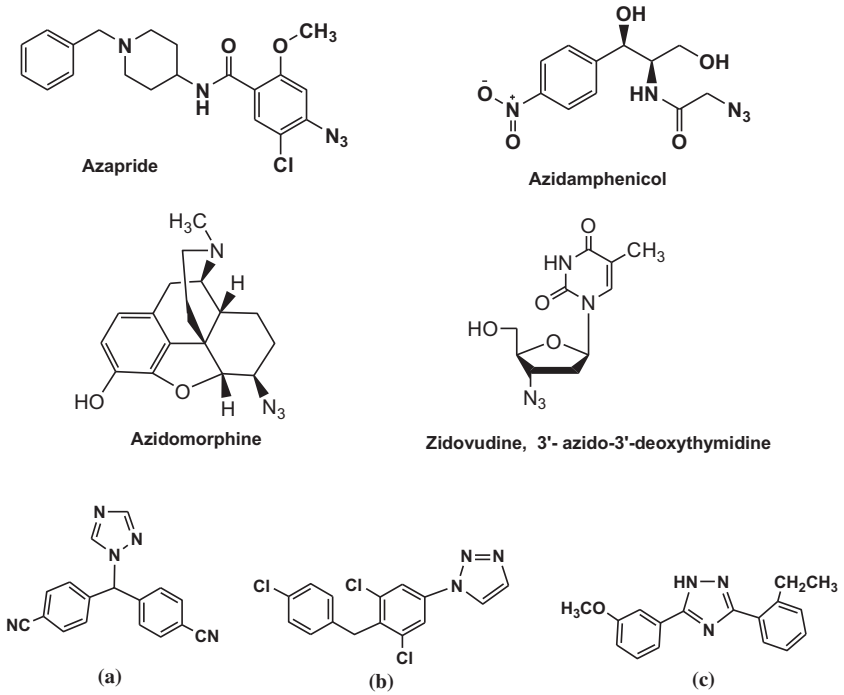
2.1.1. Synthesis of 5-(chloromethyl)-3-(4-substituted phenyl)-1,2,4-oxadiazoles (*3a*–*k*)

The aromatic monoamidoximes **2a**–**k** obtained from corresponding nitriles and hydroxyl amine were treated with chloroacetyl chloride at the reflux temperature of benzene in the presence of triethylamine to give 5-(chloromethyl)-1,2,4-oxadiazoles **3a**–**k** in excellent yields and identified by means of spectral/physical data (Scheme 2) [33].

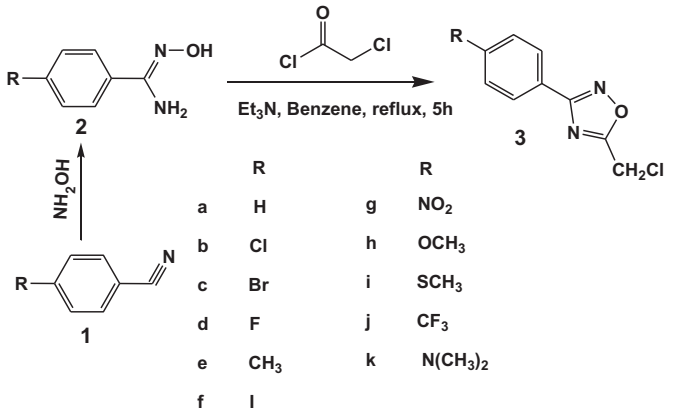
In addition to the synthesis and identification of the chloromethyl oxadiazoles, a linear Hammett correlation has been established between the carbon chemical shifts of the number three carbon of oxadiazole ring and Hammett σ_p substituent constants. As shown in Fig. 1, electron-withdrawing subtituents, CF_3 and NO_2 are positioned more downfield while the electron-donating subtituents, namely, OMe, NMe_2 and Me lie less downfield of the plot. Electron-withdrawing groups move the carbon chemical shifts to the more deshielded region while electron-releasing groups cause shielding. Due to the both inductive and mesomeric effects, the impact of halogens in deshielding or shielding is not significant, however, they still appear to cause deshielding when compared to that of electron-donating groups.

2.1.2. Synthesis of 5-(azidomethyl)-3-(4-substituted phenyl)-1,2,4-oxadiazoles (*4a*–*k*)

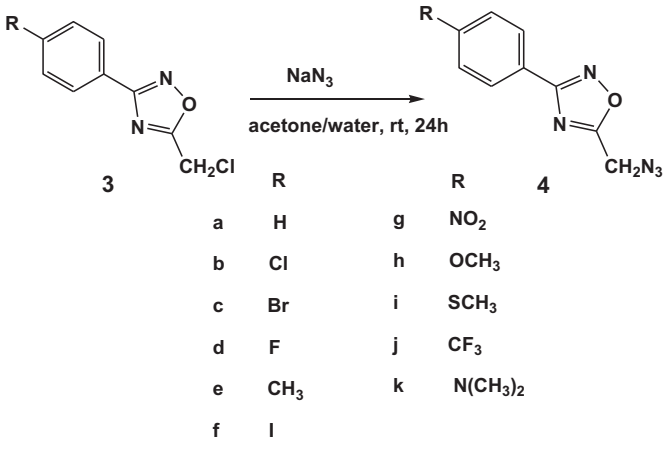
5-(Chloromethyl)-1,2,4-oxadiazoles **3a**–**k** were reacted with sodium azide at room temperature in acetone/water mixture to give 5-(azidomethyl)-1,2,4-oxadiazoles **4a**–**k,** all of which are novel



Scheme 1. Some important bioactive molecules carrying azide and triazole functionalities.



Scheme 2. Synthesis of 5-(chloromethyl)-1,2,4-oxadiazoles **3a**–**k**.



Scheme 3. Synthesis of 5-(azidomethyl)-1,2,4-oxadiazoles **4a**–**k**.

compounds (Scheme 3) [34]. When the reaction was carried out in DMSO the yields have been found to be quite low (Table 1). The 5-(azidomethyl)-1,2,4-oxadiazoles (**4a**–**k**) were identified by IR, NMR, Mass spectra, m.p., and R_f characteristics. The primary indicative proof for the generation of the 5-(azidomethyl)-1,2,4-oxadiazole is the appearance of the N=N=N absorption between 2100 and 2270 cm^{-1} in the IR spectrum. The mass spectrum is the another evidence of the 5-azidomethyl-1,2,4-oxadiazoles. All of the compounds showed corresponding molecular ions $[M]^+$ as base peaks.

In addition to the synthesis of azidomethyl-1,2,4-oxadiazoles, we also attempted to make a linear correlation between the Hammett σ_p substituent constants and carbon chemical shifts of the number three carbon of the oxadiazole ring. As shown in Fig. 2, there is a linear correlation between these values; the electron-withdrawing substituents, CF_3 and NO_2 lie less downfield, while the electron-donating substituents; OMe, NMe_2 and Me lie more downfield area of the plot. Electron-withdrawing groups cause to shift the carbon chemical shifts to the more deshielded region while electron-releasing groups cause to shift somewhat shielded positions as similarly found in the case of chloromethyl oxadiazoles.

*2.1.3. Synthesis of 3aS,6aR)-5-phenyl-1-((3-(4-substituted phenyl)-1,2,4-oxadiazol-5-yl)methyl)-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-diones (**5a**–**k**)*

To the best of our knowledge, there are many examples of cycloaddition reactions of organic azides with alkenes, but, however, organic azides **4a**–**k** bearing 1,2,4-oxadiazole ring have not been reported previously. The 5-(azidomethyl)-1,2,4-oxadiazoles **4a**–**k** were subjected to 1,3-dipolar cycloaddition reactions with the electron-deficient dipolarophile, *N*-phenyl maleimide, in benzene under reflux to afford eleven novel 1,2,4-oxadiazolo-1,6a-dihydropyrrolo[1,2,3]triazole-(3,5)-diones **5a**–**k** in good yields (Scheme 4).

Chemical structures of 1,6a-dihydropyrrolo[1,2,3]triazole-(3,5)-diones **5a**–**k** were identified by IR, NMR, both low and high resolution MS spectra, m.p. and R_f characteristics. The primary indication for the generation of the 1,6a-dihydropyrrolo[1,2,3]triazole-(3,5)-dione is the disappearance of the N=N=N absorption of the corresponding azides **4a**–**k** at the range of 2119- 2098 cm^{-1} and the appearance of the strong C=O absorption between 1725 and 1705 cm^{-1} in the IR spectrum. The carbon chemical shifts in the ^{13}C NMR spectra appeared as four different carbons between δ 160–180 ppm provide strong support for the structures of the cycloadducts 1,6a-dihydropyrrolo[1,2,3]triazole-(3,5)-diones **5a**–**k**. Two of them were assigned as two carbonyl C atoms between δ 180-170 ppm. The other two carbon signals were assigned the iminic carbons; C=N on the oxadiazole ring, one of which is closer to the oxygen resonates at lower field region in comparison to the carbon of azomethine attached to the phenyl ring, *ca* δ 170 ppm. In addition, in the ^1H NMR spectra, the H_a and H_b protons which are expected to appear as one doublet signal, have been observed as doublet of doublets at around δ 4.0–6.0 ppm. Because of N=N of the [1–3] triazole ring, H_a is the more deshielded than H_b. Therefore, H_a chemical shift appears at approximately δ 6.0 ppm and H_b chemical

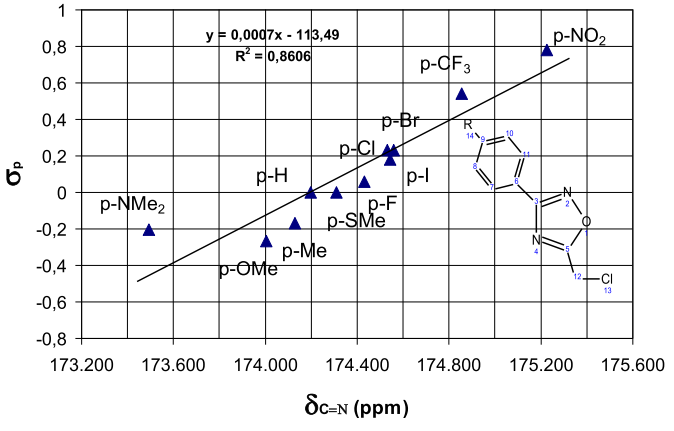


Fig. 1. Hammett correlation of imine carbon (number 3) chemical shifts of chloromethyl 1,2,4-oxadiazoles **3a**–**k** and σ_p substituent constants.

Table 1
Optimization of the preparation of azides **4a**–**k**.

Compound	*R*	Yield (%) in DMSO	Yield (%) in acetone/water mixture
4a	H	55	99
4b	Cl	34	100
4c	Br	25	100
4d	F	47	100
4e	CH_3	41	97
4f	I	12	73
4g	NO_2	26	99
4h	OCH_3	43	100
4i	SCH_3	52	100
4j	CF_3	64	93
4k	$N(CH_3)_2$	55	99

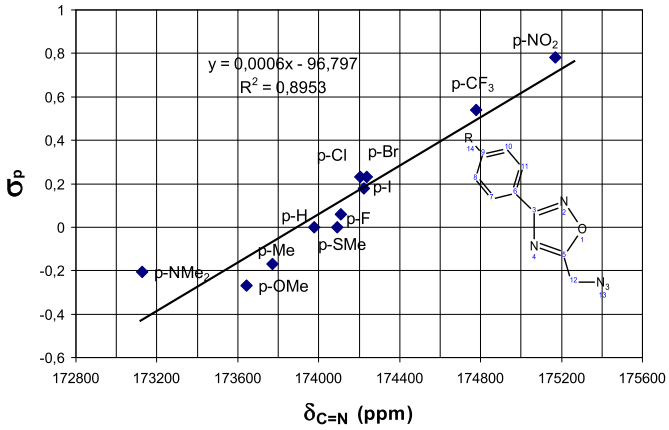
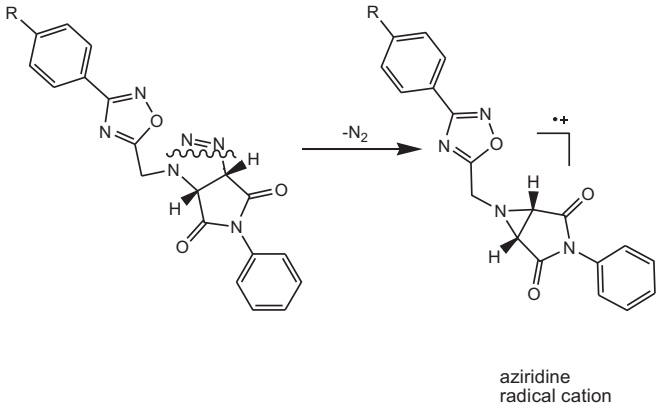


Fig. 2. Hammett correlation between Hammett σ_p substituent constants and carbon chemical shifts of the 1,2,4-oxadiazole ring of the azides **4a**–**k**.



Scheme 5. The mass spectral fragmentations of the compounds **5a**–**k**.

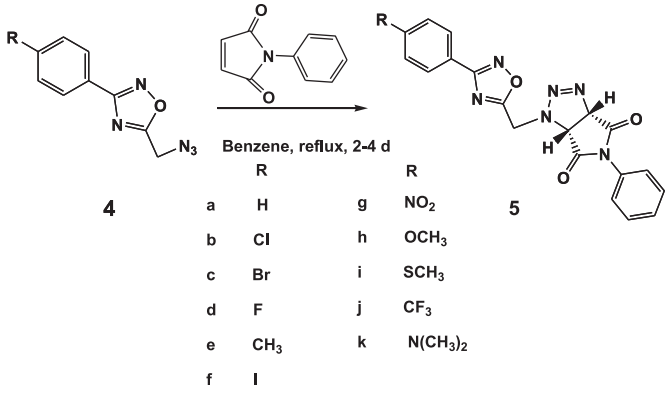
shift appears at approximately δ 4.0 in the ^{1}H NMR spectra. The methylene protons of 5-(chloromethyl)-1,2,4-oxadiazole and 5-(azidomethyl)-1,2,4-oxadiazole appear as a singlet signal, but the methylene protons of cycloadducts appear as doublets (d) or quartets (q) depending on solvent used for running NMR spectra. In $CDCl_3$+DMSO-d_6 mixture, methylene protons appear one doublet of doublet (dd) signal whereas methylene protons appear as two doublet (d) signals in the $CDCl_3$. Assignment of these chemical shifts and patterns are in accordance with the literature values reported for a similar cycloaddition reaction product different azide derivative has been used to react with *N*-phenyl maleimide [35].

On investigation of the mass spectra of these novel heterocycles showed some interesting features. Peaks related to the molecular ions $[M]^+$ of the cycloadducts were not observed in the mass spectra. But, instead, we have found major peaks at m/z $[M - N_2]^+$, which can easily be attributed to the loss of N_2 from the parent molecules, and depending on the running conditions there are also the peaks related to the clusters of $[M + K]^+$, and $[M + MeCNH]^+$ in the TOF AP^+ and TOF ES^+ MS (Scheme 5). All experimental data show that the reaction between 5-(azidomethyl)-1,2,4-oxadiazole and *N*-phenyl maleimide yields only *endo* products.

2.2. Bioactivity assessments

In vitro anti-protozoal activity of synthesized dihydropyrrolotriazoles (**5a**–**k**) was determined against *T. b. rhodesiense* (bloodstream forms), *T. cruzi* (intracellular amastigotes in L6 rat skeletal myoblasts), *L. donovani* (axenic amastigotes) and *P. falciparum* (blood stage forms of multi-drug resistant K1 strain). Interestingly, all compounds showed activity against all four parasitic species with variable potencies (Table 2). The most active compound against *T. b. rhodesiense* was **5a** (IC_{50} value 7.0 μg/mL), followed by **5e** and **5g** with similar IC_{50} values (8.4 and 8.0 μg/mL). The remaining compounds exhibited moderate activities with IC_{50} values ranging between 12.3 and 18.8 μg/mL. The growth inhibitory potential was only modest towards the American trypanosomes (*T. cruzi*) and the IC_{50} values were within the range of 36.6–62.4 μg/mL. *L. donovani* appeared to be the most susceptible protozoan towards the triazoles synthesized herein. The parent compound **5a**, as well as **5g** and **5e** appeared to be equally active against this parasite (IC_{50} values 1.6, 2.0 and 2.2 μg/mL, respectively). The remaining triazoles were also significantly potent with IC_{50} values within the range of 3–4 μg/mL. Only moderate activity was observed against drug-resistant *P. falciparum* strain, where **5e**, **5g** and **5j** displayed the best antimalarial potential (IC_{50} values between 13.2 and 15.0 μg/mL). Selective toxicity (i.e. selectivity index) of all triazoles was determined against L6 cells, a primary cell line derived from rat skeletal myoblasts. As shown in Table 2, the majority of the compounds exhibited low or no cytotoxic potential against these cells. One exception was **5e**, which showed a low IC_{50} value (15.2 μg/mL) towards L6 cells.

These results point out some interesting structure–activity relationships related to anti-protozoal activity and selectivity.



Scheme 4. Synthesis of 3aS,6aR)-5-phenyl-1-((3-(4-substituted phenyl)-1,2,4-oxadiazol-5-yl)methyl)-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-diones (**5a**–**k**).

Table 2
In vitro anti-protozoal and cytotoxic activities of **5a**–**k**.

Compound	*Trypanosoma b. rhodesiense*	*Trypanosoma cruzi*	*Leishmania donovani*	*Plasmodium falciparum*	Cytotoxicity L6 cells
5a	**7.0**	46.1	**1.6**	22.6	47.3
5b	17.2	49.0	3.2	24.9	49.5
5c	18.2	54.9	3.5	26.9	58.6
5d	**12.3**	62.4	4.1	23.6	>100
5e	**8.4**	38.9	2.2	13.2	**15.2**
5f	18.8	47.2	3.6	20.4	48.6
5g	**8.0**	41.1	**2.0**	14.7	>100
5h	16.9	54.0	3.4	17.0	>100
5i	16.3	48.5	3.5	20.6	>100
5j	13.9	51.4	3.7	15.0	44.4
5k	13.1	**36.6**	4.5	21.0	>100
Standard	0.005[a]	0.464[b]	0.171[c]	0.073[d]	0.007[e]

The IC_{50} vales are in μg/ml. Standard compounds.
[a] Melarsoprol.
[b] Benznidazole.
[c] Miltefosine.
[d] Chloroquine.
[e] Podophyllotoxin.

The best overall anti-protozoal activity was displayed by the parent compound **5a**, as well as **5e** and **5g**. However, the cytotoxicity observed for **5e** against L6 cells might indicate that its antiparasitic activity might rather be a general toxicity but not specific to parasites of interest. This also indicates that an alkyl (CH_3) substitution at *para* position of the disubstituted phenyl ring, as found in **5e**, is not favoured for selectivity. The parent compound **5a** has promising activity, although it also bears some low cytotoxic potential, narrowing its therapeutic window. The most intriguing compound hence appears to be **5g**, with a nitro substitution at the para position of the disubstituted phenyl ring, which has a strong anti-protozoal activity and no toxic potential even at the highest test concentrations (100 μg/mL). A careful inspection of Table 2 also suggests that halogen substitution at the *para* position of the disubstituted phenyl ring makes little impact in overall anti-protozoal activity, but in toxicity, as the fluorinated derivatives (**5f**) are non-toxic. The same trend applies to the remaining compounds with OCH_3 (**5h**), SCH_3 (**5i**), CF_3 (**5j**) and $N(CH_3)_2$ (**5k**) substitutions, where all have similar activities but only the fluoro derivative (**5j**) has some cytotoxic potential.

3. Conclusion

In summary, we have firstly demonstrated an efficient new synthetic route for the preparation of eleven novel oxadiazolyl pyrrolo triazoles by the 1,3-dipolar cycloaddition of organic azides bearing substituted-phenyl-1,2,4-oxadiazole ring, all of which have also been reported here, and *N*-phenyl maleimide in moderate to excellent yields. In the selection of *para* substituents, both electron donating and electron-withdrawing groups were provided. In addition to the synthesis of the precursors of cycloadducts, Hammett linear correlation was established between carbon chemical shifts and σ_p substituent constants.

The 1,2,4-oxadiazole-, triazole- and pyrrole-type of compounds are known to exert various biological activities, including anti-infective. Especially recently some have been explored for their *in vitro* and *in vivo* anti-protozoal effects [24–28]. Inspired by these studies, we herein hypothesized that compounds possessing all these three substructures will exhibit broad-spectrum antiparasitic potential. Hence the novel heterocyclic compounds (**5a**–**k**) were efficiently synthesized and assayed for their anti-protozoal activity as well as for general cytotoxicity. The synthesized compounds showed the highest susceptibility against *L. donovani* at low μg/mL range (2.0–4.5 μg/mL), but *in vitro* activities against other prozotan parasites were quite moderate. Although low therapeutic index appears to be an issue for a few compounds and a limiting factor in the development of such compounds as antiparasitic agents, several compounds were proved to be active without being toxic to mammalian cells. Our future medicinal chemistry studies will involve synthesis and biological evaluation of diverse derivatives of these selective compounds.

4. Experimental

4.1. General

The ^1H and ^{13}C NMR spectra were recorded on BRUKER Avance spectrometer (400 MHz for ^1H; 100 MHz for ^{13}C). IR spectra were recorded on a SHIMADZU FTIR 8400-S instrument (KBr pellet). Low resolution mass spectra were run on ThermoElectron Navigator Mass Spectrometer (ESI-interface). High resolution accurate mass measurements were performed on Waters Q-TOF Ultima Global andwere performed on Fisons VG Platform II and Micromass Q-TOF Micro spectrometers. Melting points were determined on a Meltemp apparatus and uncorrected. Routine TLC analyses were carried out on precoated Silica gel plates with fluorescent indicator (Merck 5735). Flash column chromatography was performed on Silica Gel (Merck, 230-400 Mesh ASTM). Chromatotron 7924T rotary TLC apparatus (T-Squared Technology, Inc. San Bruno, CA, USA) was utilized for further separation and purifications. The stain solutions of potassium permanganate and iodine were used for visualization of the TLC spots. Amidoximes **2a**–**k** were synthesized according to the previously described methods in the literature [33].

4.2. Synthesis

4.2.1. General procedure for the preparation of oxadiazoles *3a–k*

*4.2.1.1. 5-(Chloromethyl)-3-phenyl-1,2,4-oxadiazole (**3a**).* A solution of chloroacetyl chloride (0.813 g, 7.2 mmol) in benzene (10 mL) was added dropwise to a solution of benzamidoxime (**2a**) (2.448 g, 18.0 mmol) in benzene (100 mL) and the mixture was heated under reflux for 10 h. The reaction was monitored by TLC. The reaction mixture was concentrated *in vacuo*, and the crude residue was purified by flash column chromatography (FCC) using *n*-hexane:ethyl acetate (2:1) mixture to give(**3a**) as a white solid (1.245 g, 89%). M.p.: 39–40 °C (Lit.[33a] 39–40 °C). R_f: 0.95 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3034 (Arom. C–H), 1599, 1573 (C=N), 1473, 1444, 1361, 1300, 922, 711 (C–Cl). ^1H NMR (δ_H, 400 MHz, $CDCl_3$): 8.11 (d, J = 6.4 Hz, 2H), 7.52 (m, 3H), 4.77 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$):174.3 (C=N), 168.9 (C=N), 131.6, 129.0, 127.5, 126.2 (–CH), 33.4 ($-CH_2-$).MS (*m/z*, %) = 194 (M^+, 64),119 (100), 77 (12).

*4.2.1.2. 5-(Chloromethyl)-3-(4-chlorophenyl)-1,2,4-oxadiazole (**3b**).* White solid (1.448 g, 88%). M.p.:61–63 °C. R_f: 0.93 (*n*-hexane:ethyl acetate; 1:1).IR (KBr, ν: cm^{-1}): 3022 (Arom. C–H), 1593, 1566 (C=N), 1473, 1410, 1354, 1091, 839, 740 (C–Cl).^1H NMR (δ_H, 400 MHz, $CDCl_3$): 8.04 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 8.7 Hz, 2H), 4.77 (s, 2H).^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.5 (C=N), 168.1 (C=N), 137.8, 129.3, 128.8, 124.6 (–CH), 33.3 ($-CH_2-$). MS (*m/z*, %) = 228 (M^+, 96), 153 (100), 125 (18), 90 (25), 50 (8).

*4.2.1.3. 5-(Chloromethyl)-3-(4-bromophenyl)-1,2,4-oxadiazole (**3c**).* White solid (1.309 g, 100%). M.p.:61–62 °C (Lit.[33c] 55–56 °C). R_f: 0.90 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3020 (Arom. C–H), 1600, 1558 (C=N), 1469, 1406, 1352, 1109, 835, 736 (C–Cl). ^1H NMR (δ_H, 400 MHz, $CDCl_3$): 7.98 (dt, J = 2.1, 8.5 Hz, 2H), 7.66 (dt, J = 2.1, 8.5 Hz, 2H), 4.77 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.6 (C=N), 168.2 (C=N), 132.3, 129.0, 126.2, 125.1 (–CH), 33.3 ($-CH_2-$).MS (*m/z*, %) = 274 (M^+, 96), 197 (61), 90 (44), 50 (12).

*4.2.1.4. 5-(Chloromethyl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (**3d**)[25,33c].* Yellow oil (0.786 g, 95%). R_f: 0.69 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3032 (Arom. C–H), 1610, 1583 (C=N), 1481, 1417, 1356, 1234, 1157, 844, 754 (C–Cl). ^1H NMR (δ_H, 400 MHz, $CDCl_3$): 8.12 (m, 2H), 7.21 (t, J = 6.6 Hz, 2H), 4.77 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.4 (C=N), 168.1 (C=N), 166.2(–CF–), 128.9, 116.3, 116.1, (–CH), 33.3 ($-CH_2-$). MS (*m/z*,%) = 212 (M^+, 69), 137 (100), 107 (9), 75 (16).

*4.2.1.5. 5-(Chloromethyl)-3-p-tolyl-1,2,4-oxadiazole (**3e**).* White solid (0.712 g, 95%). M.p.:46–47 °C (Lit.[33c] 40–41 °C). R_f: 0.84 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3028 (Arom. C–H), 1618, 1597 (C=N), 1479, 1411, 1361, 1284, 1151, 827, 750 (C–Cl). ^1H NMR (δ_H, 400 MHz, $CDCl_3$): 7.99 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 7.9 Hz, 2H), 4.76 (s, 2H), 2.44 (s, 3H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.1 (C=N), 168.9 (C=N), 142.0, 129.7, 127.4, 123.3 (–CH), 33.4 ($-CH_2-$), 21.6 ($-CH_3$). MS (*m/z*, %) = 208 (M^+, 100), 133 (100), 91 (26), 51 (13).

*4.2.1.6. 5-(Chloromethyl)-3-(4-iodophenyl)-1,2,4-oxadiazole (**3f**)[33d].* Yellow solid (0.926 g, 73%). M.p.:70–72 °C. R_f: 0.94 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3068 (Arom. C–H), 1695, 1562 (C=N),

1467, 1404, 1350, 1273, 1141, 846, 729 (C–Cl). ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.87 (d, J = 8.7 Hz, 2H), 7.83 (d, J = 8.7 Hz, 2H), 4.77 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.5 (C=N), 168.4 (C=N), 138.2, 129.0, 125.6, 98.4, (–CH), 33.4 ($-CH_2$). MS (*m/z*, %) = 320 (M^+, 100), 245 (48), 90 (22), 50 (8).

*4.2.1.7. 5-(Chloromethyl)-3-(4-nitrophenyl)-1,2,4-oxadiazole (**3g**).* White solid (1.321 g, 45%). M.p.:88–89 °C (Lit.[33c] 80–82 °C). R_f: 0.90 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3101 (Arom. C–H), 1612, 1573 (C=N), 1529 (N=O), 1415, 1356, 1294, 1107, 868, 721 (C–Cl). ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.25 (dd, J = 8.71, 19.52 Hz, 4H), 4.72 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 187.6 (C=N), 175.2 (C=N), 149.7, 132.0, 128.5, 124.2, (–CH), 33.2 ($-CH_2$). MS (*m/z*, %) : 239 (M^+, 100), 209 (80), 164 (44), 134 (34), 88 (44), 51 (30).

*4.2.1.8. 5-(Chloromethyl)-3-(4-methoxyphenyl)-1,2,4-oxadiazole (**3h**).* Light yellow solid (1.183 g, 100%). M.p.:39–40 °C (Lit.[25] 55–56 °C). R_f: 0.75 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3003 (Arom. C–H), 1612, 1597 (C=N), 1483, 1423, 1292, 1253, 1181, 842, 754 (C–Cl). ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.04 (d, J = 8.9 Hz, 2H), 7.01 (d, J = 8.9 Hz, 2H), 4.75 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.4 (C=N), 168.6 (C=N), 162.2(-C-), 129.1, 118.6, 114.4 (–CH), 55.4 ($-CH_3$-), 33.4 ($-CH_2-$). MS (*m/z*, %): 224 (M^+, 100), 149 (43), 106 (29), 76 (11).

*4.2.1.9. 5-(Chloromethyl)-3-(4-(methylthio)phenyl)-1,2,4-oxadiazole (**3i**).* White solid (0.556 g, 100%). M.p.:58–60 °C. R_f: 0.88 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3020 (Arom. C–H), 1664, 1595 (C=N), 1467, 1408, 1361, 1294, 1118, 825, 740 (C–Cl). ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.91 (d, J = 8.6 Hz, 2H), 7.24 (d, J = 8.6 Hz, 2H), 4.67 (s, 2H), 2.44 (s, 3H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.2 (C=N), 168.6 (C=N), 143.5, 127.7, 125.7, 122.4 (–CH), 33.4 ($-CH_2-$), 15.0 ($-CH_3$). MS (*m/z*, %): 240 (M^+, 100), 149 (15), 106 (9), 63 (9).

*4.2.1.10. 5-(Chloromethyl)-3-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazole (**3j**)[33d].* Yellow oil (1.229 g, 95%). R_f: 0.83 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3030 (Arom. C–H), 1656, 1597 (C=N), 1577, 1541, 1419, 1319, 1018, 854, 713 (C–Cl). ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.24 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 8.2 Hz, 2H), 4.79 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.9 (C=N), 167.9 (C=N), 129.6, 128.0, 126.0, 126.0 (–CH–), 125.9 ($-CF_3$-) 33.3 ($-CH_2-$). MS (*m/z*, %): 262 (M^+, 66), 187 (100), 139 (14), 109 (8),75 (9).

*4.2.1.11. 5-(Chloromethyl)-3-(4-(N,N-dimethylamino)phenyl)-1,2,4-oxadiazole (**3k**).* White solid (0.387 g, 57%). M.p.:81–83 °C. R_f: 0.84 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3022 (Arom. C–H), 1653, 1647 (C=N), 1597, 1489, 1361, 1226, 1188, 817, 746 (C–Cl). ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.95 (d, J = 9.1 Hz, 2H), 6.76 (d, J = 9.0 Hz, 2H), 4.73 (s, 2H), 3.06 (s, 6H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 183.2 (C=N), 173.5 (C=N), 169.0 (–C), 128.7, 121.1, 113.2 (–CH), 45.3 ($-CH_2-$), 39.9 ($-CH_3$-). MS (*m/z*, %): 237 (M^+, 100), 145 (42), 102 (8), 63 (5).

*4.2.2. Synthesis of azides **4a–k***

*4.2.2.1. General procedure for the preparation of azides **4a–k***

*4.2.2.1.1. 5-(Azidomethyl)-3-phenyl-1,2,4-oxadiazole (**4a**).* To a stirred solution of 5-chloromethyl-3-phenyl-1,2,4-oxadiazole (**3a**) (1.200 g, 6.18 mmol) in a 50 mL water/acetone mixture (1:4) was added NaN_3 (0.442 g, 6.80 mmol). The resulting suspension was stirred at room temperature for 24 h. Dichloromethane (DCM) was added to the mixture and the organic layer was separated. The aqueous layer was extracted with 3 × 10 mL aliquots of DCM and the combined organic layers were dried over $MgSO_4$. Solvent was removed under reduced pressure, and the crude azide was purified by flash column chromatography (FCC) (*n*-hexane:ethyl acetate; 5:1) to give(**4a**) as a yellow oil (0.685 g, 99%). R_f: 0.92 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3068 (Arom. C–H), 2108 (N=N=N), 1597, 1560 (C=N), 1475, 1446, 1346, 1288, 1114, 896, 714.^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.13 (d, J = 1.5 Hz, 1H), 8.12 (d, J = 2.0 Hz, 1H), 7.56 (m, 2H), 7.51 (m, 1H), 4.65 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.0 (C=N), 168.7 (C=N), 131.6, 129.0, 127.6, 126.5 (–CH), 45.1 ($-CH_2-$). MS (*m/z*, %): 207 (M^+, 100), 173 (16), 119 (76), 91 (36), 64 (17). HRMS-TOF-MS ES$^+$:Measured; 202.0750, Calculated for $C_9H_8N_5O$; 202.0729.

*4.2.2.1.2. 5-(Azidomethyl)-3-(4-chlorophenyl)-1,2,4-oxadiazole (**4b**).* Yellow solid (0.453 g, 100%). M.p.:40–41 °C. R_f: 0.88 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3080 (Arom. C–H), 2110 (N=N=N), 1587, 1562 (C=N), 1471, 1410, 1365, 1282, 1095, 900, 837, 744. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.06 (d, J = 8.6 Hz, 2H), 7.50 (d, J = 8.6 Hz, 2H), 4.64 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.2 (C=N), 168.0 (C=N) 137.8, 129.3, 128.9, 124.7 (–CH), 45.1 ($-CH_2-$). MS (*m/z*, %): 235 (M^+, 81), 153 (100), 123 (12), 90 (54), 50 (23).

HRMS-TOF-MS ES$^+$:Measured; 236.0341, Calculated for $C_9H_7ClN_5O$; 236.0339.

*4.2.2.1.3. 5-(Azidomethyl)-3-(4-bromophenyl)-1,2,4-oxadiazole (**4c**).* Yellow solid (0.235 g, 100%). M.p.:41–42 °C. R_f: 0.88 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3084 (Arom. C–H), 2110 (N=N=N), 1653, 1558 (C=N), 1467, 1406, 1361, 1276, 1070, 902, 829, 744. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.99 (d, J = 8.6 Hz, 2H), 7.66 (d, J = 8.6 Hz, 2H), 4.65 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 74.2 (C=N), 168.1 (C=N), 132.3, 129.0, 126.2, 125.1 (–CH), 45.1 ($-CH_2-$).MS (*m/z*, %): 281 (M^+, 94), 251 (30), 197 (76), 169 (12), 114 (16), 90 (100), 50 (28). HRMS-TOF-MS ES$^+$:Measured; 279.9846, Calculated for $C_9H_7BrN_5O$; 279.9834.

*4.2.2.1.4. 5-(Azidomethyl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (**4d**).* Yellow oil (0.290 g, 100%). R_f: 0.90 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3084 (Arom. C–H), 2104 (N=N=N), 1608, 1581 (C=N), 1483, 1419, 1367, 1234, 1157, 898, 846, 748. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.12 (m, 2H), 7.21 (m, 2H), 4.64 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.1 (C=N), 167.9 (C=N), 129.8, 122.4, 116.3, 116.0 (–CH), 45.1 ($-CH_2-$).MS (*m/z*, %): 219 (M^+, 100), 191 (18), 137 (84), 109 (85), 75 (24), 57 (17). HRMS-TOF-MS ES$^+$:Measured; 219.0562, Calculated for $C_9H_6N_5OF$; 219.0556.

*4.2.2.1.5. 5-(Azidomethyl)-3-p-tolyl-1,2,4-oxadiazole (**4e**).* Yellow oil (0.253 g, 97%). R_f: 0.89 (*n*-hexane:ethyl acetate; 1:1).IR (KBr, ν: cm^{-1}): 3034 (Arom. C–H), 2104 (N=N=N), 1653, 1595 (C=N), 1481, 1413, 1340, 1286, 1112, 895, 829, 740. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.01 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 4.64 (s, 2H), 2.45 (s, 3H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 173.8 (C=N), 168.7 (C=N), 142.0, 129.7, 127.5,123,3 (–CH), 45.1 ($-CH_2-$), 21.6 ($-CH_3$).MS (*m/z*, %): 215 (M^+, 100), 187 (10), 133 (70), 91 (25), 63 (10). HRMS-TOF-MS ES$^+$:Measured; 216.0873, Calculated for $C_{10}H_{10}N_5O$; 216.0885.

*4.2.2.1.6. 5-(Azidomethyl)-3-(4-iodophenyl)-1,2,4-oxadiazole (**4f**).* Yellow solid (0.095 g, 73%). M.p.:50–51 °C. R_f: 0.96 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3081 (Arom. C–H), 2119 (N=N=N), 1591, 1557 (C=N), 1467, 1402, 1317, 1109, 1008, 833, 738. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.86 (m, 4H), 4.65 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.2 (C=N), 168.2 (C=N), 144.2, 138.5, 135.2, 129,0 (–CH), 45.1 ($-CH_2-$). MS (*m/z*, %): 327 (M^+, 100), 299 (57), 245 (54), 90 (44), 50 (16). HRMS-TOF-MS ES$^+$:Measured; 326.9624, Calculated for $C_9H_7IN_5O$; 326.9617.

*4.2.2.1.7. 5-(Azidomethyl)-3-(4-nitrophenyl)-1,2,4-oxadiazole (**4g**).* Yellow solid (0.350g, 99%). M.p.:89–90 °C. R_f: 0.84 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3107 (Arom. C–H), 2106 (N=N=N), 1610, 1577 (C=N), 1417, 1340, 1290, 1103, 860, 723. ^{1}H NMR (δ_H, 300 MHz, $CDCl_3$): 8.34 (m, 4H), 4.87 (s, 2H). ^{13}C NMR (δ_C, 75 MHz, $CDCl_3$): 175.2 (C=N), 167.4 (C=N), 150.0, 133.8, 128.8, 124.5 (–CH), 45.3 ($-CH_2-$). MS (*m/z*, %): 246 (M^+, 100), 241 (9), 187 (100), 168

(18), 139 (22), 109 (13), 75 (14). HRMS-TOF-MS ES$^+$:Measured; 247.0562, Calculated for $C_9H_7N_6O_3$; 247.0580.

*4.2.2.1.8. 5-(Azidomethyl)-3-(4-methoxyphenyl)-1,2,4-oxadiazole (**4h**).* Yellow oil (0.445 g, 100%). R_f: 0.83 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3009 (Arom. C–H), 2108 (N=N=N), 1614, 1571 (C=N), 1483, 1425, 1342, 1174, 1008, 839, 752. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.05 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 8.6 Hz, 2H), 4.62 (s, 2H), 3.88 (s, 3H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 173.6 (C=N), 168.4 (C=N), 162.2, 129.2, 118.6, 114.4 (–CH), 55.4 ($-CH_2-$), 45.1 ($-CH_3$). MS (*m/z*, %): 231 (M$^+$, 83), 203 (97), 133 (100), 90 (46), 50 (22). HRMS-TOF-MS ES$^+$:Measured; 232.0833, Calculated for $C_{10}H_{10}N_5O_2$; 232.0834.

*4.2.2.1.9. 5-(Azidomethyl)-3-(4-(methylthio)phenyl)-1,2,4-oxadiazole (**4i**).* Yellow oil (0.213 g, 100%). R_f: 0.86 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 2989 (Arom. C–H), 2108 (N=N=N), 1593, 1558 (C=N), 1471, 1408, 1340, 1162, 1089, 831, 746. ^{1}H NMR (δ_H, 300 MHz, $CDCl_3$): 8.00 (d, J = 10.8 Hz, 2H), 7.30 (m, 2H), 4.62 (s, 2H), 2.52 (s, 3H). ^{13}C NMR (δ_C, 75 MHz, $CDCl_3$): 174.1 (C=N), 168.6 (C=N), 143.7, 128.0, 126.0, 122.5 (–CH), 45.3 ($-CH_2-$), 15.2 ($-CH_3$). MS (*m/z*, %): 247 (M$^+$, 100), 219 (67), 165 (62), 149 (88), 106 (17), 90 (12), 69 (12). HRMS-TOF-MS ES$^+$:Measured; 247.0527, Calculated for $C_{10}H_9N_5OS$; 247.0528.

*4.2.2.1.10. 5-(Azidomethyl)-3-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazole (**4j**).* Yellow oil (0.307 g, 93%). R_f: 0.88 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 2933 (Arom. C–H), 2100 (N=N=N), 1597, 1573 (C=N), 1485, 1419, 1329, 1174, 1066, 854, 759. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.24 (d, J = 8.8 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H), 4.66 (s, 2H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.8 (C=N), 168.0 (C=N), 133.7, 129.7, 128.2, 126.3 ($-CF_3$-), 45.3 ($-CH_2-$). MS (*m/z*, %): 269 (M$^+$, 100), 241 (9), 187 (100), 168 (18), 139 (22) 109 (13), 75 (14). HRMS-TOF-MS ES$^+$:Measured; 269.0519, Calculated for $C_{10}H_6N_5OF_3$; 269.0524.

*4.2.2.1.11. 5-(Azidomethyl)-3-(4-(N,N-dimethylamino)phenyl)-1,2,4-oxadiazole (**4k**).* Yellow solid (0.169 g, 99%). M.p.:61–62 °C. R_f: 0.83 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 2914 (Arom. C–H), 2098 (N=N=N), 1614, 1587 (C=N), 1491, 1435, 1375, 1276, 1197, 821, 756. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.96 (d, J = 9.1 Hz, 2H), 6.76 (d, J = 9.1 Hz, 2H), 4.60 (s, 2H), 3.06 (s, 6H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 173.1 (C=N), 168.8 (C=N), 152.4, 133.4, 128.8, 111.7 (–CH), 45.1 ($-CH_2-$), 40.1 ($-NCH_3$). MS (*m/z*, %): 244 (M$^+$, 59), 215 (65), 145 (100), 102 (13). HRMS-TOF-MS ES$^+$:Measured; 244.1070, Calculated for $C_{11}H_{12}N_6O_1$; 244.1073.

*4.2.3. Synthesis of dihydropyrrolo triazoles **5a–k***

*4.2.3.1. General procedure for the preparation of **5a–k***

*4.2.3.1.1. (3aS,6aR)-5-Phenyl-1-((3-phenyl-1,2,4-oxadiazol-5-yl) methyl)-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5a**).* A mixture of *N*-phenyl maleimide (0.044 g, 0.252 mmol) and 5-(azidomethyl)-3-phenyl-1,2,4-oxadiazole (**4a**) (0.050 g, 0.240 mmol) were stirred in benzene (25 mL) and the mixture was heated under reflux for 2-4 d. The reaction was monitored by TLC. The reaction mixture was concentrared *in vacuo*, and the crude residue was washed with *n*-hexane to give (**5a**) as a white solid (0.048 g, 54%). M.p.:162–164 °C; R_f: 0.74 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3068 (Arom. C–H), 1716 (C=O), 1597, 1500 (C=N), 1475, 1444, 1384, 1348, 1188, 933, 717.^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 8.06 (d, J = 6.8 Hz, 2H), 7.73 (m, 7H), 7.26 (m, 1H), 5.92 (d, J = 10.8 Hz, 1H), 5.65 (d, J = 17.8 Hz, 1H), 5.37 (d, J = 17.8 Hz, 1H), 4.83 (d, J = 10.8 Hz, 1H).^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 175.4 (C=O), 171.6 (C=O), 170.1 (C=N), 168.2 (C=N), 131.7, 129.2, 129.1, 129.0, 128.5, 127,5, 126.8, 100.0 (–CH), 83.8 (–CH), 58.2(–CH), 44.5 ($-CH_2-$). MS TOF AP$^+$ (*m/z*, %) = 416 (M + MeCNH$^+$, 3), 375 (M + H$^+$, 7), 117 (8), 100 (100). HRMS-TOF-MS ES$^+$:Measured; 375.1201, Calculated for $C_{19}H_{14}N_6O_3$; 375.1206.

*4.2.3.1.2. (3aS,6aR)-1-((3-(4-Chlorophenyl)-1,2,4-oxadiazol-5-yl) methyl)-5-phenyl-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5b**).* White solid (0.068 g, 78%). M.p.:164–165 °C.R_f: 0.50 (*n*-hexane:ethyl acetate; 1:1).IR (KBr, ν: cm^{-1}): 3074 (Arom. C–H), 1720 (C=O), 1653, 1593 (C=N), 1494, 1471, 1388, 1340, 1089, 850, 740.^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.91 (d, J = 8.6 Hz, 2H), 7.37 (m, 6H), 7. (dd, J = 2.8, 9.8 Hz, 2H), 5.84 (d, J = 10.8 Hz, 1H), 5.51 (d, J = 17.8 Hz, 1H), 5.35 (d, J = 17.8 Hz, 1H), 4.73 (d, J = 10.8 Hz, 1H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 175.4 (C=O), 171.1 (C=O), 169.4 (C=N), 167.6 (C=N), 137.5, 131.2, 129.2, 129.0, 128.9, 126.3, 124.7 (–CH), 83.5 (–CH), 58.3(–CH), 44.3 ($-CH_2-$).MS TOF AP$^+$ (*m/z*, %) = 450 (M + MeCNH$^+$, 9), 409 (M + H$^+$, 8), 117 (23), 100 (100). HRMS-TOF-MS ES$^+$:Measured; 409.0823, Calculated for $C_{19}H_{13}ClN_6O_3$; 409.0816.

*4.2.3.1.3. (3aS,6aR)-1-((3-(4-Bromophenyl)-1,2,4-oxadiazol-5-yl) methyl)-5-phenyl-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5c**).* White solid (0.080 g, 71%). M.p.:156–157 °C.R_f: 0.62 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3059 (Arom. C–H), 1722 (C=O), 1595, 1566 (C=N), 1498, 1471, 1383, 1342, 1190, 835, 742.^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.91 (d, J = 8.7 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 7.5 Hz, 3H), 7.25 (t, J = 7.7 Hz, 2H), 5.83 (d, J = 10.7 Hz, 1H), 5.52 (d, J = 17.6 Hz, 1H), 5.37 (d, J = 17.7 Hz, 1H), 4.72 (d, J = 10.8 Hz, 1H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 179.9 (C=O), 176.3 (C=O), 174.4 (C=N), 172.5 (C=N), 137.0, 136.9, 133.8, 131.3, 130.7, 130.0, 88.9 (–CH), 62.7 (–CH), 48.4 ($-CH_2-$). MS TOF AP$^+$ (*m/z*, %): 453 (M$^+$, 3), 117 (21), 100 (100). HRMS-TOF-MS ES$^+$:Measured; 459.0534, Calculated for $C_{19}H_{13}BrN_6O_3$; 453.0311.

*4.2.3.1.4. (3aS,6aR)-1-((3-(4-Fluorophenyl)-1,2,4-oxadiazol-5-yl) methyl)-5-phenyl-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5d**).* White solid (0.067 g, 52%). M.p.:155–157 °C.R_f: 0.52 (*n*-hexane:ethyl acetate; 1:1).IR (KBr, ν: cm^{-1}): 3059 (Arom. C–H), 1724 (C=O), 1606, 1581 (C=N), 1479, 1417, 1383, 1342, 1190, 852, 740. ^{1}H NMR (δ_H, 300 MHz, $CDCl_3$): 8.04 (m, 2H), 7.45 (m, 3H), 7.30 (m, 4H), 5.93 (d, J = 10.8 Hz, 1H), 5.59 (d, J = 17.6 Hz, 1H), 5.40 (d, J = 17.9 Hz, 1H), 4.82 (d, J = 10.8 Hz, 1H).^{13}C NMR (δ_C, 75 MHz, $CDCl_3$): 174.5 (C=O), 171.2 (C=O), 169.4 (C=N), 167.6 (C=N), 130.9, 129.9, 129.3, 129.2, 126.3, 123.4, 122.4, 116.4 (–CH), 83.4 (–CH), 57.6 (–CH), 44.4 ($-CH_2-$). MS TOF AP$^+$ (*m/z*, %): 434 (M + MeCNH$^+$, 33), 393 (M + H$^+$, 38), 117 (55), 100 (100). HRMS-TOF-MS ES$^+$:Measured; 393.1115, Calculated for $C_{19}H_{13}FN_6O_3$; 393.1111.

*4.2.3.1.5. (3aS,6aR)-5-Phenyl-1-((3-p-tolyl-1,2,4-oxadiazol-5-yl) methyl)-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5e**).* White solid (0.060 g, 68%). M.p.:138–140 °C.R_f: 0.43 (*n*-hexane:ethyl acetate; 1:1).IR (KBr, ν: cm^{-1}): 3049 (Arom. C–H), 1720 (C=O), 1597, 1572 (C=N), 1483, 1383, 1342, 1107, 740.^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.93 (d, J = 8.2 Hz, 2H), 7.45 (m, 3H), 7.28 (m, 4H), 5.88 (d, J = 10.8 Hz, 1H), 5.60 (d, J = 17.8 Hz, 1H), 5.32 (d, J = 17.8 Hz, 1H), 4.80 (d, J = 10.8 Hz, 1H), 2.44 (s, 3H).^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 173.4 (C=O), 170.7 (C=O), 168.7 (C=N), 168.5 (C=N), 130.6, 129.7, 129.4, 129.3, 128.4, 127,5, 126.2, 123.1 (–CH), 83.0 (–CH), 57.2(–CH), 44.4 ($-CH_2-$), 21.6 ($-CH_3$-). MS TOF ES$^+$ (*m/z*, %): 427 (M + K$^+$, 5), 359 (M-N$_2^+$, 6), 274 (100), 146 (90), 129 (19), 105 (33). HRMS Measured; 389.2153 Calculated for $C_{20}H_{16}N_6O_3$; 389.1362.

*4.2.3.1.6. (3aS,6aR)-1-((3-(4-Iodophenyl)-1,2,4-oxadiazol-5-yl) methyl)-5-phenyl-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5f**).* White solid (0.064 g, 84%). M.p.:161–163 °C.R_f: 0.61 (*n*-hexane:ethyl acetate; 1:1).IR (KBr, ν: cm^{-1}): 3053 (Arom. C–H), 1716 (C=O), 1689, 1558 (C=N), 1498, 1402, 1384, 1334, 1195, 837, 742. ^{1}H NMR (δ_H, 300 MHz, $CDCl_3$): 7.80 (m, 5H), 7.38 (m, 4H), 5.93 (d, J = 10.8 Hz, 1H), 5.60 (d, J = 17.6 Hz, 1H), 5.41 (d, J = 17.9 Hz, 1H), 4.81 (d, J = 10.8 Hz, 1H). ^{13}C NMR (δ_C, 75 MHz, $CDCl_3$): 174.7 (C=O), 171.2 (C=O), 169.4 (C=N), 167.9 (C=N), 138.2, 130.9, 129.3, 129.2, 129.1, 126.4, 125.6, 98.5 (–CH), 83.5 (–CH), 57.6 (–CH), 44.4 ($-CH_2-$). MS TOF ES$^+$ (*m/z*, %): 541 (M + MeCNH$^+$, 22), 521 (100), 478 (9), 391 (39). HRMS-TOF-MS ES$^+$:Measured; 501.0194, Calculated for $C_{19}H_{13}IN_6O_3$; 501.0172.

*4.2.3.1.7. (3aS,6aR)-1-((3-(4-Nitrophenyl)-1,2,4-oxadiazol-5-yl) methyl)-5-phenyl-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5g**).* White solid (0.044 g, 43%). M.p.:184–186 °C. R_f: 0.54 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3144 (Arom. C–H), 1720 (C=O), 1573, 1538 (C=N), 1385, 1334, 1190, 834, 744.^{1}H NMR (δ_H, 300 MHz, $CDCl_3$): 8.30 (dd, J = 8.3, 19.3 Hz, 4H), 7.42 (m, 5H), 5.94 (d, J = 10.8 Hz, 1H), 5.64 (d, J = 17.6 Hz, 1H), 5.48 (d, J = 17.6 Hz, 1H), 4.82 (d, J = 10.5 Hz, 1H).^{13}C NMR (δ_C, 75 MHz, $CDCl_3$): 176.6 (C=O), 172.0 (C=O), 170.4 (C=N), 167.0 (C=N), 149.8, 132.3, 131.9, 129.4, 129.3, 129.0, 127.1, 124.8 (–CH), 84.1 (–CH), 58.6 (–CH), 44.8 ($-CH_2-$). MS TOF AP$^+$ (*m/z*, %): 461 (M + $MeCNH^+$, 1), 391 (M-N_2^+, 3), 165 (7), 117 (42), 100 (100). HRMS-TOF-MS ES$^+$:Measured; 420.1050, Calculated for $C_{19}H_{13}N_7O_5$; 420.1057.

*4.2.3.1.8. (3aS,6aR)-1-((3-(4-Methoxyphenyl)-1,2,4-oxadiazol-5-yl)methyl)-5-phenyl-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5h**).* White solid (0.116 g, 94%). M.p.:141–143 °C. R_f: 0.46 (*n*-hexane:ethyl acetate; 1:1).IR (KBr, ν: cm^{-1}): 3061 (Arom. C–H), 1720 (C=O), 1610, 1595 (C=N), 1481, 1425, 1377, 1257, 1178, 844, 748. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$): 7.90 (d, J = 8.6 Hz, 2H), 7.38 (m, 4H), 6.99 (d, J = 14.7 Hz, 3H) 5.86 (d, J = 10.8 Hz, 1H), 5.53 (d, J = 17.5 Hz, 1H), 5.42 (d, J = 17.5 Hz, 1H), 4.75 (d, J = 10.8 Hz, 1H), 3.82 (s, 3H). ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$): 174.9 (C=O), 171.6 (C=O), 170.0 (C=N), 169.4 (C=N), 162.2, 131.6, 129.1, 129.1, 129.0, 126.8, 119.0, 114.6 (–CH), 83.8 (–CH), 58.2 (–CH), 55.6 ($-CH_2-$), 44.5 ($-CH_3$-). MS TOF AP$^+$ (*m/z*, %): 446 (M + $MeCNH^+$, 1), 377 (M-N_2^+, 3), 117 (38), 100 (100). HRMS-TOF-MS ES$^+$:Measured; 405.1324, Calculated for $C_{20}H_{16}N_6O_4$; 405.1311.

*4.2.3.1.9. (3aS,6aR)-1-((3-(4-(Methylthio)phenyl)-1,2,4-oxadiazol-5-yl)methyl)-5-phenyl-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5i**).* White solid (0.117 g, 91%). M.p.:156–158 °C. R_f: 0.67 (*n*-hexane:ethyl acetate; 1:1).IR (KBr, ν: cm^{-1}): 3061 (Arom. C–H), 1720 (C=O), 1593, 1556 (C=N), 1498, 1469, 1383, 1186, 1114, 829, 742. ^{1}H NMR (δ_H, 300 MHz, $CDCl_3$): 7.92 (d, J = 8.5 Hz, 2H), 7.42 (m, 3H), 7.27 (m, 4H), 5.92 (d, J = 10.5 Hz, 1H), 5.58 (d, J = 17.6 Hz, 1H), 5.38 (d, J = 17.9 Hz, 1H), 4.81 (d, J = 10.8 Hz, 1H), 2.89 (s, 3H). ^{13}C NMR (δ_C, 75 MHz, $CDCl_3$): 174.3 (C=O), 171.2 (C=O), 169.4 (C=N), 168.1 (C=N), 143.6, 131.0, 129.3, 129.2, 127.8, 126.4, 125.7, 122.2 (–CH), 83.5 (–CH), 57.6 (–CH), 44.4 ($-CH_2-$), 15.0 ($-CH_3$). MS TOF AP$^+$ (*m/z*, %): 462 (M + $MeCNH^+$, 2), 421 (M + H^+, 4), 392 (M-N_2^+, 10), 391 (40), 248 (16), 117 (76), 100 (100). HRMS-TOF-MS ES$^+$:Measured; 421.1106, Calculated for $C_{20}H_{16}N_6O_3S$; 421.1083.

*4.2.3.1.10. (3aS,6aR)-5-Phenyl-1-((3-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazol-5-yl)methyl)-1,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6(3aH,5H)-dione (**5j**).* White solid (0.056 g, 46%). M.p.:170–172 °C. R_f: 0.39 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3061 (Arom. C–H), 1718 (C=O), 1595, 1573 (C=N), 1500, 1419, 1323, 1197, 1064, 862, 746. ^{1}H NMR (δ_H, 300 MHz, $CDCl_3$): 8.19 (d, J = 8.2 Hz, 2H), 7.75 (m, 2H), 7.34 (m, 5H), 5.92 (d, J = 10.8 Hz, 1H), 5.62 (d, J = 17.6 Hz, 1H), 5.48 (d, J = 17.9 Hz, 1H), 4.81 (d, J = 10.5 Hz, 1H). ^{13}C NMR (δ_C, 75 MHz, $CDCl_3$): 175.2 (C=O), 171.2 (C=O), 169.5 (C=N), 167.2 (C=N), 132.8, 132.4, 131.0, 129.6, 129.0, 128.2, 127.9, 126.3, 125.8 (–CH), 83.5 (–CH), 57.7 (–CH), 44.3 ($-CH_2-$). MS TOF AP$^+$ (*m/z*, %): 484 (M + $MeCNH^+$, 45), 443 (M + H^+, 36), 392 (36), 391 (100), 117 (19), 100 (73). HRMS-TOF-MS ES$^+$:Measured; 443.1076, Calculated for $C_{20}H_{13}F_3N_6O_3$; 443.1079.

*4.2.3.1.11. (3aS, 6aR)-1-((3-(4-(Dimethylamino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)-5-phenyl-1,6a-dihydropyrrolo[3,4-d][1,2,3] triazole-4,6(3aH,5H)-dione (**5k**).* White solid (0.099 g, 53%). M.p.:152–154 °C. R_f: 0.53 (*n*-hexane:ethyl acetate; 1:1). IR (KBr, ν: cm^{-1}): 3074 (Arom. C–H), 1720 (C=O), 1612, 1593 (C=N), 1492, 1431, 1348, 1193, 823, 742. ^{1}H NMR (δ_H, 300 MHz, $CDCl_3$): 7.89 (d, J = 8.2 Hz, 2H), 7.36 (m, 5H), 6.75 (d, J = 7.6 Hz, 2H), 5.90 (d, J = 10.8 Hz, 1H), 5.57 (d, J = 17.9 Hz, 1H), 5.29 (d, J = 17.9 Hz, 1H), 4.81 (d, J = 10.8 Hz, 1H), 3.05 (s, 6H). ^{13}C NMR (δ_C, 75 MHz, $CDCl_3$): 173.3 (C=O) 171.6 (C=O), 170.0 (C=N), 169.4 (C=N), 162.2, 130.8, 129.5, 128.9, 126.4, 111.7, 110.0, (–CH), 83.8 (–CH), 44.6 (–CH), 40.6 ($-CH_2-$), 40.0 ($-CH_3-$). MS TOF ES$^+$ (*m/z*, %): 481 (M + $MeCNa^+$, 35), 459 (M + $MeCNH^+$, 36), 418 (M + H^+, 9), 389 (M-N_2^+, 10), 169 (14), 146 (100), 105 (31). HRMS-TOF-MS ES$^+$:Measured; 417.1549, Calculated for $C_{21}H_{19}N_7O_3$; 417.1549.

4.3. Biological activities

4.3.1. Trypanocidal activity against T. brucei rhodesiense

STIB 900 strain of *T. b. rhodesiense* and the standard drug melarsoprol were used for the assay. This stock was isolated in 1982 from a human patient in Tanzania and after several mouse passages cloned and adapted to axenic culture conditions [36]. Minimum Essential Medium (50 μL) supplemented with 25 mM HEPES, 1 g/L additional glucose, 1% MEM non-essential amino acids (100×), 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate [37]. Serial drug dilutions of seven 3-fold dilution steps covering a range from 90 to 0.123 μg/mL were prepared. Then 10^4 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 μL was added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. 10 μL of a resazurin solution (12.5 mg resazurin dissolved in 100 mL double-distilled water) was then added to each well and incubation continued for a further 2–4 h. Then the plates were read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the microplate reader software Softmax Pro (Molecular Devices Cooperation, CA, USA).

4.3.2. Trypanocidal activity against T. cruzi

Rat skeletal myoblasts (L6 cells) were seeded in 96-well microtitre plates at 2000 cells/well in 100 μL RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h the medium was removed and replaced by 100 μL per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene [38]. After 48 h, the medium was removed from the wells and replaced by 100 μL fresh medium with or without a serial drug dilution of seven 3-fold dilution steps covering a range from 90 to 0.123 μg/mL. After 96 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterility. Then the substrate CPRG/Nonidet (50 μL) was added to all wells. A color reaction developed within 2–6 h and could be read photometrically at 540 nm. Data were transferred into the graphic programme Softmax Pro (Molecular Devices), which calculated IC_{50} values. Benznidazole was the standard drug used.

4.3.3. Leishmanicidal activity against L. donovani

Amastigotes of *L. donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37 °C in SM medium at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% CO_2 in air. One hundred μL of culture medium with 10^5 amastigotes from axenic culture with or without a serial drug dilution were seeded in 96-well microtitre plates. Serial drug dilutions covering a range from 90 to 0.123 μg/mL were prepared. After 72 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 μL of a resazurin solution (12.5 mg resazurin dissolved in 100 mL double-distilled water) [39] was then added to each well and the plates incubated for another 2 h. Then the plates were read in a Spectramax Gemini XS microplate fluorometer using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the software Softmax Pro. Decrease of fluorescence (i.e. inhibition) was expressed as percentage of the fluorescence of control

cultures and plotted against the drug concentrations. From the sigmoidal inhibition curves the IC_{50} values were calculated. Miltefosine was used as a reference drug.

4.3.4. Antimalarial activity against P. falciparum

In vitro activity against erythrocytic stages of *P. falciparum* was determined by a modified [^{3}H]-hypoxanthine incorporation assay [40], using the chloroquine- and pyrimethamine-resistant K1 strain and the standard drug chloroquine. Briefly, parasite cultures incubated in RPMI 1640 medium with 5% Albumax (without hypoxanthine) were exposed to serial drug dilutions in microtiter plates. After 48 h of incubation at 37 °C in a reduced oxygen atmosphere, 0.5 μCi ^{3}H-hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted using a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. IC_{50} values were calculated from graphically plotted dose–response curves.

4.3.5. Cytotoxicity against L6 cells

Assays were performed in 96-well microtiter plates, each well containing 100 μL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4×10^4 L6 cells (a primary cell line derived from rat skeletal myoblasts). Serial drug dilutions of seven 3-fold dilution steps covering a range from 90 to 0.123 μg/mL were prepared. After 72 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 μL of a resazurin solution (12.5 mg resazurin dissolved in 100 mL distilled water) was then added to each well and the plates incubated for another 2 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analysed using the microplate reader software Softmax Pro. Podophyllotoxin was the standard drug used.

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