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Antileishmanial activity screening of 5-nitro-2-heterocyclic benzylidene hydrazides

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ABSTRACT

A series of 53 nitro derivatives rationally designed were obtained by parallel synthesis and screened against *Leishmania donovani*. Six compounds exhibited IC_{50} values lower than standard drugs. Brief SAR analysis revealed that substitution is important to the activity. Nitrothiophene analogues were more potent than the nitrofuran ones. This was attributed to the ability of sulfur atoms in accommodating electrons from nitro group, which facilitate its reduction and therefore the formation of free radicals lethal to parasites.

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1. Introduction

Leishmaniasis is defined as a cluster of vector-borne diseases with diverse clinical manifestations, caused by the obligate intracellular protozoan parasite of the genus Leishmania.[1] Its manifestations include three broad groups of disorders: visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis. These manifestations are caused by a total of about 21 leishmanial species, which are transmitted by around 30 species of phlebotomine sandflies.[2–4] Visceral leishmaniasis, the only lethal form of this parasitosis, is mainly caused by *Leishmania donovani*.[5] Mucocutaneous and cutaneous manifestations, on the other hand, can be caused by a variety of species. There are over 14 recognized species of *Leishmania* within the subgenera *Leishmania* and *Viannia*, which may produce lesions in man.[6,7]

Leishmaniasis is endemic in areas of the tropics, subtropics, and Southern Europe, in settings ranging from rain forests in the Americas to the deserts in Western Asia, and from rural to periurban areas.[6]

The treatment of leishmaniasis is far from satisfactory. Since the 1940s, the pentavalent antimony compounds sodium stibogluconate (Pentostam®, Glaxo Wellcome, UK) and meglumine antimoniate (Glucantime®, Rhône-Poulenc Rorer, France) have been the mainstays of antileishmanial therapy.[6,8] These drugs present high toxicity besides requiring parenteral administration for extended periods, especially in cases of visceral leishmaniasis. Moreover, in recent years, widespread resistance to pentavalent antimonial agents has been observed, especially in cases of *Leishmania*/HIV co-infection.[9]

These agents have been improved with the advent of new formulations or dosage regimens but the need for new compounds with better potency and toxicity profiles is still urgent.[10,11] Substantial efforts have been made towards this goal and several manuscripts on antileishmanial therapy can be found on the literature.[12–16]

This paper reports the synthesis of 53 nifuroxazide derivatives (Fig. 1 and Table 1), rationally designed and obtained by parallel synthesis methodology, and their biological screening against *L. donovani*. A brief structure–activity analysis is included as well.

2. Design and chemical discussion

Fifty-three nitro compounds were designed based on nifuroxazide structure backbone (Fig. 1), an antibacterial agent for the treatment or prevention of a wide range of infections of genitourinary tract as well as an adjunct therapy for patients with second- and third-degree burns.[17]

The core structure allows different patterns of substitution in the aromatic ring generating several analogues with different physical–chemical properties but with almost similar steric characteristics. The R groups were chosen based on two parameters—σ and π constants. These constants represent, respectively, the relative electronic and lipophilic contributions of a proper substituent (R) to the global structure. Both kinds of contributions could interfere with the nitro group reduction by unspecific nitro-

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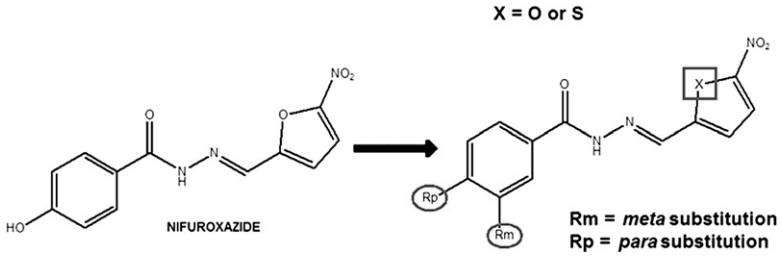


Figure 1. Nifuroxazide: lead compound and structural substitutions in the synthetic analogues.

reductases and consequent free radical formation, the most acceptable mechanism of action to nitro derivatives.[18] This criterion also allows rational development of diversity and 2D QSAR studies as well. Mono- and di-substituted analogues were synthesized and considered in this work.

Structure diversity was also obtained by using bioisosterism between nitrofuran and nitrothiophene, that is, between oxygen and sulfur since they present the same number of electrons in the valence shell but differ in several physical–chemical properties, such as van der Waals radii, electronegativity, and lipophilicity.

The compounds were prepared by solution phase parallel synthesis in three steps according to classical synthetic procedures, as indicated in Scheme 1.[19]

The compounds were obtained in moderate to high yield and their purity was determined by HPLC (Table 1).

It was observed that the main variable at parallel synthetic approach applied to obtain these compounds was the time to accomplish the kinetic equilibrium, which varied from 15 to 60 min to the considered derivatives.

3. Biological evaluation and discussion

The 53 obtained analogues were tested, in vitro, against *L. donovani* promastigote forms at concentrations ranging from 10 to 0.01 μg/mL, as described in Section 5. The obtained IC_{50} and IC_{90} values are summarized in Table 2.

The nitroderivatives exhibited very promising activities when compared to the standard drugs pentamidine and amphotericin B. The nitrothiophene analogues **2/S** (4-Cl), **6/S** (4-OH), **8/S** (4-CF_3), and **18/S** (3,4-Cl), and the nitrofuran analogues **18/O** (3,4-Cl) and **28/O** (3-NH_2; 4-CH_3) exhibited activity higher than or close to the standard drugs.

These analogues can be tested in *Leishmania* amastigote forms and are good candidates to antileishmanial in vivo assay as well.

Hansch analysis (2D-QSAR) studies were performed by applying BuildQSAR[†] and BilinProgram[‡] softwares. Linear, parabolic, and bilinear models were taken into account but no statistically acceptable model could be established when considering classical electronic and lipophilic parameters (e.g., Log*P*, σ, π, and MR). This lack of correlation may indicate that the chosen parameters were not the most appropriate or that parameters related with steric components could be of greater importance.

Nonetheless, qualitative analyses revealed interesting information about the biological behaviour of the derivatives, as will be described below.

The non-substituted derivatives showed low activity profiles when compared to the substituted ones. Only four substituted nitrofuran analogues (**14/O**, **25/O**, 26/O, and **32/O**) presented lower activity than the non-substituted analogue (**1/O**) and only three substituted nitrothiophene analogues (**3/S**, 11/S, and **32/S**) presented lower activity than the non-substituted analogue (**1/S**). Apparently, substitution is important to improve the antileishmanial activity of the compounds. However, it was not possible to observe any standard behaviour or quantitative relationship between the physical–chemical parameters chosen and the biological activity studied.

Compounds **4/O**, **4/S**, 12/S, and **27/O** have not shown any activity at tested concentrations probably because of a solubility problem. Analogues **4/O**, 4/S, and **12/S** are all dimethylamine substituted derivatives, and could not have exhibited activity as a result of protonation at pH culture medium, lowering their availability. It is noteworthy that compound **12/O**, although also a dimethylamine substituted derivative, showed to be active. Other factors might have contributed to its activity and deserves further studies.

Biological activity can also be displayed as histogram plots as shown in Figure 2.

Those histogram plots show that nitrothiophene analogues exhibited much higher activities than the nitrofuran analogues. A hypothesis, which has been advanced to explain this effect, is that sulfur free d atomic orbitals from nitrothiophene ring can accommodate more electrons than permitted by the octet theory. Being adjacent to a nitro-linked carbon, the sulfur free d orbitals could accept the nitro group electrons thus increasing the susceptibility of the latter to reduction by unspecific nitroreductases.

4. Conclusions

In conclusion, a series of novel 5-nitroheterocyclic benzohydrazide derivatives were designed and synthesized. The antileishmanial screening revealed the following six promising analogues, which exhibited IC_{50} values lower than standard drugs pentamidine and amphotericin B: **2/S** (4-Cl), **6/S** (4-OH), **8/S** (4-CF_3), **18/S** (3,4-Cl), **18/O (3,4-Cl),** and **28/O** (3-NH_2; 4-CH_3). They are good candidates for further in vivo antileishmanial assays. Nitrothiophene derivatives showed better activity profile than the nitrofuran analogues. Further studies applying CoMFA and CoMSIA are being performed by our group to explore the possibility of existing quantitative structure–activity relationships when considering steric contributions.

5. Experimental

5.1. Parallel synthetic procedures and analytical details[19]

Mono- and di-substituted benzoic methyl esters were classically obtained from the corresponding benzoic acid in the presence of excess methanol and sulfuric acid as catalyst, after 4–6 h reflux.

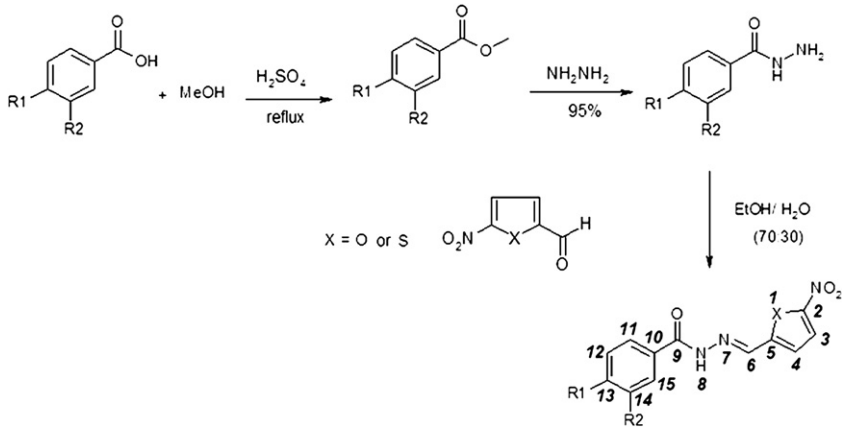
[†] Developed by Oliveira, D.B and Gáudio, A.C. from Universidade Federal do Espírito Santo—Brazil freeware (http://tau.cce.ufes.br/dfis/anderson/htmL/buildqsar_ing.htm).

[‡] Developed by Kubinyi (http://home.t-online.de/home/kubinyi).

Table 1
Synthetic library obtained by parallel synthesis

Nitrofuran derivatives					Nitrothiophene derivatives				
	R groups		Yield (%)	Purity[a] (%)		R groups		Yield (%)	Purity[a] (%)
	R_{para}	R_{meta}				R_{para}	R_{meta}		
1/O	H	H	85	100	**1/S**	H	H	91	99
2/O	Cl	H	95	100	**2/S**	Cl	H	87	100
3/O	OMe	H	52	98	**3/S**	OMe	H	85	99
4/O	$N(Me)_2$	H	44	98	**4/S**	$N(Me)_2$	H	61	99
5/O	NH_2	H	70	86	**5/S**	NH_2	H	93	97
6/O	OH	H	80	99	**6/S**	OH	H	86	99
7/O	Me	H	88	100	**7/S**	Me	H	93	99
8/O	CF_3	H	83	100	**8/S**	CF_3	H	87	100
9/O	NO_2	H	81	97	**9/S**	NO_2	H	84	98
10/O	H	Cl	95	99	**10/S**	H	Cl	93	99
11/O	H	OMe	73	98	**11/S**	H	OMe	85	99
12/O	H	$N(Me)_2$	60	99	**12/S**	H	$N(Me)_2$	88	99
14/O	H	OH	87	99	**14/S**	H	OH	86	98
15/O	H	Me	88	98	**15/S**	H	Me	83	99
16/O	H	CF_3	83	99	**16/S**	H	CF_3	87	99
17/O	H	NO_2	86	100	**17/S**	H	NO_2	93	91
18/O	Cl	Cl	71	99	**18/S**	Cl	Cl	73	97
19/O	F	F	81	99	**19/S**	F	F	80	99
22/O	F	Cl	90	100	**22/S**	F	Cl	85	99
23/O	NO_2	OMe	72	99	**23/S**	NO_2	OMe	60	99[b]
24/O	NO_2	OH	56	99	**24/S**	NO_2	OH	53	99
25/O	OMe	NH_2	30	98[b]	**25/S**	OMe	NH_2	85	99[b]
26/O	OH	NH_2	75	99	**28/S**	Me	NH_2	50	98
27/O	Cl	NO_2	59	98[b]	**29/S**	Me	OH	23	99[b]
28/O	Me	NH_2	49	100[b]	**30/S**	Me	OMe	39	99
30/O	Me	OMe	53	99	**32/S**	Cl	SO_2NH_2	63	98
32/O	Cl	SO_2NH_2	59	99					

[a] Purity determined by HPLC.
[b] Purity determined by HPLC after liquid chromatography purification. Code number/O = furan derivatives and code number/S = thiophene derivatives.



Scheme 1. Synthetic approach to obtain the library of compounds.

The hydrazide intermediates were obtained from reaction of the benzoic methyl ester with excess of hydrazine 90% in water and under heating (60 °C) and stirring. Crystals were obtained by cooling the reaction vessels with cold water followed by filtering the precipitate.

The final products were synthesized by coupling the hydrazides with the nitro-heterocyclic aldehyde (nitrofuran or nitrothiophene aldehydes) at equimolar proportions (1:1). The reactants, dissolved in a mixture of water/ethanol (2:5), were kept under reflux by times ranging from 15 min to 1 h.

All steps above were carried out in parallel using two different automatized apparatus. For the first two steps a First Mate Benchtop Synthesizer (Argonaut Technology) was used. It allows 16 simultaneous reactions in large volumes. The last step was accomplished using an Argonaut Quest 210 Parallel Organic Synthesizer (Argonaut Technology), which allowed up to 20 simultaneous reactions but with smaller volumes.

Since all reactions were produced in parallel, an initial screening was performed to determine the best conditions to a batch of reactions.

^{1}H NMR spectra were recorded on a Bruker 400 MHz Advance Systems spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. Chemical shifts were expressed in part per million (δ) and the first order values reported for coupling constants (J) in Hertz. Splitting patterns were described as follows: s (singlet), d (doublet), t (triplet), m (multiplet), and br s (broad singlet).

Elemental analyses were performed by a Perkin-Elmer CHN/SO Series II Analyzer and were within ±0.4% of the theoretical values. Some compounds presented one (*) or two (**) water molecules

Table 2
IC_{50} and IC_{90} values of the nitroderivative analogues

Samples	MW	IC_{50} (μM)	IC_{90} (μM)	Samples	MW	IC_{50} (μM)	IC_{90} (μM)
1/O	259.22	11.19	29.70	**1/S**	275.28	6.54	25.43
2/O	293.66	7.83	23.84	**2/S**	309.73	1.26	4.84
3/O	289.24	8.99	25.93	**3/S**	305.31	7.21	26.20
4/O	302.29	NA[a]	NA[a]	**4/S**	318.35	NA[a]	NA[a]
5/O	274.23	2.66	14.59	**5/S**	290.3	2.24	>34.45
6/O	275.22	2.40	17.80	**6/S**	291.28	0.89	4.46
7/O	273.24	10.98	28.55	**7/S**	289.31	4.49	7.95
8/O	327.22	8.86	24.45	**8/S**	343.28	0.82	1.89
9/O	304.22	6.57	22.35	**9/S**	320.28	2.81	5.31
10/O	293.66	7.83	24.18	**10/S**	309.73	2.74	6.78
11/O	289.24	10.37	26.62	**11/S**	305.31	8.52	32.75
12/O	302.29	7.94	24.15	**12/S**	318.35	NA[a]	NA[a]
14/O	275.22	10.17	27.98	**14/S**	291.28	2.75	7.55
15/O	273.24	19.76	>36.60	**15/S**	289.31	4.84	19.01
16/O	327.22	10.39	24.14	**16/S**	343.28	3.79	6.41
17/O	304.22	9.86	25.97	**17/S**	320.28	2.78	14.27
18/O	328.11	1.68	26.91	**18/S**	344	0.41	0.87
19/O	295.2	9.49	25.51	**19/S**	311	2.15	4.92
22/O	311.65	6.42	19.25	**22/S**	327.5	1.92	4.67
23/O	334.24	7.39	20.25	**23/S**	350	2.00	3.91
24/O	320.22	9.90	48.94	**24/S**	336	5.45	18.36
25/O	304.26	16.43	131.47	**25/S**	320	4.69	18.34
26/O	290.23	26.98	97.51	**28/S**	304	2.24	4.70
27/O	338.66	NA[a]	NA[a]	**29/S**	305	2.30	4.59
28/O	288.26	0.62	2.88	**30/S**	319	2.19	4.39
30/O	303.27	8.90	52.76	**32/S**	388.5	8.06	17.17
32/O	372.74	38.45	88.53				
Pentamidine		0.63	1.06				
Amphotericin B		1.1	1.19				

[a] NA = non-active at tested concentrations. Code number/O = furan derivatives and code number/S = thiophene derivatives.

adsorbed, which were considered on elemental analysis results. Molecules with high hygroscopicity could not be submitted to elemental analysis (***).

Mass spectra were recorded using Waters Alliance LC/MS Systems.

Flash chromatography, when necessary, was performed using 300 mesh silica gel and a mixture of ethyl acetate/hexane (2:1) as eluent. The yields were calculated after purification. HPLC purities were determined on an HPLC Alliance, Waters, and processed with Water Empower II software.

5.1.1. *N′*-[(5-Nitrofuran-2-yl)methyl]benzohydrazide (1/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.26 (s, 1H, H_4), 7.54–7.57 (d, J = 7.05, 2H, H_{12} and H_{14}), 7.60–7.62 (d, J = 6.55, 1H, H_{13}), 7.78–7.79 (d, J = 3.27, 1H, H_3), 7.90–7.92 (d, J = 6.30, 2H, H_{11} and H_{15}), 8.40 (s, 1H, H_6), 12.23 (s, 1H, H_8); MS (m/z): 258.08 [M−1]. Anal. Calcd for $C_{12}H_9N_3O_4$: *C, 51.94; H, 3.25; N, 15.15. Found: C, 51.64; H, 3.87; N, 15.08.

5.1.2. 4-Chloro-*N′*-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (2/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.27 (d, 1H, H_4), 7.59–7.61 (d, J = 7.55, 2H, H_{12} and H_{14}), 7.76–7.78 (d, J = 3.58, 1H, H_3), 7.91–7.94 (d, J = 6.76, 2H, H_{11} and H_{15}), 8.37 (s, 1H, H_6), 12.28 (s, 1H, H_8); MS (m/z): 292.01 [M−1]. Anal. Calcd for $C_{12}H_8ClN_3O_4$: *C, 46.20; H, 2.57; N, 13.48. Found: C, 45.08, H, 2.88; N, 12.93.

5.1.3. 4-Methoxy-*N′*-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (3/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.83 (s, 3H, OCH_3), 7.06–7.08 (d, J = 7.58, 2H, H_{12} and H_{14}), 7.25 (br s, 1H, H_4), 7.79–7.81 (d, J = 3.42,

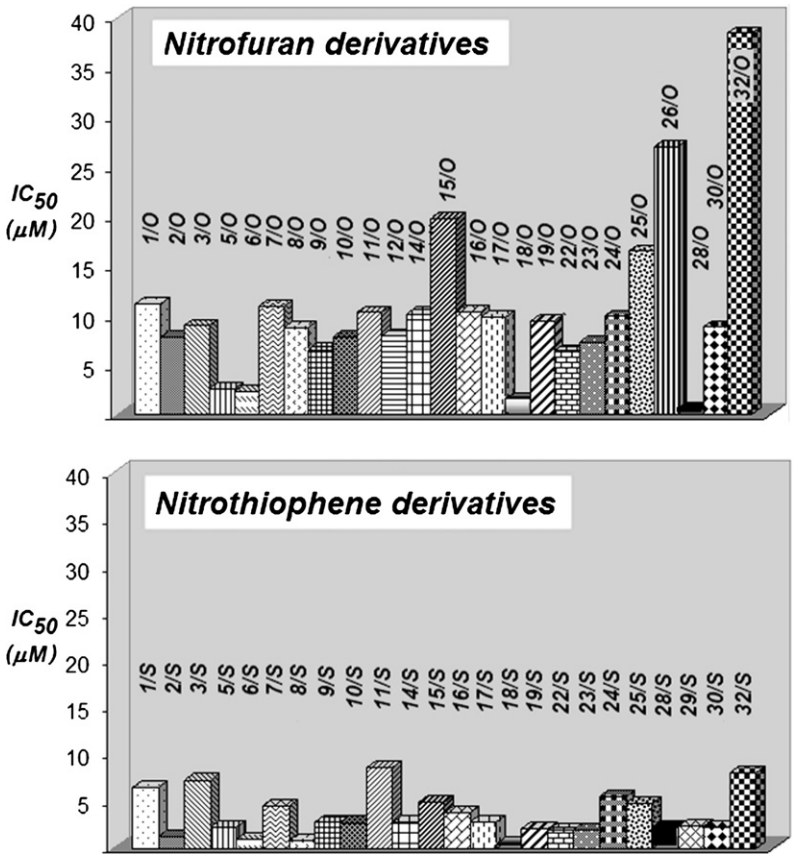


Figure 2. Histogram plot of the biological activity for nitrofuran and nitrothiophene derivatives.

1H, H_3), 7.90–7.92 (d, J = 8.31, 2H, H_{11} and H_{15}), 8.38 (s, 1H, H_6), 12.12 (s, 1H, H_8); MS (m/z): 288.14 [M−1]. Anal. Calcd for $C_{13}H_{11}N_3O_5$: C, 53.98; H, 3.83; N, 14.53. Found: C, 53.84; H, 3.58; N, 14.41.

5.1.4. 4-(Dimethylamino)-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (4/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.98 (s, 6H, $N(CH_3)_2$), 6.73–6.75 (d, J = 8.8, 2H, H_{12} and H_{14}), 7.19–7.20 (d, J = 3.91, 1H, H_4), 7.78–7.80 (m, 3H, H_3, H_{11} and H_{15}), 8.36 (s, 1H, H_6), 11.93 (s, 1H, H_8); MS (m/z): 301.16 [M−1]. Anal. Calcd for $C_{14}H_{14}N_4O_4$: °C, 52.50; H, 4.38; N, 17.50. Found: C, 52.66; H, 5.08; N, 17.68.

5.1.5. 4-Amino-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (5/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 5.89 (s, 2H, NH_2), 6.58–6.60 (d, J = 8.31, 2H, H_{12} and H_{14}), 7.19–7.21 (d, J = 3.67, 1H, H_4), 7.65–7.69 (d, J = 8.07, 2H, H_{11} and H_{15}), 7.77–7.79 (d, J = 3.42, 1H, H_3), 8.35 (s, 1H, H_6), 11.85 (s, 1H, H_8); MS (m/z): 273.04 [M−1]. Anal. Calcd for $C_{12}H_{10}N_4O_4$: C, 52.55; H, 3.65; N, 20.44. Found: C, 52.06; H, 3.52; N, 19.70.

5.1.6. 4-Hydroxy-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (6/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 6.84–6.86 (d, J = 8.31, 2H, H_{12} and H_{14}), 7.21 (br s, 1H, H_4), 7.77–7.80 (m, 3H, H_3, H_{11} and H_{15}), 8.35 (s, 1H, H_6), 10.21 (br s, 1H, OH), 12.01 (s, 1H, H_8); MS (m/z): 274.17 [M−1]. Anal. Calcd for $C_{12}H_9N_3O_5$: C, 52.36; H, 3.27; N, 15.27. Found: C, 52.28; H, 3.13; N, 15.09.

5.1.7. 4-Methyl-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (7/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.36 (s, 3H, CH_3), 7.25 (br s, 1H, H_4), 7.33–7.35 (d, J = 6.16, 2H, H_{12} and H_{14}), 7.79–7.82 (m, 3H, H_3, H_{11} and H_{15}), 8.39 (s, 1H, H_6), 12.17 (s, 1H, H_8); MS (m/z): 271.10 [M−1]. Anal. Calcd for $C_{13}H_{11}N_3O_4$: °C, 53.56; H, 3.78; N, 14.42. Found: C, 53.42; H, 4.27; N, 14.33.

5.1.8. *N*′-(5-Nitrofuran-2-yl)methylidene]-4-(trifluoromethyl)-benzohydrazide (8/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.30 (br s, 1H, H_4), 7.79 (br s, 1H, H_3), 7.91–7.93 (d, J = 7.55, 2H, H_{12} and H_{14}), 8.10–8.13 (d, J = 7.35, 2H, H_{11} and H_{15}), 8.40 (s, 1H, H_6), 12.40 (s, 1H, H_8); MS (m/z): 326.00 [M−1]. Anal. Calcd for $C_{13}H_8F_3N_3O_4$: C, 47.72; H, 2.46; N, 12.84. Found: C, 47.50; H, 2.16; N, 12.68.

5.1.9. 4-Nitro-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (9/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.31 (br s, 1H, H_4), 7.79 (br s, 1H, H_3), 8.14–8.15 (d, J = 4.97, 2H, H_{11} and H_{15}), 8.37–8.39 (d, J = 8.74, 3H, H_6, H_{12} and H_{14}), 12.49 (s, 1H, H_8); MS (m/z): 303.03 [M−1]. Anal. Calcd for $C_{12}H_8N_4O_6$: °C, 44.69; H, 2.48; N, 17.38. Found: C, 45.30; H, 2.88; N, 17.48.

5.1.10. 3-Chloro-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (10/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.26–7.29 (d, J = 9.74, 1H, H_{12}), 7.57–7.58 (d, J = 2.78, 1H, H_4), 7.68 (br s, 1H, H_3), 7.76–7.79 (d, J = 10.13, 1H, H_{13}), 7.86 (br s, 1H, H_6), 7.91–7.94 (d, J = 9.54, 1H, H_{11}), 8.35– 8.38 (d, J = 9.34, 1H, H_{15}), 12.30 (s, 1H, H_8); MS (m/z): 292.02 [M−1]. Anal. Calcd for $C_{12}H_8ClN_3O_4$: °C, 46.20; H, 2.57; N, 13.48. Found: C, 44.34; H, 3.29; N, 12.73.

5.1.11. 3-Methoxy-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (11/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.82 (s, 3H, OCH_3), 7.19 (br s, 1H, H_{13}), 7.27 (br s, 1H, H_{12}), 7.42–7.48 (m, 3H, H_3, H_4 and H_{15}), 7.79 (br s, 1H, H_{11}), 8.40 (S, 1H, H_6), 12.19 (s, 1H, H_8); MS (m/z): 287.90 [M−1]. Anal. Calcd for $C_{13}H_{11}N_3O_5$: C, 53.98; H, 3.83; N, 14.53. Found: C, 53.96; H, 3.48; N, 14.39.

5.1.12. 3-(Dimethylamino)-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (12/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.95 (s, 6H, $N(CH_3)_2$), 6.94–6.95 (d, J = 7.83, 1H, H_{13}), 7.17 (br s, 2H, H_{11} and H_{15}), 7.25 (br s, 1H, H_4), 7.31–7.33 (m, 1H, H_{12}), 7.79 (br s, 1H, H_3), 8.41 (s, 1H, H_6), 12.10 (s, 1H, H_8); MS (m/z): 300.90 [M−1]. Anal. Calcd for $C_{14}H_{14}N_4O_4$: °C, 52.45; H, 4.37; N, 17.48. Found: C, 52.78; H, 4.82; N, 17.25.

5.1.13. 3-Hydroxy-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (14/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 6.96–7.04 (d, J = 3.78, 1H, H_4), 7.24–7.34 (m, 4H, H_3, H_{12}, H_{13} and H_{15}), 7.79–7.82 (m, 1H, H_{11}), 8.40 (s, 1H, H_6), 9.84 (br s, 1H, OH), 12.17 (s, 1H, H_8); MS (m/z): 274.08 [M−1]. Anal. Calcd for $C_{14}H_{14}N_4O_4$: C, 52.37; H, 3.30; N, 15.27. Found: C, 52.11; H, 3.19; N, 15.18.

5.1.14. 3-Methyl-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (15/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.40 (s, 3H, CH_3), 7.26–7.27 (d, J = 2.5, 1H, H_4), 7.44 (br s, 2H, H_{12} and H_{13}), 7.70–7.73 (m, 2H, H_{11} and H_{15}), 7.79–7.80 (d, J = 3.5, 1H, H_3), 8.40 (s, 1H, H_6), 12.20 (s, 1H, H_8); MS (m/z): 272.17 [M−1]. Anal. Calcd for $C_{13}H_{11}N_3O_4$: C, 57.14; H, 4.03; N, 15.38. Found: C, 56.36; H, 3.94; N, 15.40.

5.1.15. *N*′-[(5-Nitrofuran-2-yl)methylidene]-3-(trifluoromethyl)-benzohydrazide (16/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.30 (br s, 1H, H_4), 7.79 (br s, 2H, H_{12} and H_3), 7.98–7.99 (d, J = 6.8, 1H, H_{13}), 8.21–8.23 (d, J = 7.6, 2H, H_{11} and H_{15}), 8.39 (s, 1H, H_6), 12.42 (s, 1H, H_8); MS (m/z): 326.01 [M−1]. Anal. Calcd for $C_{13}H_8F_3N_3O_4$: C, 47.72; H, 2.46; N, 12.84. Found: C, 47.49; H, 2.23; N, 12.80.

5.1.16. 3-Nitro-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (17/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.32 (br s, 1H, H_4), 7.80 (br s, 1H, H_3), 7.84–7.87 (t, J = 7.70, 1H, H_{12}), 8.35–8.37 (d, J = 7.09, 1H, H_{11}), 8.41 (s, 1H, H_{15}), 8.45–8.47 (d, J = 7.82, 1H, H_{13}), 8.74 (s, 1H, H_6), 12.51 (s, 1H, H_8); MS (m/z): 303.11 [M−1]. Anal. Calcd for $C_{12}H_8N_4O_6$: °C, 44.69; H, 2.48; N, 17.38. Found: C, 45.50; H, 2.75; N, 17.42.

5.1.17. 3,4-Dichloro-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (18/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.30 (br s, 1H, H_4), 7.79–7.80 (d, J = 3.9, 1H, H_{12}), 7.84–7.85 (d, J = 4.8, 1H, H_{11}), 7.89 (br s, 1H, H_{15}), 8.15 (br s, 1H, H_3), 8.37 (br s, 1H, H_6), 12.34 (s, 1H, H_8); MS (m/z): 327.85 [M−1]. Anal. Calcd for $C_{12}H_7Cl_2N_3O_4$: °C, 41.62; H, 2.02; N, 12.14. Found: C, 42.04; H, 2.51; N, 11.91.

5.1.18. 3,4-Difluoro-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (19/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.29 (br s, 1H, H_4), 7.62–7.64 (d, J = 8.8, 1H, H_{12}), 7.79 (m, 2H, H_3 and H_{15}), 7.94–7.98 (m, 1H, H_{11}), 8.36 (s, 1H, H_6), 12.27 (s, 1H, H_8); MS (m/z): 293.94 [M−1]. Anal. Calcd for $C_{12}H_7F_2N_4O_4$: °C, 46.00; H, 2.24; N, 13.42. Found: C, 46.18 H, 2.74; N, 13.46.

5.1.19. 3-Chloro-4-fluoro-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (22/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.29 (br s, 1H, H_4), 7.58–7.63 (m, 1H, H_{12}), 7.78–7.79 (d, J = 3.42, 1H, H_3), 7.89–7.99 (m, 1H, H_{11}), 8.12–8.14 (d, J = 6.34, 1H, H_{15}), 8.34 (s, 1H, H_6), 12.27 (s, 1H,

H_8); MS (*m*/*z*): 309.92 [M−1]. Anal. Calcd for $C_{12}H_7ClFN_3O_4$: *C, 43.70; H, 2.12; N, 12.75. Found: C, 43.89; H, 2.66; N, 13.51.

5.1.20. 3-Methoxy-4-nitro-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (23/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 4.01 (s, 3H, OCH_3), 7.32 (br s, 1H, H_4), 7.61–7.63 (d, J = 7.34, 1H, H_{11}), 7.78 (s, 1H, H_{15}), 7.80 (br s, 1H, H_3), 8.03–8.05 (d, J = 7.80, 1H, H_{12}), 8.41 (s, 1H, H_6), 12.38 (s, 1H, H_8); MS (*m*/*z*): 332.94 [M−1]. Anal. Calcd for $C_{12}H_7ClFN_3O_4$: C, 46.70; H, 2.99; N. 16.78. Found: C, 46.67; H, 2.88; N, 16.68.

5.1.21. 3-Hydroxy-4-nitro-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (24/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.29 (br s, 1H, H_4), 7.42–7.44 (d, J = 7.8, 1H, H_{11}), 7.59 (br s, 1H, H_{15}), 7.78 (br s, 1H, H_3), 8.00–8.02 (d, J = 8.1, 1H, H_{12}), 8.38 (s, 1H, H_6), 12.37 (s, 1H, H_8); MS (*m*/*z*): 318.95 [M−1]. Anal. Calcd for $C_{12}H_8N_4O_7$: *C, 42.60; H, 2.37; N, 16.57. Found: C, 42.59; H, 2.93; N, 16.37.

5.1.22. 3-Amino-4-methoxy-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (25/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.83 (s, 3H, OCH_3), 4.97 (s, 2H, NH_2), 6.88–6.90 (d, J = 8.3, 1H, H_{12}), 7.17 (s, 1H, H_4), 7.19–7.22 (d, J = 9.0, 2H, H_{11} and H_{15}), 7.79–7.80 (d, J = 2.9, 1H, H_3), 8.36 (s, 1H, H_6), 12.06 (s, 1H, H_8); MS: 305.05 [M+1]. Anal. Calcd for $C_{13}H_{12}N_4O_5$: not determined.

5.1.23. 3-Amino-4-hydroxy-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (26/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 4.75 (s, 2H, NH_2), 6.71–6.73 (m,1H, H_{12}), 7.02–7.06 (m, 1H, H_{15}), 7.16–7.21 (m, 2H, H_4 and H_{11}), 7.76–7.79 (m, 1H, H_3), 8.34 (s, 1H, H_6), 11.93 (s, 1H, H_8); MS (*m*/*z*): 289.01 [M−1]. Anal. Calcd for $C_{12}H_{10}N_4O_5$: C. 49.66; H, 3.45; N, 19.31. Found: C, 48.80; H, 3.56; N, 18.07.

5.1.24. 4-Chloro-3-nitro-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (27/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.29 (br s, 1H, H_4), 7.80 (br s, 1H, H_3), 7.86–7.88 (d, J = 8.1, 1H, H_{12}), 8.05–8.07 (d, J = 8.8, 1H, H_{11}), 8.29 (s, 1H, H_{15}), 8.40 (s, 1H, H_6), 12.42 (s, 1H, H_8); MS (*m*/*z*): 336.90 [M−1]. Anal. Calcd for $C_{12}H_7ClN_4O_6$: C, 42.54; H, 2.07; N, 16.54. Found: C, 42.78; H, 2.81; N, 14.42.

5.1.25. 3-Amino-4-methyl-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (28/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.07 (s, 3H, CH_3), 5.08 (s, 2H, NH_2), 7.01 (br s, 2H, H_{12} and H_{11}), 7.14 (br s, 1H. H_{15}), 7.20 (d, 1H, H_4), 7.75–7.76 (d, J = 2.9, 1H, H_3), 8.38 (s, 1H, H_6), 12.11 (s, 1H, H_8); MS (*m*/*z*): 286.90 [M−1]. Anal. Calcd for $C_{13}H_{12}N_4O_4$: C, 49.66; H, 3.45; N, 19.31. Found: C, 49.61; H, 3.24; N, 19.56.

5.1.26. 3-Methoxy-4-methyl-*N*′-[(5-nitrofuran-2-yl)methylidene]-benzohydrazide (30/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.21 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 7.27 (br s, 1H, H_4), 7.29–7.31 (d, J = 7.58, 1H, H_{12}), 7.43–7.45 (d, J = 7.80, 2H, H_{11} and H_{15}), 7.79–7.80 (d, J = 2.9, 1H, H_3), 8.41 (s, 1H, H_6), 12.14 (s, 1H, H_8); MS (*m*/*z*): 302.01 [M−1]. Anal. Calcd for $C_{14}H_{13}N_3O_5$: C, 55.40; H, 4.29; N, 13.90. Found: C, 53.95; H, 4.30; N, 13.38.

5.1.27. 2-Chloro-5-({2-[(5-nitrofuran-2-yl)methylidene]-hydrazino}carbonyl)benzene sulfonamide (32/O)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.31 (br s, 1H, H_4), 7.77–7.80 (m, 3H, H_3 and SO_2NH_2), 7.85–7.87 (d, J = 7.83, 1H, H_{12}), 8.12–8.14 (d, J = 7.30, 1H, H_{11}), 8.41 (br s, 1H, H_{15}), 8.52 (s, 1H, H_6), 12.48 (s, 1H, H_8); MS (*m*/*z*): 370.88 [M−1]. Anal. Calcd for $C_{12}H_9ClN_4O_6S$: C, 38.66; H, 2.42; N, 15.03. Found: C, 39.20; H, 2.49; N, 15.33.

5.1.28. *N*′-[(5-Nitrothiophen-2-yl)methylidene]benzohydrazide (1/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.51–7.60 (m, 4H, H_4, H_{12}, H_{13} and H_{14}), 7.88–7.90 (d, J = 6.9, 2H, H_{11} and H_{15}), 8.12 (br s, 1H, H_3), 8.67 (s, 1H, H_6), 12.22 (s, 1H, H_8); MS (*m*/*z*): 274.00 [M−1]. Anal. Calcd for $C_{12}H_9N_3O_3S$: C, 52.31; H, 3.27; N, 15.26. Found: C, 52.21; H, 3.24; N, 15.34.

5.1.29. 4-Chloro-*N*′-[(5-Nitrothiophen-2-yl)methylidene]-benzohydrazide (2/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.60–7.62 (m, 3H, H_4, H_{12} and H_{14}), 7.91–7.93 (d, J = 7.8, 2H, H_{11} and H_{15}), 8.12 (s, 1H, H_3), 8.65 (s, 1H, H_6), 12.28 (s, 1H, H_8); MS (*m*/*z*): 307.95 [M−1]. Anal. Calcd for $C_{12}H_8ClN_3O_3S$: C, 46.49; H, 2.58; N, 13.56. Found: C, 46.22; H, 2.39; N, 13.70.

5.1.30. 4-Methoxy-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (3/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.83 (s, 3H, OCH_3), 7.05–7.07 (d, J = 8.60, 2H, H_{12} and H_{14}), 7.55–7.56 (d, J = 3.18, 1H, H4), 7.88–7.90 (d, J = 7.80, 2H, H_{11} and H15), 8.11-8.12 (d, J = 3.9, 1H, H_3), 8.66 (s, 1H, H_6), 12.10 (s, 1H, H_8); MS (*m*/*z*): 304.07 [M−1]. Anal. Calcd for $C_{13}H_{11}N_3O_4S$: C, 51.15; H, 3.61; N, 13.77. Found: C, 50.48; H, 3.42, N, 13.72.

5.1.31. 4-(Dimethylamino)-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (4/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.97 (s, 6H, $N(CH_3)_2$), 6.72–6.74 (d, J = 8.8, 2H, H_{12} and H_{14}), 7.49–7.50 (d, J = 3.9, 1H, H_4), 7.77–7.79 (d, J = 8.8, 2H, H_{11} and H_{15}), 8.09–8.10 (d, J = 4.2, 1H, H_3), 8.63 (s, 1H, H_6), 11.91 (s, 1H, H_8); MS (*m*/*z*): 317.16 [M−1]. Anal. Calcd for $C_{14}H_{14}N_4O_3S$: *C, 50.00; H, 4.17; N, 16.67. Found: C, 49.91; H, 4.51; N, 16.80.

5.1.32. 4-Amino-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (5/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 5.86 (s, 2H, NH_2), 6.56–6.58 (d, J = 8.3, 2H, H_{12} and H_{14}), 7.48–7.49 (d, J = 3.7, 1H, H_4), 7.62–7.64 (d, J = 8.3, 2H, H_{11} and H_{15}), 8.08–8.09 (d, J = 3.9, 1H, H_3), 8.59 (S, 1H, H_6), 11.82 (s, 1H, H_8); MS (*m*/*z*): 289.01 [M−1]. Anal. Calcd for $C_{12}H_{10}N_4O_3S$: *C, 49.60; H, 3.44; N, 19.29. Found: C, 49.07; H, 3.37; N, 19.26.

5.1.33. 4-Hydroxy-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (6/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 6.85–6.87 (d, J = 8.3, 2H, H_{12} and H_{14}), 7.53–7.54 (d, J = 3.9, 1H, H_4), 7.78–7.80 (d, J = 8.1, 2H, H_{11} and H_{15}), 8.11–8.12 (d, J = 4.2, 1H, H_3), 8.64 (s, 1H, H_6), 10.2 (s, 1H, OH), 12.01 (s, 1H, H_8); MS (*m*/*z*): 290.01 [M−1]. Anal. Calcd for $C_{12}H_9N_3O_4S$: *C, 49.48; H, 3.11; N, 14.43. Found: C, 49.52; H, 2.79; N, 14.35.

5.1.34. 4-Methyl-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (7/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.37 (s, 3H, CH_3), 7.33–7.35 (d, J = 7.6, 2H, H_{12} and H_{14}), 7.55–7.56 (d, J = 2.9, 1H, H_4), 7.80–7.82 (d, J = 6.6, 2H, H_{11} and H_{15}), 8.11–8.12 (d, J = 3.9, 1H, H_3), 8.67 (s, 1H, H_6), 12.15 (s, 1H, H_8); MS (*m*/*z*): 288.07 [M−1]. Anal. Calcd for $C_{13}H_{11}N_3O_3S$: *C, 50.76; H, 3.58; N, 13.67. Found: C, 51.07; H, 3.99; N, 13.64.

5.1.35. *N*′-[(5-Nitrothiophen-2-yl)methylidene]-4-(trifluoromethyl)benzohydrazide (8/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.58–7.59 (d, J = 2.77, 1H, H_4), 7.90–7.92 (d, J = 7.81, 2H, H_{12} and H_{14}), 8.08–8.11 (d, J = 8.81, 3H, H_3, H_{11} and H_{15}), 8.66 (s, 1H, H_6), 12.40 (s, 1H, H_8); MS (*m*/*z*):

341.96 [M−1]. Anal. Calcd for $C_{13}H_8F_3N_3O_3S$: C, 45.44; H, 2.33; N, 12.23. Found: C, 45.21; H, 2.10; N, 12.42.

5.1.36. 4-Nitro-*N*′-[(5-Nitrothiophen-2-yl)methylidene]-benzohydrazide (9/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.60 (br s, 1H, H_4), 8.12–8.13 (d, J = 7.1, 3H, H_3, H_{11} and H_{15}), 8.36–8.38 (d, *J* = 8.3, 2H, H_{12} and H_{14}), 8.66 (s, 1H, H_6), 12.49 (s, 1H, H_8); MS (*m*/*z*): 318.98 [M−1]. Anal. Calcd for $C_{12}H_8N_4O_5S$: C, 45.00; H, 2.52; N, 17.49. Found: C, 44.62; H, 2.27; N, 17.26.

5.1.37. 3-Hydroxy-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (10/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.55–7.59 (m, 2H, H_4 and H_{12}), 7.67–7.69 (d, *J* = 7.3, 1H, H_{13}), 7.85–7.87 (d, *J* = 7.3, 1H, H_{11}), 7.94 (s, 1H, H_{15}), 8.12–8.13 (d, *J* = 3.4, 1H, H_3), 8.65 (s, 1H, H_6), 12.29 (s, 1H, H_8);MS (*m*/*z*): 290.02 [M−1]. Anal. Calcd for $C_{12}H_9N_3O_4S$: C, 46.49; H, 2.58; N, 13.56. Found: C, 45.26; H, 2.54; N, 13.27.

5.1.38. 3-Methoxy-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (11/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.82 (s, 3H, OCH_3), 7.18 (br s, 1H, H_{13}), 7.44–7.46 (m, 3H, H_3, H_4 and H_{12}), 7.57 (br s, 1H, H_{15}), 8.12 (br s, 1H, H_{11}), 8.68 (s, 1H, H_6), 12.18 (s, 1H, H_8); MS (*m*/*z*): 304.10 [M−1]. Anal. Calcd for $C_{13}H_{11}N_3O_4S$: *C, 48.29; H, 3.41; N, 13.00. Found: C, 48.04; H, 3.84; N, 13.04.

5.1.39. 3-(Dimethylamino)-*N*′-[(5-nitrothiophen-2-yl)methylidene]benzohydrazide (12/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.95 (s, 6H, $N(CH_3)_2$), 6.93–6.95 (d, *J* = 8.3, 1H, H_{13}), 7.16 (br s, 2H, H_{11} and H_{15}), 7.30–7.34 (t, *J* = 8.3, 1H, H_{12}), 7.55–7.56 (d, *J* = 3.9, 1H, H_4), 8.11–8.13 (d, *J* = 4.4, 1H, H_3), 8.70 (s, 1H, H_6), 12.10 (s, 1H, H_8); MS (*m*/*z*): 317.12 [M−1]. Anal. Calcd for $C_{14}H_{14}N_4O_3S$: *C, 50.00; H, 4.17; N, 16.67. Found: C, 49.29; H, 4.63; N, 16.60.

5.1.40. 3-Hydroxy-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (14/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.00 (br s, 1H, H_4), 7.28–7.33 (m, 3H, H_{12}, H_{13} and H_{15}), 7.57 (br s, 1H, H_3), 8.13 (s, 1H, H_{11}), 8.66 (s, 1H, H_6), 12.15 (s, 1H, H_8); MS (*m*/*z*): 290.04 [M−1]. Anal. Calcd for $C_{12}H_9N_3O_4S$: C, 49.44; H, 3.09; N, 14.42. Found: C, 49.28; H, 2.85; N, 14.49.

5.1.41. 3-Methyl-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (15/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.38 (s, 3H, H_{16}), 7.41 (br s, 2H, H_{12} and H_{13}), 7.56 (br s, 1H, H_4), 7.69 (br s, 2H, H_{11} and H_{15}), 8.11 (br s, 1H, H_3), 8.66 (s, 1H, H_6), 12.18 (s, 1H, H_8); MS (*m*/*z*): 288.12 [M−1]. Anal. Calcd for $C_{13}H_{11}N_3O_3S$: *C, 50.81; H, 3.58; N, 13.68. Found: C, 50.78, H, 4.13; N, 13.85.

5.1.42. *N*′-[(5-Nitrothiophen-2-yl)methylidene]-3-(trifluoromethyl)benzohydrazide (16/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.61-7.62 (d, *J* = 3.4, 1H, H_4), 7.78–7.82 (t, *J* = 7.6, 1H, H_{12}), 7.98–8.00 (d, *J* = 7.3, 1H, H_{13}), 8.13–8.14 (d, *J* = 3.4, 1H, H $_3$), 8.20–8.23 (m, 2H, H_{11} and H_{15}), 8.67 (s, 1H, H_6), 12.40 (s, 1H, H_8); MS (*m*/*z*): 341.96 [M−1]. Anal. Calcd for $C_{13}H_8F_3N_3O_3S$: *C, 43.18; H, 2.21; N, 11.63. Found: C, 43.38; H, 2.57; N, 11.53.

5.1.43. 3-Nitro-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (17/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.59–7.60 (d, *J* = 3.3, 1H, H_4), 7.82–7.86 (t, *J* = 8.1, 1H, H_{12}), 8.11–8.12 (d, *J* = 4.0, 1H, H_3), 8.32–8.34 (d, *J* = 7.6, 1H, H_{11}), 8.43–8.45 (d, *J* = 7.8, 1H, H_{13}), 8.66 (s, 1H, H_{15}), 8.72 (s, 1H, H_6), 12.50 (s, 1H, H_8); MS (*m*/*z*): 319.03 [M−1]. Anal. Calcd for $C_{12}H_8N_4O_5S$: **C, 44.17; H, 2.45; N, 17.18. Found: C, 44.76; H, 2.44; N, 16.75.

5.1.44. 3,4-Dichloro-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (18/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.63–7.64 (d, *J* = 4.4, 1H, H_4), 7.85–7.87 (d, *J* = 8.3, 1H, H_{12}), 7.89–7.91 (d, *J* = 8.8, 1H, H_{11}), 8.17 (br s, 2H, H_3 and H_{15}), 8.66 (s, 1H, H_6), 12.37 (s, 1H, H_8); MS (*m*/*z*): 343.87 [M−1]. Anal. Calcd for $C_{12}H_7Cl_2N_3O_3S$: C, 41.86; H, 2.03; N, 12.21. Found: C, 41.16; H, 2.06; N, 11.81.

5.1.45. 3,4-Difluoro-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (19/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.61–7.67 (m, 2H, H_4 and H_{12}), 7.80 (br s, 1H, H_{15}), 7.94–7.98 (t, *J* = 9.8, 1H, H_{11}), 8.12–8.13 (d, *J* = 2.0, 1H, H_3), 8.65 (s, 1H, H_6), 12.27 (s, 1H, H_8); MS (*m*/*z*): 309.93 [M−1]. Anal. Calcd for $C_{12}H_7F_2N_3O_3S$: C, 46.30; H, 2.25; N, 13.50. Found: C, 47.43; H, 2.18; N, 13.67.

5.1.46. 3-Chloro-4-fluoro-*N*′-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide(22/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.62–7.64 (d, *J* = 8.3, 2H, H_4 and H_{12}), 7.95 (br s, 1H, H_3), 8.13–8.15 (br s, 2H, H_{11} and H_{15}), 8,65 (s, 1H, H_6), 12,31 (s, 1H, H_8); MS (*m*/*z*): 324.90 [M−1]. Anal. Calcd for $C_{12}H_7ClFN_3O_3S$: *C, 43.97; H, 2.14; N, 12.82. Found: C, 41.95; H, 2.66; N, 12.33.

5.1.47. 3-Methoxy-4-nitro-*N*′-[(5-nitrothiophen-2-yl)methylidene]benzohydrazide (23/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.99 (s, 3H, H_{16}), 7.57–7.62 (m, 2H, H_4 and H_{11}), 7.75 (br s, 1H, H_{15}), 8.01–8.03 (d, *J* = 7.8, 1H, H_{12}), 8.14 (br s, 1H, H_3), 8.68 (s, 1H, H_6), 12.37 (s, 1H, H_8); MS (*m*/*z*): 348.96 [M−1]. Anal. Calcd for $C_{13}H_{10}N_4O_6S$: C = 44.60; H, 2.86; N, 16.00. Found: C, 44.32; H, 2.93; N, 15.76.

5.1.48. 3-Hydroxy-4-nitro-*N*′-[(5-nitrothiophen-2-yl)-methylidene]benzohydrazide (24/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.42–7.44 (d, *J* = 8.3, 1H, H_{15}), 7.59–7.61 (m, 2H, H_4 and H_{11}), 8.00–8.02 (d, *J* = 8.3, 1H, H_{12}), 7.13–8.14 (d, *J* = 3.7, 1H, H_3), 8.67 (s, 1H, H_6), 12.37 (s, 1H, H_8); MS (*m*/*z*): 334.93 [M−1]. Anal. Calcd for $C_{12}H_8N_4O_6S$: *C, 40.68; H, 2.26; N, 15.82. Found: C, 41.68; H, 2.73; N, 15.74.

5.1.49. 3-Amino-4-methoxy-*N*′-[(5-nitrothiophen-2-yl)-methylidene]benzohydrazide (25/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.83 (s, 3H, OCH_3), 4.99 (S, 2H, NH_2), 6.89–6.91 (d, *J* = 8.1, 1H, H_{12}), 7.15–7.19 (m, 2H, H_{11} and H_{15}), 7.53–7.54 (d, *J* = 3.9, 1H, H_4), 8.12-8.13 (d, *J* = 3.9, 1H, H_3), 8.64 (s, 1H, H_6), 11.99 (s, 1H, H_8); MS (*m*/*z*): 318.99 [M−1]. Anal. Calcd for $C_{13}H_{12}N_4O_4S$: not determined.

5.1.50. 3-Amino-4-hydroxy-*N*′-[(5-nitrothiophen-2-yl)-methylidene]benzohydrazide (26/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 4.75 (br s, 2H, NH_2), 6.71-6.74 (d, *J* = 8.3, 1H, H_{12}), 7.06–7.08 (d, *J* = 8.1, 1H, H_{15}), 7.17 (br s, 1H, H_{11}), 7.50–7.51 (d, *J* = 4.3, 1H, H_4), 8.10–8.11 (d, *J* = 4.3, 1H, H_3), 8.64 (s, 1H, H_6), 11.96 (s, 1H, H_8); MS (*m*/*z*): 304.90 [M−1]. Anal. Calcd for $C_{12}H_{10}N_4O_4S$: not determined.

5.1.51. 3-Amino-4-methyl-*N*′-[(5-nitrothiophen-2-yl)-methylidene]benzohydrazide (28/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.10 (s, 3H, CH_3), 5.11 (s, 2H, NH_2), 7.00–7.06 (m, 2H, H_{12} and H_{11}), 7.13 (br s, 1H, H_{15}), 7.53–7.54 (d, *J* = 3.7, 1H, H_4), 7.75–7.76 (d, *J* = 3.9, 1H, H_3), 8.98 (s, 1H,

H_6), 12.00 (s, 1H, H_8); MS: 305.05 [M+1]. Anal. Calcd for $C_{13}H_{12}N_4O_3S$: C, 45.90; H, 3.53; N, 16.47. Found: C, 45.18, H, 3.33, N, 17.45.

5.1.52. 3-Hydroxy-4-methyl-*N*′-[(5-nitrothiophen-2-yl)-methylidene]benzohydrazide (29/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.16 (s, 3H, CH_3), 7.17–7.91 (d, J = 7.8, 1H, H_{12}), 7.26–7.28 (d, J = 7.8, 1H, H_{11}), 7.32 (s, 1H, H_{15}), 7.52–7.53 (d, J = 3.9, 1H, H_4), 8.10–8.12 (d, J = 4.2, 1H, H_3), 8.64 (s, 1H, H_6), 9.72 (s, 1H, OH), 12.13 (s, 1H, H_8); MS (m/z): 303.98 [M−1]. Anal. Calcd for $C_{13}H_{11}N_3O_4S$: not determined.

5.1.53. 3-Methoxy-4-methyl-*N*′-[(5-nitrothiophen-2-yl)-methylidene]benzohydrazide (30/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 2.21 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 7.27–7.30 (d, J = 7.34, 1H, H_{12}), 7.42 (br s, 2H, H_{11} and H_{15}), 7.56–7.57 (d, J = 3.7, 1H, H_4), 8.14–8.16 (d, J = 3.9, 1H, H_3), 8.70 (s, 1H, H_6), 12.13 (s, 1H, H_8); MS (m/z): 317.97 [M−1]. Anal. Calcd for $C_{14}H_{13}N_3O_4S$: C, 52.70; H, 4.08; N, 13.17. Found: C, 52.72; H, 3.97; N, 12.94.

5.1.54. 2-Chloro-5-({2-[(5-nitrothiophen-2-yl)methylidene]-hydrazino}carbonyl) benzene sulfonamide (32/S)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 7.60–7.61 (d, J = 2.7, 1H, H_4), 7.76 (br s, 2H, SO_2NH_2), 7.84–7.86 (d, J = 8.1, 1H, H_{12}), 8.11–8.14 (m, 2H, H_3 and H_{11}), 8.50 (s, 1H, H_{15}), 8.68 (s, 1H, H_6), 12.50 (s, 1H, H_8); MS (m/z): 386.85 [M−1]. Anal. Calcd for $C_{12}H_9ClN_4O_5S_2$: C, 37.07; H, 2.32; N, 14.41. Found: C, 37.35; H, 2.24; N, 14.62.

5.2. Antileishmanial assays

Antileishmanial activity of the compounds was tested in vitro against a culture of *L. donovani* promastigotes. The parasites were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco Chem Co.) at 26 °C. A 3-day-old culture was diluted to 5×10^5 promastigotes/mL. Samples were tested at concentrations from 50 to 3.1 μg/mL. Drug dilutions were prepared directly in cell suspension in 96-well plates and were incubated at 26 °C for 48 h and growth of *Leishmania* promastigotes was determined by Alamar Blue assay.[20] Standard fluorescence was measured on a Fluostar Galaxy plate reader (BMG Lab Technologies) at excitation wavelength of 544 nm and emission wavelength of 590 nm. Pentamidine and amphotericin B were used as the standard antileishmanial agents. Percentual growth was calculated and plotted versus test concentration for computing the IC_{50} values and IC_{90}.

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