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Original article

Synthesis and evaluation of monoamidoxime derivatives: Toward new antileishmanial compounds

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ABSTRACT

A new series of monoamidoxime derivatives was synthesized using manganese(III) acetate by microwave irradiation. Several amidoximes (**27**–**31**, **33**, **38**) showed valuable *in vitro* activities toward *Leishmania donovani* promastigotes, exhibiting IC_{50} values between 5.21 and 7.89 μM. In parallel, the cytotoxicity of these compounds was evaluated on murine J774A.1 cells, revealing the corresponding selectivity index (SI). Among the 13 tested compounds, 4 monoamidoximes (**27**–**30**) exhibited an SI more than 20 times better than pentamidine. Moreover, monoamidoxime **28** (4-[5-Benzyl-3-(4-fluorophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]-N′-hydroxybenzimidamide) is 40 times more selective than pentamidine, and 1.6 times more than amphotericin B, used as reference drug compounds.

1. Introduction

Leishmaniasis is one of the most widespread parasitic diseases. It is transmitted by the bite of a sand-fly contaminated by a flagellate protozoan belonging to the genus *Leishmania*. The disease is endemic in 89 countries and its visceral form, caused by *Leishmania donovani*, leads to 5,00,000 new cases and 50,000 deaths per year [1].

1,5-Bis(4-amidinophenoxy)pentane (pentamidine, Scheme 1) is a well-known antiprotozoan aromatic diamidine [2]. It is commonly used against various infections such as leishmaniasis [3,4], trypanosomiasis [3,5] and HIV-related *Pneumocystis jirovecii* opportunistic pneumonia [6]. In spite of its wide antiparasitic spectrum, the use of pentamidine remains limited by its high toxicity, in particular nephro-, cardio- and neurological toxicities [2,7]. Furthermore, at physiological pH, amidine groups are salified, decreasing membrane permeability and thus necessitating parenteral administration [8].

Arylamidines are known to bind to the minor groove of AT DNA sequences along the phosphodiester backbone [9,10]. Two amidinium end groups appear to be necessary for this interaction [11] while the central part of the drug inserts into the minor groove. Among the series of synthetic arylamidines, pafuramidine (Scheme 1) has shown interesting therapeutic activities against *L. donovani* and offers good oral bioavailability due to the replacement of amidines by methoxyamidoximes [12].

In the course of our ongoing work on the preparation of antiparasitic compounds [13], we have previously reported the synthesis of diarylamidoxime derivatives, yielding a 2,3-dihydrofuran instead of the furan scaffold of pafuramidine [14]. Several of these diamidoxime derivatives exhibited good activity against *L. donovani*. Surprisingly, monoamidoxime derivatives also had good activity, whereas with amidoxime, two scaffolds appeared to be necessary for antiparasitic potential. We present herein a complementary study in which we synthesized various substituted monoamidoximes, in order to confirm and further characterize this newly-observed activity.

2. Results and discussion

2.1. Chemistry

Several β-ketosulfones (**1**–**13**) were synthesized using a previously reported microwave-irradiated method [15].

Sodium sulfite, sodium bicarbonate and sulfonyl chlorides in water were irradiated at 500 W for 20 min in a microwave oven. An ethanolic solution of the corresponding acetophenone was then

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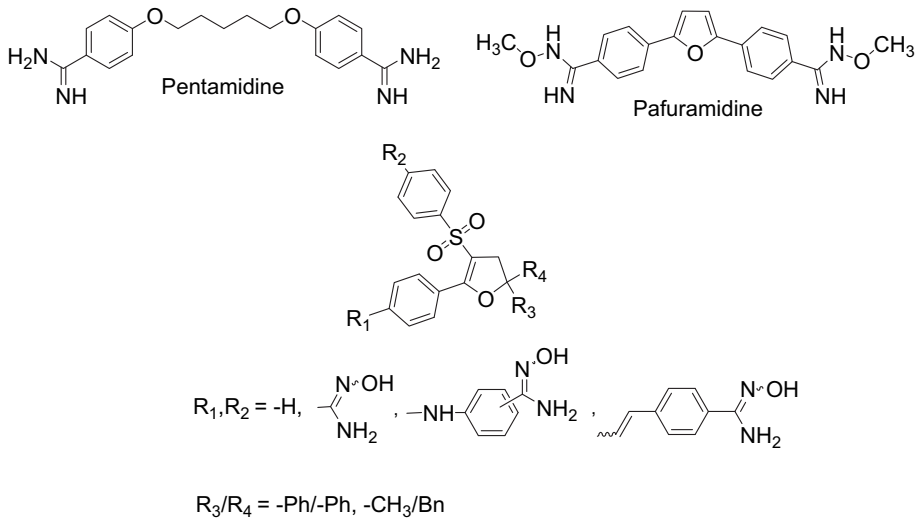


Scheme 1.

added to sodium sulfinate and the reaction mixture was irradiated for 10 min to produce sulfones in good yields (**1**–**13**) (Scheme 2).

Following a previously described procedure [16], manganese(III) acetate was added to glacial acetic acid, and the suspension was irradiated in a microwave oven at 80 °C for 15 min until solubilization. To this solution, β-ketosulfone **1**–**13** and (2-methylallyl)benzene were added and the mixture was irradiated (200 W) for 60 min.

The desired 2,3-dihydrofuran derivatives (**14**–**26**) were obtained (Scheme 3) in low to good yields. Yields highly depend on the R_1 substituent on the acetophenone moiety.

Finally, 10 equivalents of hydroxylamine hydrochloride and potassium *tert*-butoxide were added to previously synthesized nitrile derivative (**14**–**26**) in DMSO (Scheme 4) [17]. Corresponding monoamidoximes (**27**–**39**) were obtained in good yields (Table 1.).

2.2. Biology

Synthesized amidoximes **27**–**39** were evaluated *in vitro* for their activity against promastigotes of *L. donovani* strain MHOM/IN/00/DEVI and for their cytotoxicity toward mouse J774A.1 macrophages that serve as an *in vitro* host cell model for screening antileishmanial drugs [18]. The results of the evaluation are summarized in Table 2.

2.2.1. Antiparasitic activity

All monoamidoxime derivatives tested exhibited antileishmanial activity. One of them, monoamidoxime **30**, showed better antileishmanial activity than that of pentamidine (IC_{50} = 5.21 μM *versus* 6.29 μM). Moreover, 6 other monoamidoximes **27**–**29**, **31**, **33**, **38** (Scheme 5) had antileishmanial activities close to that of pentamidine (IC_{50} between 6.29 and 7.89 μM).

Substituents such as halogen, trifluoromethyl or methoxy groups improved antileishmanial activity on monoamidoximes. The best activities were obtained for substituents carried on the sulfone moiety with amidoxime carried on the other benzenic moiety. Given the IC_{50} of monoamidoximes **32** and **39**, nitro groups seem to decrease antileishmanial activities.

2.2.2. Cytotoxicity

Compared to the cytotoxicity of pentamidine (IC_{50} = 1.03 μM) and amphotericin B (IC_{50} = 3.14 μM), the cytotoxicity of monoamidoximes ranged from low to moderate (50.65–7.05 μM).

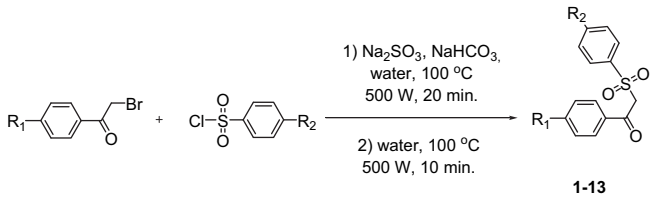
For the most active compounds, cytotoxicity values of monoamidoximes **27**–**30** were low (20.07–45.53 μM). Cytotoxicity seems to be increased by iodo and methoxy substituents when amidoxime is carried on the sulfone moiety.

By comparing antileishmanial activity and cytotoxicity we determined a selectivity index. This SI is better than that of pentamidine (0.16) for all of our molecules. 4 monoamidoximes (**27**–**30**) are over 20 times more selective than pentamidine (3.71–6.37), and the most interesting monoamidoxime, **28**, which bears a fluoro-substituent on the sulfone moiety (Scheme 6), is 40 times more selective than pentamidine and 1.6 times more than amphotericin B.

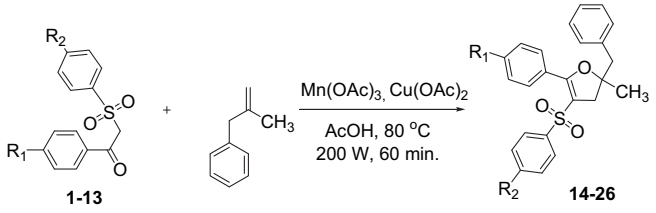
3. Conclusion

In conclusion, manganese(III)-assisted reactions allowed the synthesis of a new series of monoamidoximes. Thirteen tested products exhibited antileishmanial activity and moderate cytotoxicity with a better selectivity index compared to pentamidine, used as reference.

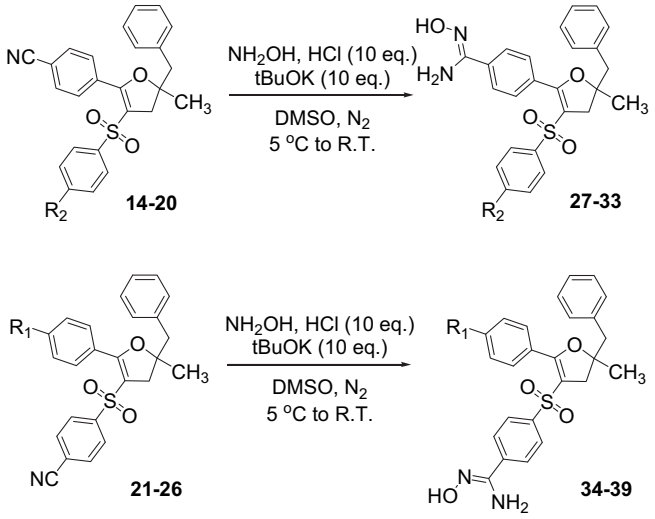
Moreover, molecule **30**, a bromo derivative, has the lowest IC_{50} against *L. donovani* promastigote (5.21 μM *versus* 6.29 μM for pentamidine), and molecule **28**, a fluoro derivative, has an interesting activity coupled with low cytotoxicity, making it 40 times



Scheme 2.



Scheme 3.



Scheme 4.

more selective than pentamidine, and 1.6 times more than amphotericin B. With a view to refining these findings, pharmacomodulation studies are in progress, targeting substituents on position 5 of the dihydrofuran.

4. Experimental

4.1. Chemistry

4.1.1. Instruments and analyses

Microwave-assisted reactions were performed in a multimode microwave oven ETHOS Synth Lab Station (Ethos start, Milestone Inc.). Melting points were determined with a B-540 Büchi melting point apparatus. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a Bruker ARX 200 spectrometer at the Service Interuniversitaire de RMN de la Faculté de Pharmacie de Marseille. ^{1}H and ^{13}C NMR chemical shifts (δ) are reported in parts per million with respect to $CDCl_3$ 7.26 ppm (^{1}H) and 77 ppm (^{13}C). Elemental analysis was carried out at the Spectropole de la Faculté des Sciences et Techniques de Saint-Jérôme. The following adsorbent was used for flash column chromatography: silica gel 60 (Merck, particle size 0.063–0.200 nm, 70–230 mesh ASTM). TLC was performed on 5 cm × 10 cm aluminum plates coated with silica gel $60F_{254}$ (Merck) in appropriate solvent. Mass spectra were run on an API-QqToF mass spectrometer.

4.1.2. β-Ketosulfones synthesis

Compounds **1**–**13** were synthesized according to previously described procedures [14,15].

*4.1.2.1. -{2-[4-(Trifluoromethyl)phenylsulfonyl]acetyl}benzonitrile (**1**).* White solid, mp 186–187 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 4.78 (s, 2H, CH_2), 7.82 (d, J = 8.6, 2H, 2CH), 7.87 (d, J = 8.1, 2H, 2CH), 8.04 (d, J = 8.1, 2H, 2CH), 8.08 (d, J = 8.6, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 63.3 (CH_2), 117.4 (C), 117.8 (C), 122.9 (q, J = 273.3, C), 126.5 (q, J = 4.0, 2CH), 129.3 (2CH), 129.7 (2CH), 132.7 (2CH), 136.2 (q, J = 33.3, C), 138.2 (C), 141.7 (C), 186.7 (C). Anal. Calcd for $C_{16}H_{10}F_3NO_3S$ (353.32 g mol^{-1}): C, 54.39; H, 2.85; N, 3.96. Found: C, 54.43; H, 2.75; N, 3.94.

*4.1.2.2. -[2-(4-Fluorophenylsulfonyl)acetyl]benzonitrile (**2**).* White solid, mp 160–161 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 4.74 (s, 2H, CH_2), 7.21–7.30 (m, 2H, 2CH), 7.82 (d, J = 8.4, 2H, 2CH), 7.88–7.94 (m, 2H, 2CH), 8.08 (d, J = 8.4, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 63.7 (CH_2), 116.8 (d, J = 22.7, 2CH), 117.5 (C), 117.7 (C), 129.7 (2CH), 131.6 (d, J = 9.9, 2CH), 132.7 (2CH), 134.2 (d, J = 3.3, C), 138.3 (C), 166.3 (d, J = 258.3, C), 187.0 (C). Anal. Calcd for $C_{15}H_{10}FNO_3S$ (303.31 g mol^{-1}): C, 59.40; H, 3.32; N, 4.62. Found: C, 59.35; H, 3.35; N, 4.59.

*4.1.2.3. 4-[2-(4-Chlorophenylsulfonyl)acetyl]benzonitrile (**3**).* White solid, mp 146–148 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 4.75 (s, 2H, CH_2), 7.54 (d, J = 8.4, 2H, 2CH), 7.78–7.82 (m, 4H, 4CH), 8.06 (d, J = 8.4, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 63.5 (CH_2), 117.4 (C), 117.6 (C), 129.6 (2CH), 129.7 (2CH), 130.0 (2CH), 132.6 (2CH), 136.6 (C), 138.3 (C), 141.4 (C), 186.9 (C). Anal. Calcd for $C_{15}H_{10}ClNO_3S$ (319.76 g mol^{-1}): C, 56.34; H, 3.15; N, 4.38. Found: C, 55.94; H, 3.10; N, 4.30.

*4.1.2.4. 4-[2-(4-Bromophenylsulfonyl)acetyl]benzonitrile **(4)** [14]*

*4.1.2.4.1. 4-[2-(4-Iodophenylsulfonyl)acetyl]benzonitrile (**5**).* White solid, mp 179 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 4.72 (s, 2H, CH_2), 7.57 (d, J = 8.6, 2H, 2CH), 7.82 (d, J = 8.5, 2H, 2CH), 7.94 (d, J = 8.6, 2H, 2CH), 8.07 (d, J = 8.5, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 63.6 (CH_2), 102.9 (C), 117.5 (C), 117.7 (C), 129.7 (2CH), 129.8 (2CH), 132.7 (2CH), 137.8 (C), 138.3 (C), 138.7 (2CH), 186.9 (C). Anal. Calcd

Table 1
Synthesis of amidoximes from nitriles.

Compound	R_1	R_2	Yield (%)[a]
27		$-CF_3$	91
28		$-F$	85
29		$-Cl$	79
30		$-Br$	89
31		$-I$	83
32		$-NO_2$	80
33		$-OCH_3$	89
34	$-CF_3$		81
35	$-F$		93
36	$-Cl$		91
37	$-Br$		56
38	$-I$		83
39	$-NO_2$		55

[a] Yield of isolated product based on the corresponding 2,3-dihydrofuran.

Table 2
In vitro activity/cytotoxicity of compounds **27**–**39**.

Compound	*Leishmania donovani*, promastigotes IC_{50} (μM) ± SD	Cytotoxicity J774A.1 IC_{50} (μM) ± SD	Selectivity Index (SI)
27	7.89 ± 2.23	30.52 ± 2.23	3.87
28	7.15 ± 2.17	45.53 ± 0.28	6.37
29	7.40 ± 0.13	27.43 ± 4.30	3.71
30	5.21 ± 0.55	20.07 ± 5.24	3.85
31	6.89 ± 0.47	11.84 ± 2.47	1.72
32	19.40 ± 9.74	50.65 ± 1.16	2.61
33	7.49 ± 1.04	9.03 ± 2.84	1.21
34	8.46 ± 0.30	11.86 ± 4.59	1.40
35	30.67 ± 11.37	36.19 ± 13.87	1.18
36	9.87 ± 1.43	7.05 ± 0.37	0.71
37	8.95 ± 0.28	9.78 ± 0.06	1.09
38	6.63 ± 0.31	8.08 ± 2.10	1.22
39	18.83 ± 1.33	18.46 ± 8.77	0.98
Pentamidine	6.29 ± 0.07	1.03 ± 0.40	0.16
Amphotericin B	0.77 ± 0.03	3.14 ± 0.45	4.08
Doxorubicin		0.02 ± 0.01	

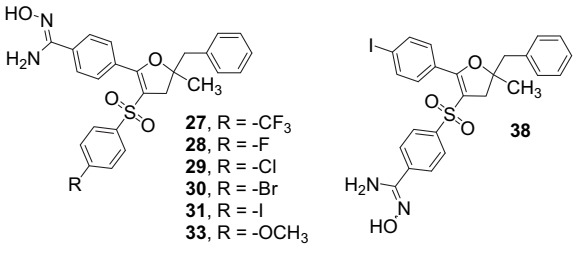
for $C_{15}H_{10}INO_3S$ (411.21 g mol^{-1}): C, 43.81; H, 2.45; N, 3.41. Found: C, 43.60; H, 2.45; N, 3.43.

*4.1.2.4.2. 4-[2-(4-Nitrophenylsulfonyl)acetyl]benzonitrile (**6**).* Yellow solid, mp 188–190 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 4.81 (s, 2H, CH_2), 7.84 (d, J = 8.5, 2H, 2CH), 8.09 (d, J = 8.2, 2H, 2CH), 8.11 (d, J = 8.6, 2H, 2CH), 8.44 (d, J = 8.6, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 63.2 (CH_2), 117.3 (C), 118.1 (C), 124.5 (2CH), 129.6 (2CH), 130.3 (2CH), 132.8 (2CH), 138.1 (C), 143.5 (C), 151.2 (C), 186.6 (C). Anal. Calcd for $C_{15}H_{10}N_2O_5S$ (330.32 g mol^{-1}): C, 54.54; H, 3.05; N, 8.48. Found: C, 54.16; H, 3.26; N, 8.13.

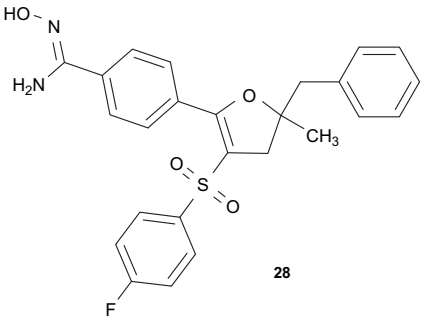
*4.1.2.4.3. 4-[2-(4-Methoxyphenylsulfonyl)acetyl]benzonitrile (**7**).* White solid, mp 144–145 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 3.89 (s, 3H, CH_3), 4.71 (s, 2H, CH_2), 7.00 (d, J = 9.0, 2H, 2CH), 7.78 (d, J = 9.0, 2H, 2CH), 7.80 (d, J = 8.6, 2H, 2CH), 8.08 (d, J = 8.6, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 55.8 (CH_3), 64.1 (CH_2), 114.5 (2CH), 117.4 (C), 117.6 (C), 129.7 (C), 129.8 (2CH), 130.8 (2CH), 132.6 (2CH), 138.5 (C), 164.4 (C), 187.3 (C). Anal. Calcd for $C_{16}H_{13}NO_4S$ (315.34 g mol^{-1}): C, 60.94; H, 4.16; N, 4.44. Found: C, 60.80; H, 4.15; N, 5.04.

*4.1.2.4.4. 4-{2-oxo-2-[4-(Trifluoromethyl)phenyl]ethylsulfonyl} benzonitrile (**8**).* White solid, mp 160–162 °C (isopropyl alcohol) ^{1}H NMR (DMSO-d_6), δ: 5.65 (s, 2H, CH_2), 7.91 (d, J = 8.3, 2H, 2CH), 8.09–8.17 (m, 6H, 6CH). ^{13}C NMR (DMSO-d_6), δ: 62.2 (CH_2), 116.6 (C), 117.7 (C), 123.8 (q, J = 273.0, C), 125.9 (q, J = 3.7, 2CH), 129.1 (2CH), 129.9 (2CH), 133.5 (2CH), 133.6 (q, J = 32.2, C), 138.7 (C), 143.4 (C), 188.9 (C). HMRS (EI): m/z calcd for $C_{16}H_{10}F_3NO_3S$ M + NH_4^+: 371.0672. Found: 371.0664.

*4.1.2.4.5. 4-[2-(4-Fluorophenyl)-2-oxoethylsulfonyl]benzonitrile (**9**).* White solid, mp 168 °C (ethyl alcohol) ^{1}H NMR (DMSO-d_6), δ: 5.56 (s, 2H, CH_2), 7.37 (t, J = 8.8, 2H, CH), 8.02–8.18 (m, 6H, CH). ^{13}C NMR (DMSO-d_6), δ: 61.9 (CH_2), 115.9 (CH), 116.4 (d, J = 22.0, 2CH), 117.7 (C), 129.0 (2CH), 132.3 (d, J = 9.9, 2CH), 133.4 (2CH), 143.6 (C), 165.9 (d, J = 253.9, C), 187.2 (C). 1 (C) not observed in these conditions. Anal. Calcd for $C_{15}H_{10}FNO_3S$ (319.76 g mol^{-1}): C, 59.40; H, 3.32; N, 4.62. Found: C, 59.48; H, 3.34; N, 4.63.



Scheme 5.



IS = 6.37
IS Pentamidine = 0.16
IS Amphotericin B = 4.08

Scheme 6.

*4.1.2.4.6. 4-[2-(4-Chlorophenyl)-2-oxoethylsulfonyl]benzonitrile (**10**).* White solid, mp 205–206 °C (isopropyl alcohol) ^{1}H NMR (DMSO-d_6), δ: 5.56 (s, 2H, CH_2), 7.61 (d, J = 8.6, 2H, 2CH), 7.97 (d, J = 8.6, 2H, 2CH), 8.10 (d, J = 8.9, 2H, 2CH), 8.15 (d, J = 8.9, 2H, 2CH). ^{13}C NMR (DMSO-d_6), δ: 61.9 (CH_2), 116.5 (C), 117.7 (C), 129.0 (2CH), 129.1 (2CH), 131.0 (2CH), 133.5 (2CH), 134.4 (C), 139.6 (C), 143.6 (C), 183.3 (C). Anal. Calcd for $C_{15}H_{10}ClNO_3S$ (319.76 g mol^{-1}): C, 56.34; H, 3.15; N, 4.38. Found: C, 56.24; H, 3.14; N, 4.31.

*4.1.2.5. 4-[2-(4-Bromophenyl)-2-oxoethylsulfonyl]benzonitrile (**11**) [14]*

*4.1.2.5.1. 4-[2-(4-Iodophenyl)-2-oxoethylsulfonyl]benzonitrile (**12**).* Yellow solid, mp 218 °C (isopropyl alcohol) ^{1}H NMR (DMSO-d_6), δ: 5.53 (s, 2H, CH_2), 7.70 (d, J = 8.4, 2H, CH), 7.93 (d, J = 8.4, 2H, CH), 8.11–8.13 (m, 4H, CH). ^{13}C NMR (DMSO-d_6), δ: 61.8 (CH_2), 103.8 (C), 116.5 (C), 117.7 (C), 129.0 (2CH), 130.7 (2CH), 133.4 (2CH), 134.9 (C), 137.9 (2CH), 143.5 (C), 188.9 (C). Anal. Calcd for $C_{15}H_{10}INO_3S$ (411.21 g mol^{-1}): C, 43.81; H, 2.45; N, 3.41. Found: C, 44.21; H, 2.43; N, 3.57.

*4.1.2.5.2. 4-[2-(4-Nitrophenyl)-2-oxoethylsulfonyl]benzonitrile (**13**).* Yellow solid, mp 175–176 °C (isopropyl alcohol) ^{1}H NMR (DMSO-d_6), δ: 5.66 (s, 2H, CH_2), 8.09–8.19 (m, 4H, 4CH), 8.17 (d, J = 8.9, 2H, 2CH), 8.33 (d, J = 8.9, 2H, 2CH). ^{13}C NMR (DMSO-d_6), δ: 62.4 (CH_2), 116.7 (C), 117.7 (C), 124.0 (2CH), 129.1 (2CH), 130.6 (2CH), 133.6 (2CH), 140.1 (C), 143.4 (C), 150.7 (C), 188.7 (C). Anal. Calcd for $C_{15}H_{10}N_2O_5S$ (330.32 g mol^{-1}): C, 54.54; H, 3.05; N, 8.48. Found: C, 54.58; H, 3.06; N, 8.38.

4.1.3. General procedure for $Mn(OAc)_3$-mediated reaction of β-ketosulfones with alkenes

A solution of manganese(III) acetate dihydrate (6.87 mmol, 1.84 g) and copper(II) acetate (3.27 mmol, 0.59 g) in 30 mL of glacial acetic acid was heated under microwave irradiation (200 W, 80 °C) for 15 min, until dissolution. Then, the reaction mixture was cooled to 50 °C, and a solution of **1**–**5** (3.27 mmol) and 1.1-diphenylethene (9.81 mmol) in 5 mL acetic acid was added. The mixture was heated under microwave irradiation (200 W, 80 °C) for 45 min. The reaction mixture was poured into 200 mL of cold water, and extracted with chloroform (3 × 40 mL). The organic extracts were collected and washed with saturated aqueous $NaHCO_3$ (3 × 40 mL) and dried ($MgSO_4$). Solvent evaporation was followed by column chromatography (gradient, from chloroform/petroleum ether (1/1) to chloroform/petroleum ether/diethyl ether (5/3/2)), and the product obtained was recrystallized from the appropriate solvent.

*4.1.3.1. 4-{5-Benzyl-5-methyl-3-[4-(trifluoromethyl)phenylsulfonyl]-4,5-dihydrofuran-2-yl}benzonitrile (**14**).* White solid, mp 152–153 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 1.54 (s, 3H, CH_3), 2.78–3.08 (m, 4H, $2CH_2$), 7.02–7.07 (m, 2H, 2CH), 7.13–7.20 (m, 3H, 3CH), 7.56 (d, J = 8.4, 2H, 2CH), 7.64–7.69 (m, 6H, 6CH). ^{13}C NMR ($CDCl_3$), δ: 27.7 (CH_3), 41.2 (CH_2), 46.4 (CH_2), 89.3 (C), 110.7 (C), 114.6 (C), 118.0 (C), 123.1 (q, J = 273.0, C), 126.2 (q, J = 3.7, 2CH), 127.1 (CH), 127.2 (2CH), 128.3 (2CH), 130.0 (2CH), 130.2 (2CH), 131.6 (2CH), 132.8 (C), 134.5 (q, J = 32.9, C), 135.1 (C), 144.6 (C), 161.6 (C). Anal. Calcd for $C_{26}H_{20}F_3NO_3S$ (483.50 g mol^{-1}): C, 64.59; H, 4.17; N, 2.90. Found: C, 64.53; H, 4.37; N, 2.89.

*4.1.3.2. 4-[5-Benzyl-3-(4-fluorophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]benzonitrile (**15**).* Yellow solid, mp 147–148 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 1.51 (s, 3H, CH_3), 2.81–3.07 (m, 4H, $2CH_2$), 7.03–7.11 (m, 4H, 4CH), 7.20–7.24 (m, 2H, 2CH), 7.45–7.52 (m, 3H, 3CH), 7.67–7.72 (m, 4H, 4CH). ^{13}C NMR ($CDCl_3$), δ: 27.4 (CH_3), 41.6 (CH_2), 46.4 (CH_2), 89.0 (C), 111.6 (C), 114.3 (C), 116.3 (d, J = 22.7, 2CH), 118.1 (C), 127.1 (CH), 128.3 (2CH), 129.5 (d, J = 9.5, 2CH), 130.0 (2CH), 130.3 (2CH), 131.5 (2CH), 133.0 (C), 135.3 (C), 137.3 (d, J = 3.3, C), 160.4 (C), 165.2 (d, J = 255.8, C). Anal. Calcd for $C_{25}H_{20}FNO_3S$ (433.49 g mol^{-1}): C, 69.27; H, 4.65; N, 3.23. Found: C, 68.96; H, 4.77; N, 3.19.

*4.1.3.3. 4-[5-Benzyl-3-(4-chlorophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]benzonitrile (**16**).* White solid, mp 144–146 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 1.52 (s, 3H, CH_3), 2.79–3.06 (m, 4H, $2CH_2$), 7.04–7.08 (m, 2H, 2CH), 7.20–7.23 (m, 3H, 3CH), 7.35 (d, J = 8.8, 2H, 2CH), 7.40 (d, J = 8.8, 2H, 2CH), 7.64–7.72 (m, 4H, 4CH). ^{13}C NMR ($CDCl_3$), δ: 27.5 (CH_3), 41.5 (CH_2), 46.4 (CH_2), 89.1 (C), 111.3 (C), 114.4 (C), 118.1 (C), 127.1 (CH), 128.1 (2CH), 128.3 (2CH), 129.3 (2CH), 130.1 (2CH), 130.2 (2CH), 131.5 (2CH), 132.9 (C), 135.2 (C), 139.5 (C), 139.7 (C), 160.7 (C). Anal. Calcd for $C_{25}H_{20}ClNO_3S$ (449.95 g mol^{-1}): C, 66.73; H, 4.48; N, 3.11. Found: C, 66.51; H, 4.67; N, 3.02.

*4.1.3.4. 4-[5-Benzyl-3-(4-bromophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]benzonitrile (**17**) [14]*

*4.1.3.4.1. 4-[5-Benzyl-3-(4-iodophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]benzonitrile (**18**).* White solid, mp 176–177 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 1.52 (s, 3H, CH_3), 2.79–3.06 (m, 4H, $2CH_2$), 7.02–7.07 (m, 2H, 2CH), 7.15–7.26 (m, 5H, 5CH), 7.64–7.72 (m, 4H, 4CH), 7.75 (d, J = 8.6, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 27.5 (CH_3), 41.4 (CH_2), 46.4 (CH_2), 89.1 (C), 100.5 (C), 111.2 (C), 114.4 (C), 118.1 (C), 127.1 (CH), 128.1 (2CH), 128.3 (2CH), 130.1 (2CH), 130.2 (2CH), 131.5 (2CH), 132.9 (C), 135.2 (C), 138.3 (2CH), 140.8 (C), 160.7 (C). Anal. Calcd for $C_{25}H_{20}INO_3S$ (541.40 g mol^{-1}): C, 55.46; H, 3.72; N, 2.59. Found: C, 55.18; H, 3.85; N, 2.53.

*4.1.3.4.2. 4-[5-Benzyl-5-methyl-3-(4-nitrophenylsulfonyl)-4,5-dihydrofuran-2-yl]benzonitrile (**19**).* Yellow solid, mp 189–190 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 1.55 (s, 3H, CH_3), 2.78–3.11 (m, 4H, $2CH_2$), 7.05–7.21 (m, 5H, 5CH), 7.57 (d, J = 8.9, 2H, 2CH), 7.66 (d, J = 8.8, 2H, 2CH), 7.72 (d, J = 8.8, 2H, 2CH), 8.20 (d, J = 8.9, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 27.8 (CH_3), 41.1 (CH_2), 46.4 (CH_2), 89.5 (C), 110.1 (C), 114.7 (C), 117.9 (C), 124.3 (2CH), 127.1 (CH), 127.8 (2CH), 128.3 (2CH), 130.0 (2CH), 130.3 (2CH), 131.6 (2CH), 132.5 (C), 135.1 (C), 146.7 (C), 150.0 (C), 162.3 (C). Anal. Calcd for $C_{25}H_{20}N_2O_5S$ (460.50 g mol^{-1}): C, 65.20; H, 4.38; N, 6.08. Found: C, 65.46; H, 4.47; N, 5.77.

*4.1.3.4.3. 4-[5-Benzyl-3-(4-methoxyphenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]benzonitrile (**20**).* Yellow solid, mp 155–156 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 1.47 (s, 3H, CH_3), 2.79–3.08 (m, 4H, $2CH_2$), 3.86 (s, 3H, CH_3), 6.87 (d, J = 8.9, 2H, 2CH), 7.04–7.11 (m, 2H, 2CH), 7.18–7.23 (m, 3H, 3CH), 7.46 (d, J = 8.9, 2H, 2CH), 7.66 (d, J = 8.8, 2H, 2CH), 7.71 (d, J = 8.8, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 27.0 (CH_3), 41.9 (CH_2), 46.4 (CH_2), 55.6 (CH_3), 88.7 (C), 112.5 (C), 114.1 (C), 114.2 (2CH), 118.2 (C), 127.0 (CH), 128.3 (2CH), 129.0 (2CH), 130.1 (2CH), 130.3 (2CH), 131.4 (2CH), 132.8 (C), 133.2 (C), 135.4 (C), 159.2 (C), 163.1 (C). Anal. Calcd for $C_{26}H_{23}NO_4S$ (445.53 g mol^{-1}): C, 70.09; H, 5.20; N, 3.14. Found: C, 69.76; H, 5.43; N, 3.52.

*4.1.3.4.4. 4-{5-Benzyl-5-methyl-2-[4-(trifluoromethyl) phenyl]-4,5-dihydrofuran-3-ylsulfonyl}benzonitrile (**21**).* White solid, mp 198–200 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 1.55 (s, 3H, CH_3), 2.80–3.11 (m, 4H, $2CH_2$), 7.08–7.12 (m, 2H, 2CH), 7.18–7.24 (m, 3H, 3CH), 7.50 (d, J = 8.1, 2H, 2CH), 7.62–7.66 (m, 6H, 6CH). ^{13}C NMR ($CDCl_3$), δ: 27.1 (CH_3), 41.2 (CH_2), 46.4 (CH_2), 89.2 (C), 109.7 (C), 116.4 (C), 117.2 (C), 123.6 (q, J = 273.0, C), 124.9 (q, J = 3.7, 2CH), 127.1 (CH), 127.2 (2CH), 128.3 (2CH), 129.7 (2CH), 130.3 (2CH), 131.8 (C), 132.7 (2CH), 135.3 (C), 145.5 (C), 162.9 (C). 1 (C) not observed in these conditions. HMRS (EI): m/z calcd for $C_{26}H_{20}F_3NO_3S$ M + H$^+$: 484.1189. Found: 484.1189.

*4.1.3.4.5. 4-[5-Benzyl-2-(4-fluorophenyl)-5-methyl-4,5-dihydrofuran-3-ylsulfonyl]benzonitrile (**22**).* Yellow oil ^{1}H NMR ($CDCl_3$), δ: 1.52 (s, 3H, CH_3), 2.83 (d, J = 14.0, 1H, CH_2), 2.88 (d, J = 14.5, 1H, CH_2), 3.05 (d, J = 14.5, 1H, CH_2), 3.06 (d, J = 14.0, 1H, CH_2), 7.03–7.13 (m, 4H, 4CH), 7.19–7.25 (m, 3H, 3CH), 7.47 (d, J = 8.4, 2H, 2CH), 7.54–7.64 (d, 4H, 4CH). ^{13}C NMR ($CDCl_3$), δ: 27.6 (CH_3), 41.3 (CH_2), 46.4 (CH_2), 88.6 (C), 108.2 (C), 115.2 (d, J = 22.0, 2CH), 116.1 (C), 117.3 (C), 124.3 (d, J = 3.3, C), 127.0 (3CH), 128.3 (2CH), 130.4 (2CH), 131.6 (d, J = 8.8, 2CH), 132.6 (2CH), 135.4 (C), 145.9 (C), 163,5 (C), 164.3 (d, J = 252.9, C). HMRS (EI): m/z calcd for $C_{25}H_{20}FNO_3S$ M + H$^+$: 434.1221. Found: 434.1225.

*4.1.3.4.6. 4-[5-Benzyl-2-(4-chlorophenyl)-5-methyl-4,5-dihydrofuran-3-ylsulfonyl]benzonitrile (**23**).* Yellow oil, ^{1}H NMR ($CDCl_3$), δ: 1.52 (s, 3H, CH_3), 2.79–3.10 (m, 4H, $2CH_2$), 7.07–7.12 (m, 2H, 2CH), 7.20–7.25 (m, 2H, 2CH), 7.29–7.41 (m, 3H, 3CH), 7.46–7.54 (m, 4H, 4CH), 7.63 (d, J = 8.4, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 27.6 (CH_3), 41.4 (CH_2), 46.4 (CH_2), 88.7 (2C), 108.6 (C), 116.2 (C), 117.3 (C), 126.6 (CH), 127.0 (C), 127.1 (2CH), 128.3 (2CH), 130.4 (2CH), 130.6 (2CH), 131.6 (2CH), 132.7 (2CH), 135.4 (C), 145.9 (C), 163.4 (C). HMRS (EI): m/z calcd for $C_{25}H_{20}ClNO_3S$ M + H$^+$: 450.0925. Found: 450.0917.

*4.1.3.5. 4-[5-Benzyl-2-(4-bromophenyl)-5-methyl-4,5-dihydrofu–ran-3-ylsulfonyl]benzonitrile (**24**) [14]*

*4.1.3.5.1. 4-[5-Benzyl-2-(4-iodophenyl)-5-methyl-4,5-dihydrofuran-3-ylsulfonyl]benzonitrile (**25**).* Yellow solid, mp 72 °C (isopropyl alcohol) ^{1}H NMR ($CDCl_3$), δ: 1.51 (s, 3H, CH_3), 2.81 (d, J = 14.0, 1H, CH_2), 2.86 (d, J = 14.6, 1H, CH_2), 3.01 (d, J = 14.6, 1H, CH_2), 3.05 (d, J = 14.0, 1H, CH_2), 7.06–7.11 (m, 2H, 2CH), 7.20–7.30 (m, 5H, 5CH), 7.48 (d, J = 8.5, 2H, 2CH), 7.63 (d, J = 8.5, 2H, 2CH), 7.74 (d, J = 8.5, 2H, 2CH). ^{13}C NMR ($CDCl_3$), δ: 27.6 (CH_3), 41.3 (CH_2), 46.4 (CH_2), 88.8 (C), 98.1 (C), 108.6 (C), 116.1 (C), 117.3 (C), 127.0 (CH), 127.1 (2CH), 127.7 (C), 128.2 (2CH), 130.3 (2CH), 130.7 (2CH), 132.7 (2CH), 135.3 (C), 137.1 (2CH), 145.7 (C), 163.5 (C). HMRS (EI): m/z calcd for $C_{25}H_{20}INO_3S$ M + H$^+$: 542.0281. Found: 542.79.

*4.1.3.5.2. 134-[5-Benzyl-5-methyl-2-(4-nitrophenyl)-4,5-dihydrofuran-3-ylsulfonyl]benzonitrile (**26**).* Yellow oil ^{1}H NMR (DMSO-d_6), δ: 1.49 (s, 3H, CH_3), 2.80–3.04 (m, 4H, $2CH_2$), 7.14–7.27 (m, 5H, 5CH), 7.62–7.74 (m, 4H, 4CH), 8.02 (d, J = 8.0, 2H, 2CH), 8.30 (d, J = 8.0, 2H, 2CH). ^{13}C NMR (DMSO-d_6), δ: 25.6 (CH_3), 41.8 (CH_2), 45.5 (CH_2), 90.1 (C), 110.6 (C), 115.8 (C), 117.7 (C), 123.3 (2CH), 126.9 (CH), 127.3 (2CH), 128.1 (2CH), 128.3 (C), 130.4 (2CH), 130.7 (2CH), 133.8 (2CH), 135.7 (C), 144.7 (C), 148.8 (C), 161.5 (C). HMRS (EI): m/z calcd for $C_{25}H_{20}N_2O_5S$ M + H$^+$: 461.1166. Found: 461.1161.

4.1.4. General procedure for amidoxime synthesis from nitriles

A suspension of hydroxylamine hydrochloride (1.7 mmol, 0.12 g, 10 eq.) in 8 mL of DMSO was stirred under inert atmosphere and cooled to 5 °C. Then, potassium tertiobutylate (1.7 mmol, 0.19 g, 10 eq.) was slowly added. The reaction mixture was stirred at room temperature for 30 min, and the corresponding nitrile (0.17 mmol,

1 eq.) was added. The reaction mixture was stirred for 12 h and poured into 100 mL of cold water. The precipitate thus formed was crystallized from the appropriate solvent.

*4.1.4.1. 4-{5-Benzyl-5-methyl-3-[4-(trifluoromethyl)phenylsulfonyl]-4,5-dihydrofuran-2-yl}-N′-hydroxy-benzimidamide (**27**).* White solid, mp 73–76 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 2.54 (s, 3H, CH_3), 2.80–3.03 (m, 4H, $2CH_2$), 5.93 (bs, 2H), 7.07–7.17 (m, 5H, 5CH), 7.47 (d, *J* = 8.4, 2H, 2CH), 7.67 (d, *J* = 8.3, 2H, 2CH), 7.73 (d, *J* = 8.4, 2H, 2CH), 7.90 (d, *J* = 8.3, 2H, 2CH), 9.86 (bs, 1H). ^{13}C NMR (DMSO-d_6), δ: 27.1 (CH_3), 40.8 (CH_2), 45.4 (CH_2), 89.0 (C), 108.5 (C), 123.6 (q, *J* = 273.0, C), 124.9 (2CH), 126.7 (CH), 126.8 (2CH), 127.4 (2CH), 128.1 (2CH), 128.7 (C), 129.0 (2CH), 130.4 (2CH), 132.8 (q, *J* = 32.2, C), 135.8 (C), 135.9 (C), 145.2 (C), 150.3 (C), 163.2 (C). HMRS (EI): *m*/*z* calcd for $C_{26}H_{23}F_3N_2O_4S$ M + H^+: 517.1403. Found: 517.1400.

*4.1.4.2. 4-[5-Benzyl-3-(4-fluorophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]-N′-hydroxybenzimi-damide (**28**).* White solid, mp 139–140 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.40 (s, 3H, CH_3), 2.73–2.98 (m, 4H, $2CH_2$), 5.87 (bs, 2H), 7.07–7.19 (m, 5H, 5CH), 7.26–7.35 (m, 2H, 2CH), 7.44 (d, *J* = 8.6, 2H, 2CH), 7.49–7.53 (m, 2H, 2CH), 7.69 (d, *J* = 8.4, 2H, 2CH), 9.80 (bs, 1H). ^{13}C NMR (DMSO-d_6), δ: 26.9 (CH_3), 41.4 (CH_2), 45.5 (CH_2), 88.5 (C), 109.4 (C), 116.7 (d, *J* = 22.7, 2CH), 124.9 (2CH), 126.8 (CH), 128.2 (2CH), 128.8 (C), 129.0 (2CH), 129.5 (d, *J* = 9.9, 2CH), 130.5 (2CH), 135.6 (C), 136.0 (C), 137.9 (d, *J* = 2.9, C), 150.3 (C), 162.0 (C), 165.0 (d, *J* = 251.8, C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}FN_2O_4S$ M + H^+: 467.1435. Found: 467.1434.

*4.1.4.3. 4-[5-Benzyl-3-(4-chlorophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]-N′-hydroxybenzimi-damide (**29**).* White solid, mp 89–91 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.44 (s, 3H, CH_3), 2.84–3.01 (m, 4H, $2CH_2$), 5.91 (bs, 2H), 7.12–7.22 (m, 5H, 5CH), 7.46–7.49 (m, 4H, 4CH), 7.59 (d, *J* = 8.7, 2H, 2CH), 7.73 (d, *J* = 8.4, 2H, 2CH), 9.84 (bs, 1H). ^{13}C NMR (DMSO-d_6), δ: 27.0 (CH_3), 41.3 (CH_2), 45.4 (CH_2), 88.7 (C), 109.1 (C), 124.9 (2CH), 126.8 (CH), 128.1 (2CH), 128.3 (2CH), 128.8 (C), 129.0 (2CH), 129.7 (2CH), 130.4 (2CH), 135.7 (C), 136.0 (C), 138.1 (C), 140.3 (C), 150.3 (C), 162.3 (C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}ClN_2O_4S$ M + H^+: 483.1140. Found: 483.1143.

*4.1.4.4. 4-[5-Benzyl-3-(4-bromophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]-N′-hydroxybenzimi-damide (**30**).* White solid, mp 93–95 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.44 (s, 3H, CH_3), 2.77–3.03 (m, 4H, $2CH_2$), 5.91 (bs, 2H), 7.11–7.22 (m, 5H, 5CH), 7.38–7.50 (m, 4H, 4CH), 7.71–7.73 (m, 4H, 4CH), 9.83 (bs, 1H). ^{13}C NMR (DMSO-d_6), δ: 27.0 (CH_3), 41.2 (CH_2), 45.4 (CH_2), 88.7 (C), 109.0 (C), 124.9 (2CH), 126.8 (CH), 127.2 (C), 128.1 (2CH), 128.4 (2CH), 128.8 (C), 129.0 (2CH), 130.4 (2CH), 132.6 (2CH), 135.7 (C), 135.9 (C), 140.7 (C), 150.3 (C), 162.4 (C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}BrN_2O_4S$ M + H^+: 527.0635. Found: 527.0643.

*4.1.4.5. 4-[5-Benzyl-3-(4-iodophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]-N′-hydroxybenzimi-damide (**31**).* White solid, mp 68–70 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.44 (s, 3H, CH_3), 2.75–3.02 (m, 4H, $2CH_2$), 5.90 (bs, 2H), 7.08–7.26 (m, 7H, 7CH), 7.48 (d, *J* = 8.4, 2H, 2CH), 7.72 (d, *J* = 8.4, 2H, 2CH), 7.91 (d, *J* = 8.5, 2H, 2CH), 9.83 (bs, 1H). ^{13}C NMR (DMSO-d_6), δ: 26.9 (CH_3), 41.6 (CH_2), 45.4 (CH_2), 88.6 (C), 101.7 (C), 109.0 (C), 124.9 (2CH), 126.8 (CH), 128.0 (2CH), 128.1 (2CH), 128.8 (C), 129.0 (2CH), 130.4 (2CH), 135.7 (C), 135.9 (C), 138.4 (2CH), 141.1 (C), 150.3 (C), 162.4 (C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}IN_2O_4S$ M + H^+: 575.0496. Found: 575.0492.

*4.1.4.6. 4-[5-Benzyl-5-methyl-3-(4-nitrophenylsulfonyl)-4,5-dihydrofuran-2-yl]-N′-hydroxybenzimi-damide (**32**).* White solid, mp 167–168 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.46 (s, 3H, CH_3), 2.82–3.05 (m, 4H, $2CH_2$), 5.95 (bs, 2H), 7.14–7.18 (m, 5H, 5CH), 7.48 (d, *J* = 8.3, 2H, 2CH), 7.70–7.76 (m, 4H, 4CH), 8.31 (d, *J* = 8.8, 2H, 2CH), 9.86 (bs, 1H). ^{13}C NMR (DMSO-d_6), δ: 27.0 (CH_3), 35.9 (CH_2), 45.5 (CH_2), 89.2 (C), 108.2 (C), 124.9 (2CH), 125.0 (2CH), 126.8 (CH), 128.0 (2CH), 128.1 (2CH), 128.6 (C), 129.0 (2CH), 130.4 (2CH), 135.8 (C), 135.9 (C), 146.7 (C), 150.0 (C), 150.4 (C), 163.7 (C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}N_3O_6S$ M + H^+: 494.1380. Found: 494.1378.

*4.1.4.7. 4-[5-Benzyl-3-(4-methoxyphenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl]-N′-hydroxybenzimi–damide (**33**).* White solid, mp 79–83 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.40 (s, 3H, CH_3), 2.71–2.99 (m, 4H, $2CH_2$), 3.84 (s, 3H, CH_3), 5.92 (bs, 2H), 7.03 (d, *J* = 8.9, 2H, 2CH), 7.09–7.23 (m, 5H, 5CH), 7.45 (d, *J* = 8.9, 2H, 2CH), 7.50 (d, *J* = 8.6, 2H, 2CH), 7.71 (d, *J* = 8.6, 2H, 2CH), 9.84 (bs, 1H). ^{13}C NMR (DMSO-d_6), δ: 26.7 (CH_3), 41.7 (CH_2), 45.4 (CH_2), 55.9 (CH_3), 88.2 (C), 110.3 (C), 114.7 (2CH), 124.8 (2CH), 126.8 (CH), 128.2 (2CH), 128.7 (2CH), 129.0 (2CH), 129.1 (C), 130.5 (2CH), 133.2 (C), 135.5 (C), 136.1 (C), 150.4 (C), 160.8 (C), 162.8 (C). HMRS (EI): *m*/*z* calcd for $C_{26}H_{26}N_2O_5S$ M + H^+: 479.1635. Found: 479.1632.

*4.1.4.8. 4-{5-Benzyl-5-methyl-2-[4-(trifluoromethyl)phenyl]-4,5-dihydrofuran-3-ylsulfonyl}-N′-hydroxy-benzimidamide (**34**).* White solid, mp 92 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.45 (s, 3H, CH_3), 2.85–3.01 (m, 4H, $2CH_2$), 6.00 (bs, 2H), 7.09–7.19 (m, 5H, 5CH), 7.48–7.52 (m, 2H, 2CH), 7.65–7.69 (m, 2H, 2CH), 7.81–7.85 (m, 4H, 4CH), 10.00 (s, 1H) ^{13}C NMR (DMSO-d_6), δ: 27.0 (CH_3), 41.2 (CH_2), 45.5 (CH_2), 89.4 (C), 110.9 (C), 125.2 (q, *J* = 3.7, 2CH), 126.4 (2CH), 126.5 (2CH), 126.9 (CH), 128.2 (2CH), 129.5 (q, *J* = 272.6, C), 130.2 (2CH), 130.5 (2CH), 130.8 (q, *J* = 31.5, C),132.8 (C), 135.9 (C), 137.9 (C), 141.0 (C), 149.9 (C), 160.8 (CH). HMRS (EI): *m*/*z* calcd for $C_{26}H_{23}F_3N_2O_4S$ M + H^+: 517.1403. Found: 517.1392.

*4.1.4.9. 4-[5-Benzyl-2-(4-fluorophenyl)-5-methyl-4,5-dihydrofuran-3-ylsulfonyl]-N′-hydroxybenzimi-damide (**35**).* White solid, mp 88 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.42 (s, 3H, CH_3), 2.75–3.00 (m, 4H, $2CH_2$), 5.98 (bs, 2H), 7.11–7.27 (m, 7H, 7CH), 7.44–7.51 (m, 4H, 4CH), 7.79 (d, *J* = 7.8, 2H, 2CH), 10.02 (s, 1H). ^{13}C NMR (DMSO-d_6), δ: 26.9 (CH_3), 41.4 (CH_2), 45.5 (CH_2), 88.6 (C), 109.4 (C), 115.3 (d, *J* = 22.3, 2CH), 126.2 (d, *J* = 3.3, C), 126.3 (2CH), 126.4 (2CH), 126.9 (CH), 128.2 (2CH), 130.5 (2CH), 131.8 (d, *J* = 8.8, 2CH), 134.0 (C), 137.7 (C), 141.4 (C), 149.9 (C), 161.3 (C), 163.5 (d, *J* = 249.0, C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}FN_2O_4S$ M + H^+: 467.1435. Found: 467.1436.

*4.1.4.10. 4-[5-Benzyl-2-(4-chlorophenyl)-5-methyl-4,5-dihydrofuran-3-ylsulfonyl]-N′-hydroxybenzimi-damide (**36**).* White solid, mp 56–58 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.38 (s, 3H, CH_3), 2.71–2.96 (m, 4H, $2CH_2$), 5.92 (bs, 2H), 7.03–7.14 (m, 5H, 5CH), 7.40–7.50 (m, 4H, 4CH), 7.68–7.82 (m, 4H, 4CH), 9.99 (s, 1H). ^{13}C NMR (DMSO-d_6), δ: 27.1 (CH_3), 41.5 (CH_2), 45.6 (CH_2), 89.1 (C), 109.9 (C), 125.8 (C), 126.5 (2CH), 126.6 (2CH), 126.7 (CH), 127.1 (C), 128.4 (2CH), 128.5 (2CH), 130.6 (2CH), 131.2 (2CH), 136.1 (C), 137.9 (C), 141.4 (C), 150.1 (C), 161.4 (C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}ClN_2O_4S$ M + H^+: 483.1140. Found: 483.1134.

*4.1.4.11. 4-[5-Benzyl-2-(4-bromophenyl)-5-methyl-4,5-dihydrofuran-3-ylsulfonyl]-N′-hydroxyben–zimidamide (**37**).* White solid, mp 107 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.43 (s, 3H, CH_3), 2.75–3.01 (m, 4H, $2CH_2$), 5.99 (bs, 2H), 7.10–7.17 (m, 5H, 5CH), 7.41–7.51 (m, 4H, 4CH), 7.64–7.68 (m, 2H, 2CH), 7.80–7.84 (m, 2H, 2CH), 10.00 (s, 1H). ^{13}C NMR (DMSO-d_6), δ: 26.8 (CH_3), 41.4 (CH_2), 45.4 (CH_2), 88.8 (C), 109.8 (C), 124.6 (C), 126.3 (2CH), 126.4 (2CH), 126.8 (CH), 127.9 (C), 128.2 (2CH), 130.4 (2CH), 131.1 (2CH), 131.2

(2CH), 135.9 (C), 137.8 (C), 141.2 (C), 149.8 (C), 161.1 (C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}BrN_2O_4S$ M + H^+: 527.0635. Found: 527.0632.

*4.1.4.12. 4-[5-Benzyl-2-(4-iodophenyl)-5-methyl-4,5-dihydrofuran-3-ylsulfonyl]-N′-hydroxybenzimi-damide (**38**).* White solid, mp 102 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.42 (s, 3H, CH_3), 2.74–2.99 (m, 4H, $2CH_2$), 5.99 (bs, 2H), 7.09–7.28 (m, 7H, 7CH), 7.48 (d, J = 8.2, 2H, 2CH), 7.80–7.84 (m, 4H, 4CH), 10.02 (s, 1H). ^{13}C NMR (DMSO-d_6), δ: 26.9 (CH_3), 41.4 (CH_2), 45.5 (CH_2), 88.9 (C), 98.4 (C), 109.7 (C), 126.3 (2CH), 126.4 (2CH), 126.9 (CH), 128.2 (2CH), 128.7 (C), 130.4 (2CH), 131.1 (2CH), 135.9 (C), 137.0 (2CH), 137.8 (C), 141.3 (C), 149.9 (C), 161.5 (C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}IN_2O_4S$ M + H^+: 575.0496. Found: 575.0489.

*4.1.4.13. 4-[5-Benzyl-5-methyl-2-(4-nitrophenyl)-4,5-dihydrofuran-3-ylsulfonyl]-N′-hydroxybenzimida-mide (**39**).* White solid, mp 94 °C (ethyl alcohol/water, (1/1)) ^{1}H NMR (DMSO-d_6), δ: 1.42 (s, 3H, CH_3), 2.75–3.00 (m, 4H, CH_2), 5.97 (bs, 2H), 7.04–7.14 (m, 5H, CH), 7.47 (d, J = 8.4 Hz, 2H, 2CH), 7.68 (d, 2H, J = 8.6 Hz, 2CH), 7.79 (d, J = 8.4 Hz, 2H, 2CH), 8.25 (d, J = 8.6 Hz 2H, 2CH), 10.00 (s, 1H). ^{13}C NMR (DMSO-d_6), δ: 27.2 (CH_3), 41.5 (CH_2), 45.8 (CH_2), 89.9 (C), 112.0 (C), 123.5 (2CH), 126.7 (2CH), 126.8 (2CH), 127.2 (CH), 128.5 (2CH), 130.7 (2CH), 131.0 (2CH), 135.2 (C), 136.1 (C), 138.3 (C), 141.0 (C), 149.0 (C), 150.2 (C), 160.2 (C). HMRS (EI): *m*/*z* calcd for $C_{25}H_{23}N_3O_6S$ M + H^+: 494.1380. Found: 494.80.

4.2. Biology

4.2.1. General

Reagents for cell culture media and compound screening included Schneider's medium (Invitrogen), RPMI 1640 (PAA), fetal calf serum (FCS) (PAA), L-glutamine-penicillin-streptomycin solution (Sigma), thiazolyl blue tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) (Sigma). Stock solutions of amidoxime derivatives and reference drugs (doxorubicin, amphotericin B and pentamidine, all from Sigma) were prepared in DMSO.

4.2.2. Cytotoxic assays on mouse monocyte macrophages J774A.1

Cytotoxicity was assessed on mouse monocyte macrophages J774A.1 (ECACC, Salisbury, UK) by a colorimetric assay based on the mitochondrial reduction of MTT as described previously [19]. J774A.1 cells routinely cultured in RPMI 1640 (without phenol red) supplemented with 10% FCS, 2 mM L-glutamine and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) were seeded in sterile 96-well plate at an average density of 5 × 10^4 cell/mL with a range of compound concentrations in duplicate (final DMSO concentration less than 0.5% *v*/*v*). Appropriate controls treated with or without solvent (DMSO), and various concentrations of doxorubicin (positive control), pentamidine and amphotericin B were added to each set of experiments. After 72 h incubation at 37 °C and 6% CO_2, well supernatant was removed. The formation of formazan was measured by adding MTT (0.5 mg/mL in RPMI 1640, 100 μL/well) and incubating plates for 2 h at 37 °C. The supernatant was subsequently removed. The pellet was dissolved in 100 μL of DMSO and the absorbance measured in a plate reader at 570 nm. Inhibitory concentration 50% (IC_{50} J774A.1) was defined as the concentration of drug required to inhibit by 50% the metabolic activity of J774A.1 cells compared to the control. IC_{50} was calculated by non-linear regression analysis processed on dose–response curves, using TableCurve 2D V5 software. IC_{50} values represent the mean value calculated from three independent experiments.

4.2.3. Antileishmanial activity

The effects of the tested compounds on the growth of *L. donovani* (strain MHOM/IN/00/DEVI, CNR *Leishmania*, Montpellier, France) promastigotes were assessed by MTT assay Briefly, promastigotes in late log-phase in Schneider's medium supplemented with 20% FCS, 2 mM L-glutamine and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) were incubated at an average density of 10^6 parasites/mL in sterile 96-well plates with various concentrations of compounds dissolved in DMSO (final concentration less than 0.5% *v*/*v*) incorporated in duplicate. Appropriate controls treated by DMSO, pentamidine or amphotericin B (reference drugs) were added to each set of experiments. After a 72-h incubation period at 27 °C, parasite metabolic activity was determined. Plates were centrifuged at 900 *G* for 10 min and the supernatant removed. After the addition of MTT (0.5 mg/mL in RPMI 1640, 100 μL/well), plates were incubated for 6 h at 27 °C. The plates were subsequently centrifuged at 900 *G* for 10 min and the supernatant removed. The pellet was dissolved in 100 μL of DMSO and the absorbance measured in a plate reader at 570 nm. Inhibitory concentration 50% (IC_{50} *Ld*) was defined as the concentration of drug required to inhibit by 50% the metabolic activity of *Leishmania* promastigotes compared to the control. IC_{50} was calculated by non-linear regression analysis processed on dose–response curves, using TableCurve 2D V5 software. IC_{50} values represent the mean value calculated from three independent experiments.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.04.026.

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