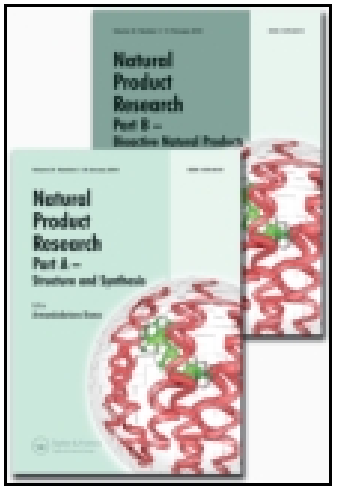

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Leishmanicidal potential of N-substituted morpholine derivatives: Synthesis and structure-activity relationships

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Leishmanicidal potential of N-substituted morpholine derivatives: Synthesis and structure–activity relationships

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A series of N-substituted morpholines **2**–**20** was synthesised by reacting various acid chlorides and alkyl halides with morpholine (**1**). All of the synthesised compounds **2**–**20** were screened for their leishmanicidal effects using amphotericin B ($IC_{50} = 0.24\,\mu g\,L^{-1}$) and pentamidine ($IC_{50} = 2.56\,\mu g\,mL^{-1}$) as standards and a structure–activity relationship (SAR) study was established. The compounds **2** ($IC_{50} = 48\,\mu g\,mL^{-1}$), **3** ($IC_{50} = 30.0\,\mu g\,mL^{-1}$), **10** ($IC_{50} = 41.0\,\mu g\,mL^{-1}$), **15** ($IC_{50} = 33.0\,\mu g\,mL^{-1}$), **16** ($IC_{50} = 35.0\,\mu g\,mL^{-1}$) and **20** ($IC_{50} = 47.0\,\mu g\,mL^{-1}$) showed weak leishmanicidal activities.

Keywords: N-substituted morpholine; synthesis; anti-Leishmanial activity; SAR

1. Introduction

Leishamaniasis is one of the major parasitic diseases, causes enormous suffering in many parts of the tropical and subtropical regions of the world, and contributes to serious health problems (World Health Organisation (WHO), 2001). This disease manifests in different clinical forms, including cutaneous, mucocutaneous and visceral leishmaniasis. The disease is caused by a parasitic protozoa (*Kinetoplastida*: Trypanosomatideae) (Olliaro & Bryceson, 1993; WHO, 2001), which is transmitted as metacyclic flagellated promastigote forms from host to host by the bite of infected sand flies. This species has been isolated from patients with visceral disease or with post-Kala Azar dermal leishmaniasis (Barral et al., 1991; Leon, Machado, Carvalho-Paes, & Grimaldi, 1990).

Therapy of patients with leishmaniasis remains a serious problem. The drugs for the treatment of all clinical forms of leishmaniasis are sodium stibogluconate (pentostam) and meglumine antimonate (glucantime), although they exhibit renal and cardiac toxicity (Raht et al., 2003). Alternative drugs such as pentamidine, amphotericin B and some azo-derivatives are also very toxic, with serious side effects (Mcgregor, 1998). Miltefosine, a phosphocholine analogue, originally developed as an anticancer agent, has been found to be highly effective against leishmaniasis *in vitro* and *in vivo*. Presently, this compound is the

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only oral agent effective against both cutaneous (Soto et al., 2004) and visceral (Prasad, Kumar, Jaiswal, & Singh, 2004) leishmaniasis, although causing severe gastrointestinal problems (Sangraula, Sharma, Rijal, Dwivedi, & Koirala, 2003). Since the chemotherapy against leishmaniasis is still inefficient and with toxic side effects, there is an urgent need for the development of new, efficient and non-toxic drugs for the treatment of this disease (Carvalho, Arribas, & Ferreira, 2000).

In a continuation of research work on bioactive molecules, and as a part of a programme to study potential leishmanicidal agents (Khan et al., 2003, 2008), 19 morpholine derivatives **2**–**20** (Scheme 1) were synthesised and randomly screened for their leishmanicidal potential. These studies may provide lead compounds for future drug development.

2. Results and discussion

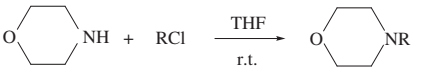
2.1. Chemistry

A series of morpholine derivatives **2**–**20** (Scheme 1) were synthesised by treating various acid chlorides with morpholine (**1**) in tetrahydrofuran at room temperature. The structures of the resultant products **2**–**20** were determined spectroscopically, including UV, IR, NMR, and MS. CHN analyses were found in good agreement with the calculated values.

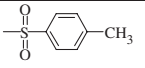
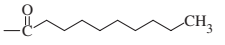
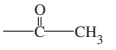
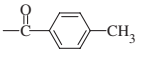
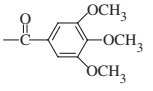
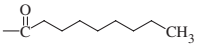
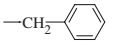
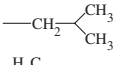
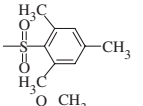
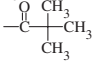
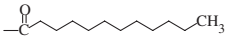
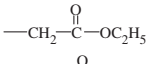
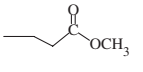
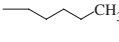
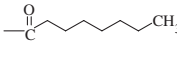
2.2. Biology

Several reports (Ram & Nath, 1996) describing the leishmanicidal activity of nitrogen-containing compounds suggested random evaluation of the leishmanicidal potential of the synthetic N-substituted morpholines in the present series. The testing was performed at the $100\,\mu g\,mL^{-1}$ level according to a literature protocol (Atta-ur-Rahman, Choudhary, & Thomsen, 2001) in duplicate. Amphotericin B and pentamidine were used as the positive controls, having an IC_{50} of 0.24 and $2.56\,\mu g\,mL^{-1}$, respectively.

All of the synthetic derivatives **2**–**20** of morpholine (**1**) were tested for their effects against *Lesihmania major* and the results of the leishmanicidal activity are collected in Table 1. The compounds **3** and **16** were found to be the most active compounds in the present series, having IC_{50} values of 30.0 and $33.0\,\mu g\,mL^{-1}$, respectively. Compounds **3**, **10**, **16**, and **20**, which were long, straight chain aliphatic amides, showed IC_{50} values of 30.0, 41.0, 47.0, and $35.0\,\mu g\,mL^{-1}$, respectively. However, the aliphatic amide **4** exhibited an IC_{50} value of $80.0\,\mu g\,mL^{-1}$. The difference in activities of compounds may be explained on the basis of their structures and suggested that the IC_{50} values of these compounds depends on the length of the carbon chain attached to the amide moiety. A nine-carbon chain attached to the amide residue, as in compound **3**, was found to be the most suitable for leishmanicidal activity. The slight decline in activity of compound **20**, having an IC_{50} value of $47.0\,\mu g\,mL^{-1}$, may be due to the reduction of one or two carbons in the carbon chain. Unfortunately, a compound with 10 carbons could not be prepared, but compound **16** ($IC_{50} = 35.0\,\mu g\,mL^{-1}$), with an 11-carbon chain, showed a slight decline in activity, demonstrating that either an increase or a decrease in carbon chain affects the leishmanicidal activity. This hypothesis also establishes that the relative IC_{50} value of compound **4** ($IC_{50} = 80.0\,\mu g\,mL^{-1}$), which has only one carbon attached to the amide residue, with compound **15** ($IC_{50} = 35.0\,\mu g\,mL^{-1}$) may be explained on the basis of its branched carbon chain. The aromatic amides **5**, **6** and **9** showed very weak activity, which



1 **2–20**

Compounds	R	Yield (%)	Physical data
2		87	Off white solid; m.p. 160–162°C; R*f* = 0.47 (1:1, Ethyl acetate/*n*-hexane).
3		72	Colourless liquid; R_f = 0.57 (1:1, Ethyl acetate/*n*-hexane).
4		81	Yellow liquid; R_f = 0.47 (1:1, Ethyl acetate/*n*-hexane).
5		89	Brown liquid; R_f = 0.47 (1:1, Ethyl acetate/*n*-hexane).
6		88	Yellow liquid; R_f = 0.47 (1:1, Ethyl acetate/*n*-hexane).
7		81	Colourless crystals; m.p. 150–152°C; R*f* = 0.42 (1:1, Ethyl acetate/*n*-hexane).
8		74	Yellow liquid; R_f = 0.47 (1:1, Ethyl acetate/*n*-hexane).
9		80	Yellow liquid; R_f = 0.52 (1:1, Ethyl acetate/*n*-hexane).
10		77	Yellow liquid; R_f = 0.56 (1:1, Ethyl acetate/*n*-hexane).
11		86	Light yellow crystals; m.p. 170–172°C; R_f = 0.48 (1:1, Ethyl acetate/*n*-hexane).
12		79	White powder; m.p. 155–157°C; R_f = 0.53 (1:1, Ethyl acetate/*n*-hexane).
13		79	Colourless liquid; R_f = 0.51 (1:1, Ethyl acetate/*n*-hexane).
14		86	Pale yellow crystals; m.p. 180–182°C; R_f = 0.57 (1:1, Ethyl acetate/*n*-hexane).
15		79	Brown liquid; R_f = 0.45 (1:1, Ethyl acetate/*n*-hexane).
16		81	Colourless crystals; m.p. 163–165°C; R_f = 0.45 (1:1, Ethyl acetate/*n*-hexane).
17		80	Colourless crystals; m.p. 187–189°C; R_f = 0.54 (1:1, Ethyl acetate/*n*-hexane).
18		89	Yellow liquid; R_f = 0.46 (1:1, Ethyl acetate/*n*-hexane).
19		88	Yellow liquid; R_f = 0.46 (1:1, Ethyl acetate/*n*-hexane).
20		81	Colourless crystals; m.p. 172–174°C; R_f = 0.46 (1:1, Ethyl acetate/*n*-hexane).

Scheme 1. Synthetic route and physical data of compounds **2–20**.

indicate that an aromatic linkage with an amide residue in this type of compound is not desirable for leishmanicidal activity.

The sulphonamides **2**, **12** and **14** were also evaluated for leishmanicidal activity, having IC_{50} values 48.0, 51.0 and 100.0 $\mu g\,mL^{-1}$, respectively. The difference in activities

Table 1. Leishmanicidal activity of compounds **2–20**.

Compounds	IC_{50} ($\mu g\,mL^{-1}$)
2	48.0
3	30.0
4	80.0
5	83.0
6	100.0
7	100.0
8	80.0
9	100.0
10	41.0
11	100.0
12	51.0
13	100.0
14	100.0
15	35.0
16	35.0
17	100.0
18	100.0
19	100.0
20	47.0
Amphotericin B	0.24
Pentamidine	2.56

may again be explained on the basis of their structures. Compound **2**, which was a 4-methyl phenyl sulphonamide, was found to be the most active sulphonamide, whereas methane sulphonamide showed a slight decline in activity, suggesting that aromatic sulphonamides with suitable substitution may have better potential for leishmanicidal activity as compared to aliphatic sulphonamides. The marked reduction in activity of 2,4,6-trimethylphenyl sulphonamide (**14**) may be explained on the basis of the steric hindrances of the sulphonamide.

The compounds **7**, **8**, **11**, **13** and **17–19**, which were tertiary amine derivatives of morpholine, showed only weak potential for leishmanicidal activity. Only compound **8** showed activity of $<80.0\,\mu g\,mL^{-1}$, while all other compounds showed $100.0\,\mu g\,mL^{-1}$. The enhanced activity of compound **8** may be due to the presence of a small carbon chain (ethyl) on the nitrogen atom of morpholine (**1**), while compounds **11**, **13** and **17–19** showed a decrease in activity due to an unsuitable carbon chain attached to the nitrogen atom.

In conclusion, aliphatic, straight carbon chain amides enhance the leishmanicidal activity of morpholine (**1**), while an aliphatic, branched chain amide linkage at the nitrogen atom of morpholine (**1**) also increased the leishmanicidal potential of morpholine (**1**). For the sulphonamides, it was concluded that a suitable substitution on an aromatic sulphonamide may enhance the activity of morpholine (**1**) and that a small carbon chain sulphonamide of morpholine (**1**) might play an important role in increasing leishmanicidal activity. Further research on the tertiary amides of compound **1** may also produce good results, either using aliphatic, straight carbon chains with some functional groups like amidine, or hydrazino residues, which may enhance the activity,

as in the case of pentamidine and acyl hydrazides. This research suggested that morpholine may act as possible lead compound for further research for potential leishmanicidal agents.

3. Experimental

3.1. General

Melting points were determined using a Büchi 434 melting point apparatus and are uncorrected. NMR spectroscopy was performed on a Bruker AVANCE 400 MHz. Elemental analyses were carried out on a Carlo Erba Strumentazion-Mod-1106, Italy. UV and IR spectra were recorded on a Perkin-Elmer Lambda-5 UV/VIS and a JASCO IR-A-302 spectrophotometer, respectively. A Finnigan MAT-311A (Germany) spectrometer was used to record mass spectra. Thin layer chromatography (TLC) was performed on pre-coated silica gel glass plates (Kieselgel 60, 254, E. Merck, Germany) and chromatograms were visualised by UV light at 254 and 365 nm or iodine vapours.

3.2. General procedure for the preparation of compounds 2–20

To a mixture of morpholine (**1**, 1 mmol) dissolved THF (10 mL) was added a mixture of potassium carbonate (2 mmol), and the resultant mixture was stirred at room temperature for 15 min and then various acid chlorides (1 mmol) were added. The mixture was stirred for 48 h and the reaction was monitored by TLC analysis. On completion, the solid was filtered with a sintered glass funnel. The filtrate was evaporated on a rotary evaporator, the residue was dried on a two-stage pump (high vacuum) and the resultant product, if solid, was recrystalised and dried in a desiccator, while the liquid products were purified by vacuum distillation. The compounds were characterised using different spectroscopic techniques, including UV, IR, NMR and MS, elemental analyses, to determine the purity.

3.3. Leishmanicidal bioassay (in vitro)

Leishmania major were grown in bulk in modified NNN biphasic medium by using normal physiological saline. *Leishmania* promastigotes were cultured with RPMI 1640 medium, supplemented with 10% heat-inactivated foetal bovine serum (FBS). Parasites at log phase were centrifuged at 2000 rpm for 10 min, and washed three times with saline at the same speed and time. Parasites were diluted with fresh culture medium to a final density of 10^6 cells per millilitre.

In a 96-well microtiter plate, 180 μL of medium was added in the first row and 100 μL of medium was added in others wells. A total of 20 μL of the experimental compound was added in medium and serially diluted. A total of 100 μL of the parasite culture was added in all wells. Two rows were left for negative and positive controls. Negative controls received only medium while the positive control contained varying concentrations of standard anti-leishmanial compounds amphotericin B and petamidine. The plate was incubated 21–22°C for 72 h. The culture was examined microscopically on an improved Neubauer counting chamber and IC_{50} values of compounds were calculated by software EZfit 5.03 (Perella Scientific). All assays were performed in duplicate (Atta-ur-Rahman, Choudhary, & Thomsen, 2001).

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