Nitroimidazooxazoles# Part XXIV, Search for Antileishmanial Agents: 2,3-Dihydro-6-nitroimidazo[*2,1-b*]oxazoles as Potential Antileishmanial Agents

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A number of mono and bicyclic nitroimidazoles were screened for *in vitro* antileishmanial activity. Among these, compounds belonging to the class of nitroimidazo[*2,1-b*]oxazoles showed moderate to good activity. This class of compounds had been reported previously to have pronounced antitubercular activity, particularly CGI17341 (5a). In the present study (5a) and (5d) and (7) were found to be more potent antileishmanials *in vitro* than the standard and less toxic in relation to a reference compound. (7) Was earlier formulated to have the phenyl group located on C-2(5b).

Key words: Nitroimidazoles, antileishmanial activity, nitroimidazooxazoles, satranidazole

Nitroimidazoles, known to have a wide antimicrobial spectrum, are particularly potent against amoeba, giardia and trichomonas[1,2]. Our earlier extensive foray into the medicinal chemistry of nitroimidazoles resulted in the development of satranidazole (satrogyl®), a potent antiamoebic and antitrichomonal drug with clear superiority over the standard drug metronidazole (3a)[3]. (1a) and its congeners had also potent antianaerobic activity superior to that of (3a)[4]. A further outcome of our research was the discovery of significant antitubercular activity in a series of 2,3-dihydro-6-nitroimidazo[*2,1-b*]oxazoles among which CGI17341 (5a) was the most potent[5,6] that inspired the development of two molecules, which are in clinical trials now, nitroimidazooxazole

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OPC67683, delamanid[7,8] and nitroimidazooxazine, PA824[9,10].

Leishmaniasis is a worldwide disease caused by protozoan parasites of the genus of *leishmania*, which cause a range of diseases in humans ranging from disfiguring cutaneous lesions (CL) to visceral leishmaniasis (VL)[11]. Despite the widespread occurrence and severity of the disease, suitable drugs with a good toxicity profile are not available[12]. In a compounds mining effort, the availability of a library of nitroimidazoles and their ring condensed versions with us attracted the attention of Drugs for Neglected Diseases (DNDi) who undertook to explore their potential for activity against leishmaniasis. Accordingly, about 70 compounds were screened for this activity.

The collection included mainly those that had been investigated for antiamoebic activity earlier and described in our structure–activity relationship paper[3], having the general structures (1-4) and (8-10) (fig. 1). Specially to be mentioned are satranidazole (1a), its 4-nitro analogue (2a), standard antiamoebic nitroimidazoles, secnidazole (3b), ornidazole (3c) and tinidazole (3d). Another group of particular interest for this communication are 2,3-dihydro-6-nitroimidazo[*2,1-b*]oxazoles (5) to which the antitubercular compound (5a) belongs.

The isomeric 2,3-dihydro-3-phenyl-5-nitroimidazo [*2,1-b*]oxazole (6a) and two examples of the corresponding imidazothiazoles (6b) and (6c) formed part of this study. Also tested were five derivatives of 2-amino-6-nitrobenzothiazole. The collection had further the following compounds: four mono and dinitropyrazoles and 2,5-dinitrophenyl piperazines having CH_3, $CO_2C_2H_5$ or $CON(C_2H_5)_2$ group on N(4).

Nitroimidazoles (5a) and (7) previously formulated as (5b) were also resythesised by the earlier procedure[5].

Compound (5a), melting point (m.p.) 160–161°, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (*DMSO-d*$_6$, 8.15) (s, 1H), 5.27 (q, *J*=7 Hz, 1H), 4.37 (dd, *J*=10.2, 8.3 Hz, 1H), 3.93 (dd, *J*=10.2,7.4, 1H), 1.9–2.1 (m, 2H), 1.08 (t, *J*=7.4, 3H); ^{13}C NMR (100.6 MHz, *DMSO-d6*); δ 156.6, 146.2, 116.5, 89.6, 48.4, 27.2, 9.1; m$^+$ 184.16

Compound (7), m.p. 198-200°, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45–7.5 (m, 3H), 7.42 (*DMSO-d*$_6$, 8.20) (s, 1H), 7.2-7.3 (m, 1H), 5.58 (t, *J*=8 Hz, 1H), 5.36 (t, *J*=8.6 Hz, 1H), 4.84 (t, *J*=8.6, 8 Hz, 1H); ^{13}CNMR (100.6 MHz, *DMSO-d6*); δ 157.1, 146.2, 127.0, 129.6, 129.7,116.5, 82.2, 59.4; m+ 232.2.

The resynthesis was undertaken for detailed biological studies and also for structure confirmation. This was required because their interesting antitubercular and antileishmanial activity made them important leads for developing new drugs and their route of synthesis indicated uncertainty in the location of the nitro and alkyl/aryl substituents[5]. We carried out extensive ^{1}H, ^{13}C and HMBC spectral studies. While the location of the NO_2 group at position 6 in both molecules seemed to be secure, that of the C_2H_5 group in (5a) and of the phenyl group in the analogue could not be established due to lack of correlation information in the Heteronuclear multiple-bond correlation (HMBC) spectra. Single crystal X-ray studies (T.N. Guru Row and Sajesh Thomas. Private Communication) confirmed the structure of (5a) (C_2H_5group on C-2) while they revealed that the phenyl group had to be located on C-3 as in (7) and not on C-2 as in (5b) as postulated earlier[5].

Activity against *Leishmania donovani* (axenic) was determined according to the method of Cunningham[13] and *L. donovani* (macrophage) according to the method of Yang *et al*.[14]. Miltefosine was the reference drug. Cytotoxicity was assessed by the procedure described by Page *et al*.[15], the comparative drug being podophyllotoxin. Compounds showing less than 50% inhibition of the axenic culture at the screening dose of 0.8 µg/ml were considered negligibly active and hence of no interest. From the medicinal chemistry point of view, we note that satranidazole (1a), its 4-nitroisomer (2a), other standard antiamoebic drugs, secnidazole (3b), ornidazole (3c), tinidazole (3d) and the 4-nitroisomer, (4a) fall into this category. The IC_{50} of eight compounds passing the screening test and cytotoxicity are recorded (Table 1) and *in vivo* data activity against *L. donovani* for 5a is provided in Table 2.

Compounds 2b (analogue of satranidazole), (6c) [2,3-dihydroimidazo(*2,1-b*)thiazole dioxide], (8)(R=1-benzimidazolyl) and (8)(R=2-methyl-1,3,4-thiadiazolyl-2-amino) had modest IC_{50} values in the 'axenic' test but were less potent than miltefosine. Five compounds having the nitroimidazooxazole scaffold had appreciable activity but among these, (5c) and (5e) were less active than the standard. Compound

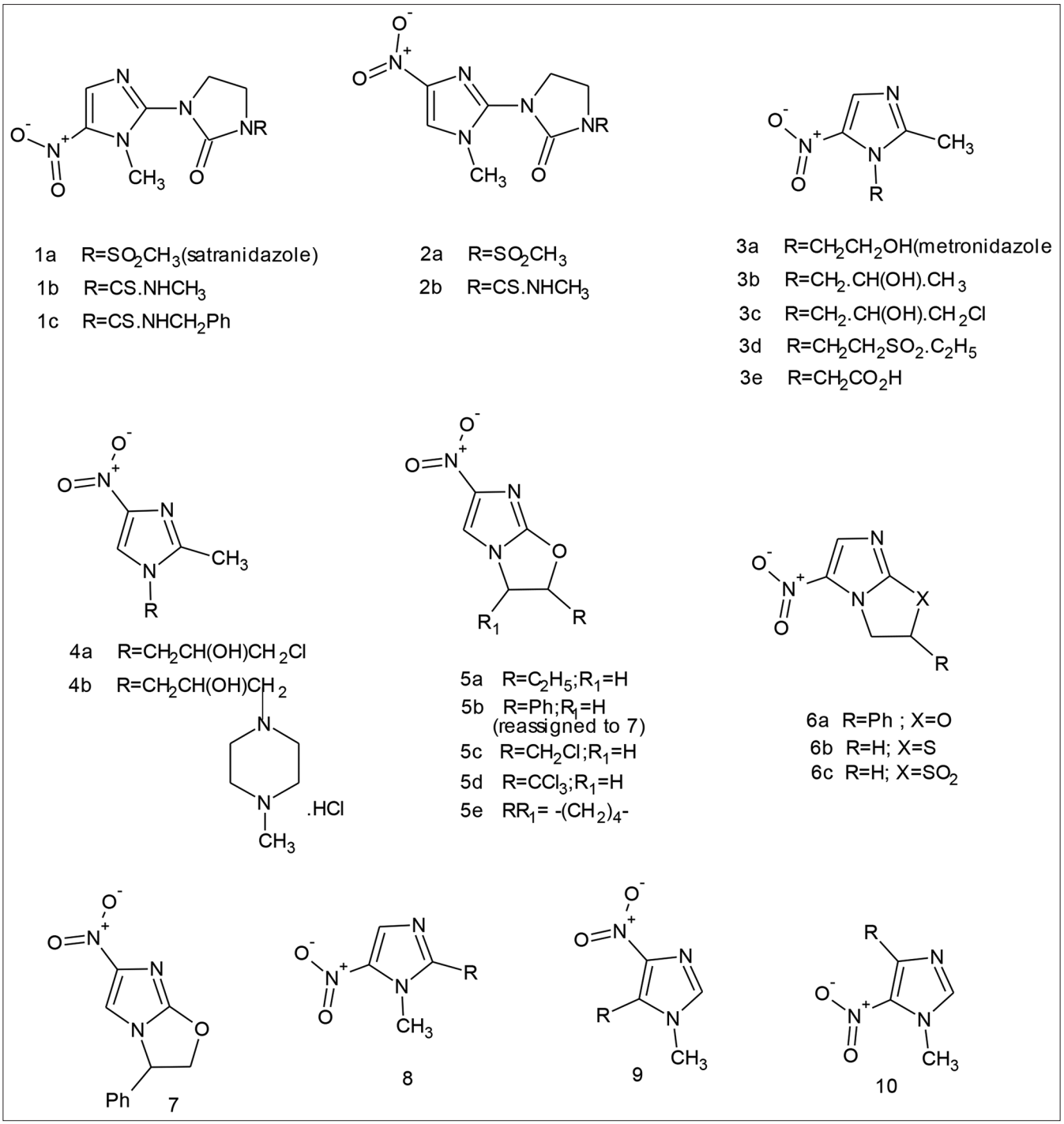

Fig. 1: Structures of nitroimidazole derivatives.

(5a) with an ethyl group and (5d) with a CCl_3 group were about 3.5 times more potent than standard while (7) with a phenyl group at C-3 was twice as active. In the macrophage assay, (5a) had 20 times the potency of the standard, (5d) about 3.5 times and (7) about 2 times. (5a) and (7) were not cytotoxic below 90 µg/ml compared with a figure of 0.009 for podophyllotoxin while the IC_{50} of (5d) in this test was 73.3. These three nitroimidaoxazoles have been taken up for *in vivo* and genotoxicity studies. The results will be published elsewhere.

The present study reveals that 2,3-dihydro-6-nitroimidazo[*2,1-b*]oxazoles like the antitubercular CGI 17341(5a) represent important novel leads for antileishmanial activity.

TABLE 1: *IN VITRO* ACTIVITY AGAINST *L. DONOVANI* AND CYTOTOXICITY

Compound	*L. donovani* (axenic) IC_{50} (µg/ml)	*L. donovani* (macrophage) IC_{50} (µg/ml)	Cytotoxicity $L6IC_{50}$ (µg/ml)>90
2b	0.318		>90
5a	0.048	0.041	>90
5c	0.594		88.3
5d	0.045	0.22	73.3
5e	0.591		>90
6c	0.406		1.405
7	0.081	0.42	>90
8(R=1-benzimidazolyl)	0.476		>90
8(R=2-methyl-1, 3, 4-thiadiazolyl-2-amino)	0.235		1.19
Miltefosine	0.169	0.8	-
Podophyllotoxin	-	-	0.009

The IC50 of eight compounds passing the screening test and cytotoxicity

TABLE 2: *IN VIVO* ACTIVITY AGAINST *L. DONOVANI*

Compound	Dose	% Inhibition	Group weight % change
5a	50 mg/kg i.p.×5	43.9	1.6
SbV (pentostan)	15 mg/kg s.c.×5	47.2	9.2

SbV=Pentavalent antimonials, i.p=intraperitoneal injection, s.c=subcutaneous injection

ACKNOWLEDGEMENTS

The authors are grateful to Marcel Kaiser and Rito Brun, Swiss TPH, Basel for their help in biological screening and to Sandesh Jatindranath and B. Ugarkar for 1H and 13C NMR data.

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Accepted 30 December 2013
Revised 24 December 2013
Received 16 October 2013
Indian J Pharm Sci 2014;76(1):92-95