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Nitroimidazoles XXI** 2,3-dihydro-6-nitroimidazo [2,1-*b*] oxazoles with antitubercular activity***

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nitroimidazoles / antimycobacterial activity

Introduction

Among chemotherapeutic drugs, the group of nitroimidazoles is characterized by a remarkably broad spectrum of antimicrobial properties including antibacterial activity [1, 2]; but there are few reports of a member of this class possessing antitubercular activity. Nitroimidazooxazole **3a** [3, 4] is reported to have radiosensitizing properties [3]. During a routine screening of nitroimidazoles for antimicrobial activity, especially antiamoebic-antitrichomonal properties, which led to the development of satranidazole (C 10213-GO) [5, 6], we discovered that **3a** had potent *in vitro* and *in vivo* antitubercular activities, thus marking this compound one of the few leads besides the quinolone antibacterials [7] for this indication, regarding which the last major discovery of clinical utility was rifampicin in 1965 [8]. This prompted us to synthesize several analogues of **3a** for an expanded study. These new compounds are reported in this note.

Chemistry

The title compounds were obtained from the reaction of 2,4-dinitroimidazole **1** [4] with substituted oxiranes **2**. The reaction was complex and usually gave rise to 3 identifiable products, the title compounds **3**, the 5-nitroisomers **4** and the open chain alcohols **5**. In such reactions **3** was presumably formed from **5** by elimination of nitrous acid. Alcohols **6**, isomeric with **5**, were also evidently formed but were totally converted to the cyclic products **4**. Imidazooxazoles **3** were the major products in most cases and often crystallised out of the reaction mixture. Chromatography of the products from the mother liquors first gave **4**, then more of **3** and finally alcohols **5**. In those cases where the reaction failed to give **3** directly, chromatography was resorted to with the already mentioned results. The 2,4-dinitroimidazolyl ethanols **5** underwent facile cyclisation in the presence of sodium hydride or sodium acetate to afford **3** in moderate to high yields. Our attention was almost exclusively devoted to the synthesis and study of the 6-nitroimidazooxazoles **3**, since very early on in our work we found that only this series exhibited *in vivo* antitubercular activity.

Compounds of this study were characterized by modern spectroscopic techniques (Table I) and by elemental analysis (Scheme 1). The preferred attack by **1** at the less substituted carbon atom of the oxiranes **2** (R = H) has been established earlier [4]. The 6-nitroimidazooxazoles **3** and 2,4-dinitroimidazolylethanols **5** were easily differentiated from the 5-nitro derivatives **4** and **6** respectively by the larger difference in chemical shifts of the imidazole protons ($\Delta\delta$C-5H 0.53–0.75) in the ^{1}H NMR spectra of **3** and **5** in DMSO-d_6 and $CDCl_3$, compared to $\Delta\delta$ of − 0.04 to 0.29 for C-6 H in **4** and C-4H in **6**. In the case of **3p**, in the $CDCl_3$ NMR spectrum the signal of the proton adjacent to the ether function was seen at 4.41 ppm as a large triplet (J 13 Hz) split further into a doublet (J 4 Hz); this corresponds to 2 a,a and one a,e couplings, indicative of a *trans* fusion of the oxazole and cyclohexane ring which is theoretically expected. The signal due to the other bridgehead proton adjacent to the nitrogen atom at 3.82 ppm had, as expected, a similar splitting pattern.

The mass spectra of nitroimidazooxazoles in general exhibited highly intense molecular ions which fragmented by loss of O, then NO, followed by loss of CO to presumably form a 2-alkylimidazole radical. The latter loses CH_2 groups progressively to form an imidazolyl radical cation (m/z 67). Thus the fragments for a typical case like **3b** are: 183 (M^+ 100%), 167 (10%), 137 (10%), 109 (5%), 95

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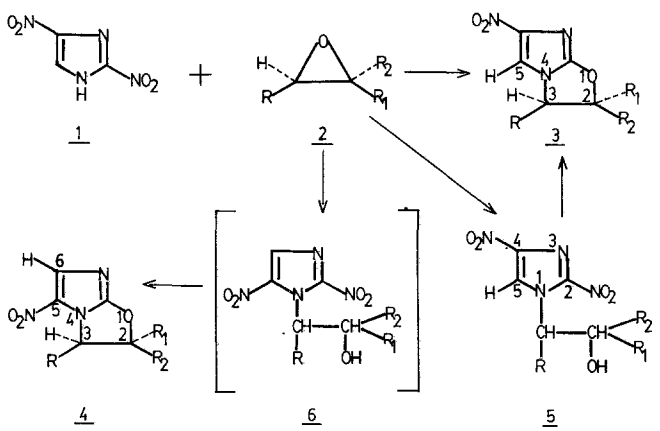
Table I. Nitroimidazooxazoles **3,4** and dinitroimidazolylethanols **5**.

Compound No.	R	R_1	R_2	Crystallised from*	Yield %	m.p. °C	Molecular formula	^{1}H NMR-δ imidazole H			Antitubercular activity	
								DMSO-d_6	$CDCl_3$	Δδ	*In vitro* MIC (r/ml)	*In vivo* mg/kg *p.o.* (ED_{50})
3a	H	CH_3	H	A	35	152–154	$C_6H_7N_3O_3$	8.11	7.54	0.57	1.95	25
4a	H	CH_3	H	D	11	70–72	$C_6H_7N_3O_3$	7.84	7.67	0.17	250	–
3b	H	C_2H_5	H	D	35	160–161	$C_7H_9N_3O_3$	8.11	7.54	0.57	0.06	10
3c	H	CH_2Cl	H	D	9	168–170	$C_6H_6ClN_3O_3$	8.10	7.56	0.54	0.12	30–100
4c	H	CH_2Cl	H	G	6	94–97	$C_6H_6ClN_3O_3$	7.60	7.64	–0.04	250	–
3d	H	Ph	H	A	27	191–192	$C_{11}H_9N_3O_3$	8.13	7.53	0.60	0.95	ø at 100
4d	H	Ph	H	E	2	145–147	$C_{11}H_9N_3O_3$	7.99	7.70	0.29	1.95	ø at 100
3e	H	Bu(n)	H	B	15	142–145	$C_9H_{13}N_3O_3$	8.11	–	–	–	–
3f	H	CH_2Br	H	F	32	174–176	$C_6H_6BrN_3O_3$	8.12	7.55	0.57	0.24	30–100
3g	H	CCl_3	H	A	28	167–169	$C_6H_4Cl_3N_3O_3$	8.13	7.59	0.54	31.2	–
3h	H	$CH_2OPr(iso)$	H	B	40	108–110	$C_9H_{13}N_3O_4$	8.07	7.54	0.53	3.9	ø at 200
3i	H	$CH_2OAllyl$	H	A	21	95–97	$C_9H_{11}N_3O_4$	8.11	7.54	0.57	3.9	ø at 200
3j	H	CH_2O Ph	H	A	55	206–208 (d)	$C_{12}H_{11}N_3O_4$	8.17	insol	–	0.24	ø at 200
3k	H	Bu(n)	CH_3	A	15	135–137	$C_{10}H_{15}N_3O_3$	8.03	insol	–	0.015	–
3l	H	$C_7H_{15}(n)$	CH_3	A	10	145–146	$C_{13}H_{21}N_3O_3$	8.07	insol	–	0.0037	–
3m	H	$-(CH_2)_5-$		B	36	255–256	$C_{10}H_{13}N_3O_3$	8.09	7.50	0.59	0.03	ø at 100
3n	H	$(CH_2)_2$-CH(CH_3)-$(CH_2)_2$		H	20	252–253	$C_{11}H_{15}N_3O_3$	8.10	insol	–	0.12	–
3o	H	$-(CH_2)_6-$		I	7	210–212	$C_{11}H_{15}N_3O_3$	8.09	7.50	0.59	0.015	ø at 100
3p	–	$(CH_2)_4-$	H	B	60	160–162	$C_9H_{11}N_3O_3$	8.29	7.55	0.74	31.2	–
5a	H	CH_3	H	A	35	92–94	$C_6H_8N_4O_5$	8.68	8.00	0.68	1.95	ø at 400
5b	H	C_2H_5	H	B	30	112–114	$C_7H_{10}N_4O_5$	8.69	8.00	0.69	0.48	ø at 100
5c	H	CH_2Cl	H	B	40	120–122	$C_6H_7ClN_4O_5$	8.50	insol	–	0.97	ø at 100
5d	H	CH_2Br	H	A	2	116–120	$C_6H_7BrN_4O_5$	8.69	insol	–	–	–
5e	H-$(CH_2)_2$-CH(CH_3)-$(CH_2)_2$			C	10	130–132	$C_{11}H_{16}N_4O_5$	8.53	7.93	0.60	–	–
5f		$-(CH_2)_4-$	H	B	30	158–161	$C_9H_{12}N_4O_5$	8.95	insol	–	62.5	–

*Solvents of crystallisation: A: CH_2Cl_2 + Et_2O; B: CH_2Cl_2 + Et_2O + MeOH; C: MeOH + Et_2O; D: CH_2Cl_2 + EtOH + hexane; E: CH_2Cl_2 + hexane; F: acetone + Et_2O; G: Et_2O; H: CH_3CN + Et_2O; I: acetone.
ø: inactive at the dose stated.



Scheme 1.

(5%), 81 (5%) and 67 (80%). In the case of spiro compound **3n**, the molecular ion at 237 fragmented additionally by a different pathway to give the radical cation of 4-methylcyclohexanone (*m/z* 108; 50%). The open chain alcohols, *e.g.* **5e** (M^+ 237) seemed to cyclize in the mass spectrometer with loss of HNO_2 (– 47) and undergo fragmentation by the pathway observed for **3n**.

Antitubercular activity

Most of the compounds were tested for antitubercular activity *in vitro* and *in vivo* (Table I) as described in the experimental section. Many exhibited good *in vitro* activity, with **3a–d, f, j, k–o, 4d** and **5a–c** having MIC values of 1.95 μg/ml or less. Among these, **3b** (0.06), **3m** (0.03), **3k** and **o** (0.015) and **31** (0.0037 μg/mg) were highly active. Comparable figures for some standard antituber-

cular drugs are: isoniazid, 0.06 μg/ml; ethambutol, 0.2 μg/ml and rifampicin, 0.04 μg/ml. *In vivo* screening results showed that the 5-nitroimidazooxazole **4d** and the 2,4-nitroimidazolylethanols **5a–c** were inactive. Among the 6-nitroimidazooxazoles, **3d**, **h**, **i**, and **o** were inactive *in vivo* even at 100 mg/kg or above, while **3a–c** and **f** were active with an ED_{50} of < 100 mg/kg *per os (p.o.)*. Among these the most active, **3b**, had an ED_{50} value of 10 mg/kg *p.o.* This may be compared with ED_{50} values for isoniazid 2.5 mg/kg *p.o.* and rifampicin 5.9 mg/kg *p.o.* The approximate LD_{50} of **3b** in mice was around 500 mg/kg *p.o.*, thus giving a good therapeutic ratio. However, it was found to be mutagenic in the Ames test, discouraging us from carrying out further developments and investigations in this series, such as the *in vivo* characterization of **31** with very high *in vitro* activity. Nevertheless, the recently published work of Walsh *et al.* [10] offers the hope that further structural alterations may lead to a separation of antitubercular activity from mutagenicity and eventually to the development of a useful molecule.

Experimental protocols

All new compounds were analysed for C, H, N and the values found were within 0.4% of the theoretical values. ^1H NMR spectra were run on a Varian EM 360 spectrometer. Chemical shifts are quoted in ppm downfield from TMS as the internal standard. Mass spectra were run on a Shimadzu QP 1000 GC-MS instrument.

*Reaction of 2,4-dinitroimidazole **1** with substituted oxiranes **2***

A suspension of 44 g (0.28 mol) of **1** and 22 g of anhydrous sodium acetate in 300 ml of absolute alcohol containing 60 ml of butyleneoxide was heated at 70° for 12 h and the resultant solution left at 28° for 16 h. The precipitated **3d** was filtered off and the filtrate evaporated *in vacuo*. The residue was triturated with cold water and the solid product removed. The combined solids were crystallized from methylene chloride–ether to afford 17.5 g of **3b** (35%), mp 165–167°. Upon acidification the aqueous solution allowed recovery of unreacted **1** (12%). The mother liquor from the crystallization of **3b** was evaporated and the residue chromatographed over a column of 250 g of silica gel. Elution with chloroform gave a small quantity of impure **3c**. Chloroform 1% methanol eluted the alcohol **5b** which was crystallized from methylene chloride–methanol–ether; 18.4 g (30%). In the case of propylene oxide, epichlorhydrin and styrene oxide, chromatography gave some amounts of **4a**, **4c** and **4d** respectively; these were followed by **3** and finally by **5**.

The reaction between **1** and **2** could be carried out in the absence of a solvent when similar products were formed in a highly exothermic reaction. Distillation of volatiles and trituration of residue with ether–hexane gave **3**. For the neat reaction of **1** with cyclohexene oxide, a few drops of 50% KOH were needed as catalyst.

*Cyclization of alcohols **5** to imidazooxazoles **3***

1 g of the alcohols **5f**, 0.25 g of 50% suspension of sodium hydride in kerosene and 3 ml of dioxane were warmed together and set aside for 1/2 h. A few drops of methanol were then added followed by excess water. The precipitate of **3p** was collected and crystallized from methylene chloride–ether to afford 0.6 g (73.5%), mp 160–162°. The cyclisation could also be brought about by using sodium acetate in ethanol; yields by these methods ranged from 40–75%. Compounds **3–5** are listed in Table I.

Antitubercular screening [9]

In vitro *testing*. The compounds were dissolved in appropriate diluent (DMF, DMSO) and 2-fold serial dilutions ranging from 500–0.01 μg/ml were carried out in Kirchner's broth medium containing 0.05% of Tween 80. Each tube received 0.05 ml of 7-day-old growth *Mycobacterium tuberculosis* strain $H_{37}R_V$. All the tubes were then incubated at 37°C for 7–9 days. The results were recorded in terms of Minimal Inhibitory Concentration (MIC), defined as the lowest concentration of the drug that inhibited the growth of *M. tuberculosis* completely as compared with the drug-free control.

In vivo *testing*. Female mice (MF2 strain) weighing about 17–19 g were used. Each mouse was infected through the tail vein with $\approx 5 \times 10^6$ CFU of *M. bovis* (Ravenel) suspended in 0.2 ml of Kirchner's broth medium. Various test doses of the compounds were administered orally on days 11 and 12 post-infection to groups of 10 mice. Mortalities were observed daily. The results were recorded in terms of protective response *viz.* ED_{50} dose of the drug which protected 50% of the test animals at the time of 100% mortality in the drug free infection control group (23rd day).

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