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SYNTHESIS OF 2,3-DIHYDRO-7-NITROIMIDAZO[5,1-b]OXAZOLES AS POTENTIAL TUBERCULOSTATIC AGENTS

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Abstract: A series of 2,3-dihydro-7-nitroimidazo[5,1-b]oxazoles has been prepared in one-pot reaction. The proposed new method of their synthesis requires a treatment of 4,5-dinitroimidazole or 2-methyl-4,5-dinitroimidazole with oxiranes. Some of the obtained compounds have been tested *in vitro* for antimycobacterial activity and the spatial structure of them has been also discussed.

Keywords: nitroimidazooxazolines, 2,3-dihydro-6-nitroimidazo[2,1-b]oxazoles, 2,3-dihydro-7-nitroimidazo-[5,1-b]-oxazoles, tuberculosis, antituberculotic activity

Nitroimidazoles make a group of compounds of great commercial and chemotherapeutic importance. Clinical trials have shown that many of them have a remarkably broad spectrum of antimicrobial properties including antibacterial activity. The introduction of metronidazole which acts against anaerobic bacteria and additionally has antiprotozoal activity, has stimulated much synthetic chemistry on nitroimidazoles (1). A series of bicyclic nitroimidazooxazoles, originally investigated as radiosensitizers for use in cancer chemotherapy, have been found to be active against culture replication *M. tuberculosis* (2, 3). Compounds containing the imidazo[2,1-b][1,3]oxazine ring system have been shown to be active against tuberculosis as well. The most promising compound of this series, PA-824 (Figure 1), has the MIC of 0.06 μg/mL against *M. bovis* BCG and high activity against Mtb H_{37}Rv (4, 5).

Mycobacterium tuberculosis is the greatest single infectious cause of mortality worldwide. Estimates indicate that one-third of the world population is infected with latent *M. tuberculosis* (6). According to the World Health Organization (WHO) report, *M. tuberculosis* currently infects over 2 billion people worldwide. Each year, 30 million of new cases are reported. Tuberculosis (TB) kills roughly two million people annually (7). In most parts of the world people who suffer from TB are restricted to combinations of only five drugs for effective treatment of this disease. These are rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide (8, 9).

Hitherto, a series of 2,3-dihydro-6-nitroimidazo[2,1-b]oxazoles has been tested as to antitubercular properties. One of these compounds – CGI 17341 has been found particularly promising in clinical trials (10). This compound was originally discovered in the search for novel dinitroimidazoles that might be used as radiosensitizers. The reaction between 2,4-dinitroimidazoles and oxiranes unexpectedly gave the products of intramolecular cyclization and simultaneous loss of the 2-nitro group (2). However, the activity of 2,3-dihydro-7-nitroimidazo[5,1-b]oxazoles obtained from 4,5-dinitroimidazoles, was not discovered then.

In continuation of our work on the syntheses of pharmacologically active nitroimidazole derivatives, an effort was made at synthesis of bicyclic 2,3-dihydro-7-nitroimidazo[5,1-b]oxazoles.

Some of newly obtained and some of previously described (11, 12) nitroimidazooxazoles from [2,1-b] and [5,1-b] series were tested *in vitro* as to their tuberculostatic activity.

EXPERIMENTAL

Chemistry

All compounds were crystallized from water or 40% ethanol. Melting points were measured on a Kofler's apparatus and are uncorrected. The

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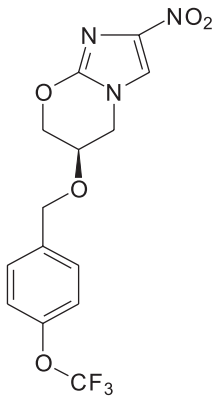


Figure 1. The structure of compound PA-824.

^{1}H-NMR spectra were recorded on a Varian Gemini 300 VT and Mercury 300 spectrometers at 300 MHz in the DMSO-d_6 solutions using TMS as internal standard. The mass spectra were recorded on a 402 AMD INTECTRA apparatus by the electron impact technique, operating at 75 eV. The HRMS were recorded on the same spectrometer. Analytical TLC was performed on Merck silica gel $60F_{254}$ plates using a mixture of methylene chloride and methanol (9:1, v/v) as eluent. The spots were observed in the ultraviolet light (λ = 254 nm).

The syntheses of a series of eight 2,3-dihydro-7-nitroimidazo[5,1-b]oxazoles were performed. These compounds were synthesized in a one-pot reaction by treating 4,5-dinitroimidazole or 2-methyl-4,5-dinitroimidazole with appropriate oxiranes (Scheme 1). This type of intramolecular cyclization was possible in the presence of a base and in alcohol as a solvent. In the syntheses of bicyclic derivatives of 4,5-dinitroimidazole, ethanol was the most satisfactory solvent. In the similar reactions of 2-methyl-4,5-dinitroimidazole, *n*-propanol was the optimal choice. In the preliminary experiment, sodium hydroxide was used as a base, however, it caused some decomposition reactions. Then, potassium carbonate was tested and proved to be smooth in activity and to lead to target compounds. Bromination with bromine in acetic acid solution in the presence of sodium acetate of compounds **7a** and **8a** gave two new derivatives as the products of electrophilic substitution reaction with the bromine atom only in the 2 position of aromatic imidazole ring.

The structures of compounds **4a**-**8b** were determined on the basis of their ^{1}H-NMR, MS and HRMS spectra. The mass spectra of nitroimidazooxazoles obtained in general showed highly abundant molecular ions which fragmented by the loss of O, then NO, followed by other groups or atoms such as Cl, Br to form an N-alkylimidazole radical. The subsequent loss of CH_2 led to imidazolyl radical cation (m/z 67). In the ^{1}H-NMR spectra of the compounds obtained the signals of the characteristic groups showed similar chemical shifts. For instance, the chemical shifts of the methyl groups at position 2 of the imidazole ring in compounds **4b**-**8b** were in the range of 1.86-2.23 ppm, whereas in the spectra of compounds **4a** and **4b**, the doublets at 1.60-1.62 ppm were assigned to the methyl group at the oxazole moiety. In the case of ethyl group in the same position in oxazole ring (compounds **5a**, **5b**) there were two signals: triplets assigned to CH_3 at 0.98-1.00 ppm and multiplets assigned to CH_2 in the range of 1.86-2.06 ppm. The signals assigned to the CH_2Cl and CH_2Br groups appeared as fragment of multiplets in the range of 3.99-4.20 ppm in the ^{1}H-NMR spectra of compounds **7a**, **7b** (11) and **8a**, **8b**. One singlet at 7.45 ppm with integral intensity for one proton was characteristic of the hydrogen atom at C-2 position in the imidazole ring (compounds **4a**, **5a**, **6a** and **8a**).

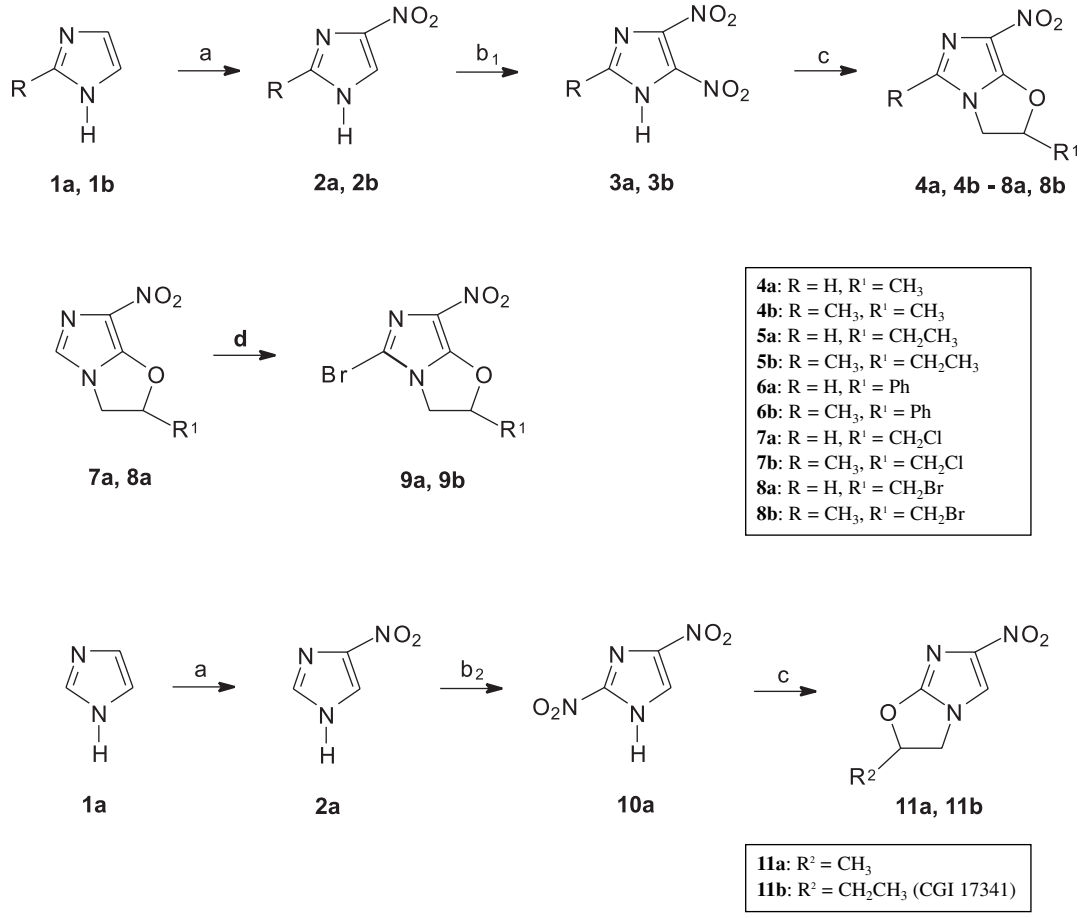
General procedures

Nitroimidazoles, dinitroimidazoles (13) and compounds **7a**, **7b** (11) (from 4,5-dinitroimidazole series), **10a** (14), **11a**, **11b** (12) (from 2,4-dinitroimidazole series) were synthesized according to known procedures.

Compounds **4a-6b**

In a typical experiment, compound **3a** or **3b** (1 equiv.) and the corresponding oxirane (3 equiv.) were dissolved in 10 mL of ethanol or *n*-propanol, respectively. One equiv. of anhydrous K_2CO_3 was then added and the mixtures were heated under reflux for about 2 h. After cooling, the mixtures were diluted with five times quantity of water, then were transferred to a separatory funnel and extracted three times with methylene chloride. The organic extracts were dried with $MgSO_4$ and concentrated in vacuum. The orange solid residues were crystallized from water with charcoal to afford pure products as white needles. Spectral and analytical data of the products obtained are as follows:

4a. Yield 25%, m.p. 141-143^{o}C, R_f = 0.66; ^{1}H-NMR δ (ppm): 7.43 (s, 1H, $H_{arom.}$), 5.90-5.78 (m, 1H, C*H*-CH_3), 4.51 (dd, *J* = 8.2; 10.2 Hz,1H, N-CH_2), 4.00 (dd, *J* = 7.9; 10.3 Hz, 1H, N-CH_2), 1.62 (d, *J* = 6.3 Hz, 3H, CH_3). MS m/z (%): 169.0 (M^+; 83.7); HRMS (ES): calcd. for $C_6H_7O_3N_3$: 169.04874, found: 169.04848.



Scheme 1. Synthesis methods of **4a**, **4b**-**8a**, **8b** and **11a**-**b**.
a: HNO_3, H_2SO_4, temp.; **b_1**: conc. HNO_3, conc. H_2SO_4, temp.; **b_2**: conc. HNO_3, CH_3COOH, $(CH_3CO)_2O$, PhCl, temp.; **c**: appropriate epoxide: 1,2-epoxypropane (**4a**, **4b**, **11a**), 1,2-epoxybutane (**5a**, **5b**, **11b**), styrene oxide (**6a**, **6b**), epichlorohydrin (**7a**, **7b**), epibromohydrin (**8a**, **8b**), K_2CO_3, alcohol, temp.; **d**: Br_2, CH_3COOH, CH_3COONa.

4b. Yield 28%, m.p. 130-131°C, R_f = 0.68; ^{1}H-NMR δ (ppm): 5.89-5.78 (m, 1H, C*H*-CH_3), 4.42 (dd, *J* = 8.0; 9.9 Hz, 1H, N-CH_2), 3.93 (dd, *J* = 7.7; 10.0 Hz, 1H, N-CH_2), 2.20 (s, 3H, CH_3 in the imidazole ring), 1.60 (d, *J* = 6.3 Hz, 3H, CH_3). MS m/z (%): 183.1 (M^+; 43.4); HRMS (ES): calcd. for $C_7H_9O_3N_3$: 183.16046, found: 183.16107.

5a. Yield 15%, m.p. 162-163°C, R_f = 0.74; ^{1}H-NMR δ (ppm): 7.45 (s, 1H, $H_{arom.}$), 5.75-5.65 (m, 1H, C*H*-C_2H_5), 4.54 (dd, *J* = 8.1; 10.1 Hz, 1H, N-CH_2), 4.00 (dd, *J* = 8.1; 10.0 Hz, 1H, N-CH_2), 2.06-1.87 (m, 2H, C*H*$_2$-CH_3), 1.00 (t, *J* = 7.4 Hz, 3H, CH_2-C*H*$_3$). MS m/z (%): 183.3 (M^+; 11.8); HRMS (ES): calcd. for $C_7H_9O_3N_3$: 183.16046, found: 183.16088.

5b. Yield 26%, m.p. 91-92°C, R_f = 0.77; ^{1}H-NMR δ (ppm): 5.74-5.64 (m, 1H, C*H*-C_2H_5), 4.40 (dd, *J* = 8.1; 10.0 Hz, 1H, N-CH_2), 4.01 (dd, *J* = 7.8; 10.0 Hz,1H, N-CH_2), 2.21 (s, 3H, CH_3 in the imidazole ring), 2.04-1.86 (m, 2H, C*H*$_2$-CH_3), 0.98 (t, *J* = 7.4 Hz, 3H, CH_2-C*H*$_3$). MS m/z (%): 197.0 (M^+; 23.0); HRMS (ES): calcd. for $C_8H_{11}O_3N_3$: 197.08005, found: 197.08031.

6a. Yield 20%, m.p. 137-139°C, R_f = 0.48; ^{1}H-NMR δ (ppm): 7.52-7.28 (m, 6H, Ph (5H) and imidazole C-2 (1H)), 5.93 (dd, *J* = 6.1; 8.5 Hz, 1H, N-CH_2), 5.77 (t, *J* = 8.8 Hz, 1H, C*H*-Ph), 5.24 (dd, *J* = 6.0; 9.2 Hz, 1H, N-CH_2). MS m/z (%): 231.0 (M^+; 50.2); HRMS (ES): calcd. for $C_{11}H_9O_3N_3$: 231.20746, found: 231.20767.

6b. Yield 23%, m.p. 138-140°C, R_f = 0.68; ^{1}H-NMR δ (ppm): 7.51-7.27 (m, 5H, Ph), 5.92 (dd, *J* = 6.1; 8.4 Hz,1H, N-CH_2), 5.76 (t, *J* = 8.8 Hz, 1H, C*H*-Ph), 5.22 (dd, *J* = 6.0; 9.1 Hz, 1H, N-CH_2), 1.86 (s, 3H, CH_3 in the imidazole ring). MS m/z (%): 245.0 (M^+; 52.4); HRMS (ES): calcd. for $C_{12}H_{11}O_3N_3$: 245.23404, found: 245.23435.

Compounds **8a, 8b**

In a typical experiment compound **3a** or **3b** (1 equiv.) and epibromohydrin (3 equiv.) were dissolved in 10 mL of ethanol or *n*-propanol, respectively. One equiv. of anhydrous K_2CO_3 was then added and the mixtures were heated under reflux for 25 min. After cooling, the mixtures were diluted with five times quantity of water. The light brown precipitates were filtered off and washed with cold water. The products were crystallized from 40% ethanol with charcoal to give pure crystals as white needles. Spectral and analytical data are as follows:

8a. Yield 60%, m.p. 155-157°C, R_f = 0.60; ^{1}H-NMR δ (ppm): 7.45 (s, 1H, $H_{arom.}$), 6.05-5.97 (m, 1H, C*H*-CH_2Br), 4.54 (dd, *J* = 8.9; 10,7 Hz, 1H, N-CH_2), 4.18-3.99 (m, 3H, N-CH_2 (1H) and CH_2Br (2H)). MS m/z (%): 247.0 (M^+; 53.9); HRMS (ES): calcd. for $C_6H_6O_3N_3Br$: 246.95926, found: 246.96008.

8b. Yield 76%, m.p. 162-165°C, R_f = 0.43; ^{1}H-NMR: δ (ppm): 6.04-5.96 (m, 1H, C*H*-CH_2Br), 4.53 (dd, *J* = 8.9; 10.7 Hz, 1H, N-CH_2), 4.20-3.99 (m, 3H, N-CH_2 (1H), CH_2Br (2H)), 2.23 (s, 3H, CH_3 in the imidazole ring). MS m/z (%): 261.0 (M^+; 62.4); HRMS (ES): calcd. for $C_7H_8O_3N_3Br$: 260.97491, found: 260.97425.

Compounds **9a, 9b**

In a typical experiment compound **7a** or **8a** (1 equiv.) and sodium acetate (3.6 equiv.) were dissolved in 10 mL of glacial acetic acid and the small excess of bromine (1.5 equiv.) was then added. After 24 h the light yellow solid was precipitated. The precipitate was filtered off and washed with cold water. To obtain the pure product, the solid was dissolved in DMSO and water was then dropped until the majority of the compound precipitated; then, the amorphous product was separated. Spectral and analytical data are as follows:

9a. Yield 53%, m.p. 176-177°C, R_f = 0.73; ^{1}H NMR δ (ppm): 6.10-6.02 (m, 1H, C*H*-CH_2Cl), 4.49 (dd, *J* = 8.9; 10.7 Hz, 1H, N-CH_2), 4.23-4.12 (m, 3H, N-CH_2 (1H) and CH_2Cl (2H)). MS m/z (%): 282.8 (M^+; 33.2); HRMS (ES): calcd. for $C_6H_5O_3N_3ClBr$: 282.91824, found: 282.91774.

9b. Yield 60%, m.p. 173-175°C, R_f = 0.91; ^{1}H NMR δ (ppm): 6.08-6.00 (m, 1H, C*H*-CH_2Br), 4.49 (dd, *J* = 8.8; 10.2 Hz, 1H, N-CH_2), 4.16-3.97 (m, 3H, N-CH_2 (1H) and CH_2Br (2H)). MS m/z (%): 326.9 (M^+; 80.6); HRMS (ES): calcd. for $C_6H_5O_3N_3Br_2$: 326.87798, found: 326.86771.

Pharmacology

Compounds **4a**, **4b**, **7a**, **7b**, **11a**, and **11b** were tested for antimycobacterial activity *in vitro*, against *M. tuberculosis* $H_{37}R_v$, M. BCG, *M. avium* and two "wild" strains, isolated from the tuberculotic patients. There were the 1676 strain, resistant to isoniazid (INH) and 456 strain, resistant to INH and rifampicin (RFP). The antitubercular activity of the compounds was determined in liquid Youmans medium containing 10% OADC (Oleic Acid Albumin Dextrose Complex). There were prepared 9 concentrations of tested compounds: 0.035; 0.07; 0.15; 0.3; 0.6; 1.2; 2.5; 5 and 10 μg/mL. Isoniazid (INH) was the control sample. The growth of strains was tested after 21 days. The lowest concentration of the compound investigated, at which no growth of strains was observed, was taken as the MIC. The results obtained are given in Table 1.

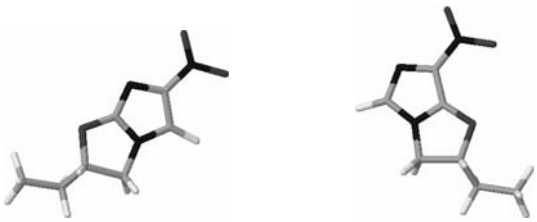
RESULTS AND DISCUSSION

Eight new 2,3-dihydro-7-nitroimidazo[5,1-b]oxazoles have been prepared. Six of them were tested as to antitubercular activity. Two compounds (**5a**, **5b**) were close structural analogues of CGI 17341 (**11b**), the most promising compound of this series.

The results of the biological tests performed proved antitubercular activity of compounds **11a** and **11b**. Additionally, at the concentrations tested, compounds **4a**, **4b**, **7a** and **7b** showed no activity as well as isoniazid, especially against *Myc.* BCG, *Myc. tbc.* 1676, *Myc. tbc.* 456 and *Myc. avium*. It is not easy to conclude about the structure – inhibition activity relationship on the basis of the results obtained. However, simple analysis of the orientation of molecules **5a** and **11b** was performed by PC GAMESS 7.0 program (15) (Figure 2). Calculations of distances between atoms were conducted for the lowest energy conformations. Comparison of these two molecules showed similarity in the distances between the oxygen atoms from oxazole rings and the alkyl chains in both of them. But the distances between the NO_2 groups and the alkyl chains as well as the NO_2 groups and the oxygen atoms from oxazole rings were different and appeared to be an important factor in antitubercular activity. It was possible that the orientation of compound **5a** and similar **4a** provided too little space for a receptor. It was known that receptors usually work according to the "host-guest" rule. Isomeric molecules **5a** and **11b** had different 3D orientations. Isomer **5a** did not fit to the binding site of the receptor just like isomer **11b**. Probably it was the reason why compounds **4a**, **4b**, **7a**, **7b** did not show as high tuberculosis inhibition activity as their structural isomers **11a** and **11b**.

Table 1. Antimycobacterial activity of the investigated compounds, as MIC values (μg/mL).

Compound	Myc. tbc. $H_{37}R_v$	Myc. BCG	Myc. avium	Myc. tbc. 456 res. INH, RMP	Myc. tbc. 1676 res. INH
4a	> 10,0	> 10,0	> 10,0	> 10,0	> 10,0
4b	> 10,0	> 10,0	> 10,0	> 10,0	> 10,0
7a	> 10,0	> 10,0	> 10,0	> 10,0	> 10,0
7b	> 10,0	> 10,0	> 10,0	> 10,0	> 10,0
11a	5.0	> 10,0	> 10,0	> 10,0	> 10,0
11b	0.15	1.2	> 10,0	5.0	> 10,0
INH	0.3	> 10,0	> 10,0	> 10,0	> 10,0



11b 5a

The distances between essential groups or atoms:

	11b	**5a**
NO_2 – O (from oxazole ring)	4.67458 Å	3.28392 Å
NO_2 – CH_3	7.11406 Å	6.05847 Å
O (from oxazole ring) – CH_3	2.98517 Å	2.97649 Å

Figure 2. The molecular structure of isomeric compounds **11b** and **5a**.

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