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SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF SELECTED NITROBENZYLOXYLATED BENZOTRIAZOLES

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Abstract: A series of 1-nitrobenzyloxybenzotriazoles was prepared by the benzylation of the respective halogenosubstituted 1-hydroxybenzotriazoles. The newly obtained compounds were tested against four *Mycobacterium* strains. Particularly high antimycobacterial activity, comparable with that of isoniazide, was found for 5,6-dichloro-1-(3,5-dinitrobenzyloxy)-1H-benzotriazole.

Keywords: 1-benzyloxybenzotriazoles; antimycobacterial activity

Tuberculosis (TB) is a growing global health problem in terms of both disease burden and resistance to chemotherapy. The infection with *Mycobacterium tuberculosis* concerns both the developing and highly developed countries. The World Health Organization (WHO) in its 2006 World Report estimated new TB cases at 9 millions and TB-related deaths – at approximately 2 millions in 2004. The number of new TB cases was stable in most WHO regions, but was on rise in Africa. Infections due to mycobacteria other than tuberculosis (MOTT), 'synergy' of mycobacterial and either HIV or mycotic infections, and mycobacterial infections in immunocompromised patients add to the complexity of the issue.

Despite enormous work done in pharmacology, genetics and molecular biology of mycobacteria, no new clinically useful drug against TB was developed over the last 40 years.

Recent studies on novel derivatives of benzimidazole ring system revealed considerable antimycobacterial activity of various derivatives modified both in the heterocyclic core and in exocyclic constituents (1-5). It is to note that antimycobacterial activity was especially enhanced by nitrobenzylsulfenyl substitution at position 2. On the other hand, most promising of the benzimidazole derivatives modified on the benzene part of the heterocyclus were those substituted with halogen atom(s) (2). These observations have inspired us to test antimycobacterial activity of structurally similar 1-hydroxybenzotriazole system (6). Below, we present the synthesis and antimycobacterial activity of several new O-nitrobenzylated derivatives of halogenosubstituted 1-hydroxybenzotriazoles. The major objective of this study was to search for novel compounds showing a promise to become useful antimycobacterial agents.

EXPERIMENTAL

All chemicals and solvents were purchased from Sigma-Aldrich. Melting points (uncorr.) were measured in open capillary tubes on a Gallenkamp-5 melting point apparatus.

^{1}H-NMR spectra (in ppm) were measured with a Varian Gemini 200 MHZ (or a Varian UNITYplus 500 MHz) spectrometer at 298^{o}K in DMSO-d_6 using tetramethylsilane as an internal standard. Flash chromatography was performed with Merck silica gel 60. Analytical TLC was carried out on precoated silica gel F_{254} (Merck) plates (0.25 mm thickness). Elemental analyses of the new compounds were within ± 0.4% of the respective theoretical values.

Synthesis

4,5,6,7-Tetrabromo-1-hydroxybenzotriazole (**2**)

The mixture contained pentabromonitrobenzene (7) (1.9 g, 4.7 mmol), ethanol (25 mL), toluene (12 mL) and hydrazine hydrate (98%, 2 g, 40 mmol,

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98%) was stirred and refluxed for 24 h. The resulting pale yellow solution was chilled and the precipitate formed was separated by filtration. Further purification was performed on a silica gel column (3 × 10 cm) with $CHCl_3$-MeOH (9:1, v/v). The product was crystallized from EtOH-H_2O (3:1, v/v). White powder, yield 480 mg (23%), m.p. 243°C (with decomposition). ^{1}H-NMR (DMSO-d_6): 7.2 (bs).

5,6-Dibromo-1-hydroxybenzotriazole (**4**)

The solution containing 1,2-dibromo-4,5-dinitrobenzene (8) (5 g, 15.3 mmol) and hydrazine hydrate (98%, 4 g, 80 mmol) in ethanol (70 mL) was stirred and refluxed for 20 h. The pale yellow solution was diluted with water (40 mL) and acidified with 1 M HCl. The mixture was kept in cold for 12 h, the precipitate separated was crystallized from 50% ethanol solution. White powder, yield 3.3 g (74%). M.p. 190°C (with decomposition). ^{1}H-NMR (DMSO-d_6): 8.26 and 8.54 (2s, 2H, arom. H), 14.0 (1H, bs).

Benzylation of substituted 1-hydroxybenzotriazoles

Method A. A biphasic mixture of water (20 mL) and methylene chloride (25 mL) containing respective 1-hydroxybenzotriazole (3 mmol), respective nitrobenzyl chloride/bromide (3 mmol), nitrosubstituted benzyl chloride (0.5 mmol) and potassium carbonate (1.12 g, 20 mmol) was stirred vigorously for 12 h. The organic phase was separated, washed with water (2 × 15 mL), dried over anhydrous magnesium sulfate and evaporated to dryness. The residue was crystallized from a mixture of ethyl acetate/petroleum ether.

Method B. To the solution of the respective 1-hydroxybenzotriazole (2 mmol) in acetone (40 mL) appropriate nitrobenzyl chloride/bromide (3 mmol) and finely powdered anhydrous potassium carbonate were added. The mixture was stirred at room temperature for 1 h (dinitrobenzyl agent) or 12 h (mononitrobenzyl agent). The solid was filtered off and washed with acetone. The washings were combined, evaporated to dryness and crystallized. Only in two cases (**4c** and **5e**) an additional purification by flash column chromatography was necessary.

Antimycobacterial evaluation

The newly obtained compounds were tested for tuberculostatic activity *in vitro* using strains of both the *M. tuberculosis* complex and MOTT: a standard strain of *M. tuberculosis* H_{37}Rv, an INH-resistant *M. tuberculosis* strain (clinical isolate), *M. avium* intercellular complex and IHN-resistant MOTT *M. kansasii* strain.

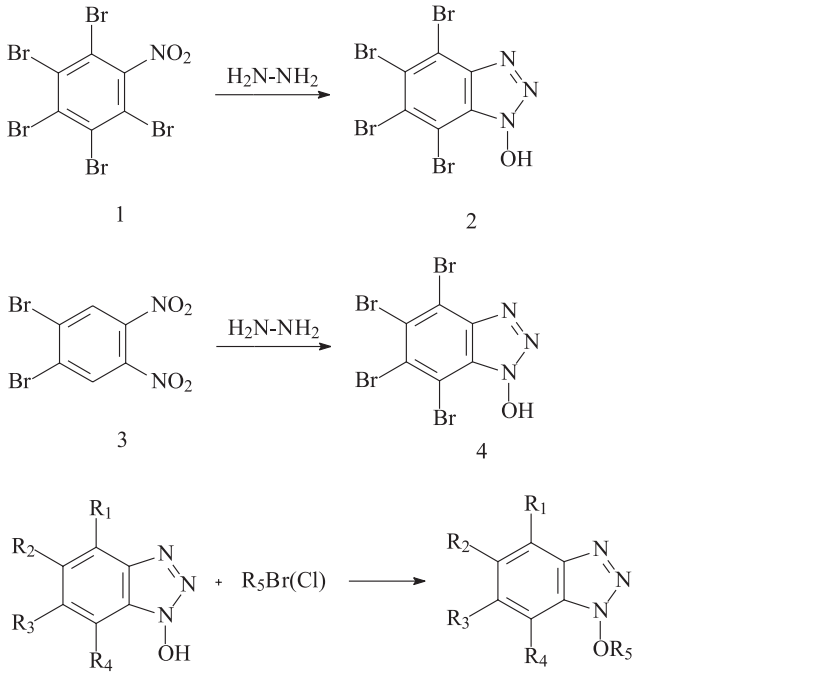
In vitro microbiological studies of the newly synthesized compounds were carried out by a classical test tube method of serial dilutions. Minimum inhibitory activity concentrations were determined in liquid Youman's medium supplemented with bovine serum (10%). The results presented are the means of three independent measurements.

RESULTS AND DISCUSSION

A number of chlorine- or bromine-substituted 1-hydroxybenzotriazoles were chosen as starting compounds for the synthesis of 1-nitrobenzyloxy derivatives. 6-Bromo- (**5**), 5,6-dichloro- (**6**) and 4,5,6,7-tetrachloro- (**7**) 1-hydroxybenzotriazoles were previously described compounds (9-11). The synthesis of additional two derivatives, namely 4,5,6,7-tetrabromo-1-hydroxybenzotriazole (**2**) and 5,6-dibromo-1-hydroxybenzotriazole (**4**), was achieved by heating, respectively, pentabromonitrobenzene (**1**) and 4,5-dibromo-1,2-dinitrobenzene (**3**) with hydrazine (Scheme1).

Benzylation was performed either by stirring 1-hydroxybenzotriazole with the respective isomer of mono- or dinitro-benzyl chloride or bromide in acetone in the presence of finely powdered potassium carbonate (procedure A), or by employing the "phase transfer" protocol (procedure B). 2-Nitro-, 3-nitro-, 4-nitro-, 2,4-dinitro-, and 3,5-dinitrobenzyl chlorides/bromides were used as the benzylating agents. In most cases, the final product was purified by crystallization from the respective reaction mixture; only the derivatives **4c** and **5e** required additional purification by flash chromatography. A series of nitrobenzyloxy derivatives was obtained both for 5,6-dichloro- (**6a-e**) and 4,5,6,7-tetrachlorobenzotriazole (**7a-e**). After preliminary antimycobacterial screening test of chlorinated derivatives, only the most promising structures were chosen for the synthesis of the respective bromine-substituted homologs (**2a**, **4a-c**, **5a,b**) (Scheme 1).

The newly synthesized compounds were characterized by elemental analysis and ^{1}H-NMR, and their purity was verified by TLC (Table 1). It is worth mentioning that the singlet signal of the benzylic -CH_2- group protons at 5.76–6.15 ppm, which was characteristic of all series of benzotriazoles studied, was shifted downfield for 3,5-dinitrobenzoxylated compounds as compared to that for their 2,4-dinitrobenzoxylated isomers. NMR analysis revealed also identical position of the -CH_2- protons' signal in the spectra of mononitrobenzoxylated 3-nitro and 4-nitro isomers (**6a-c** and **7a-c**,



2 R_{1-4} = Br
4 $R_{1,4}$ = H, $R_{2,3}$ = Br
5 $R_{1,2,4}$ = H, R_3 = Br
6 $R_{1,4}$ = H, $R_{2,3}$ = Cl
7 R_{1-4} = Cl

2a R_{1-4} = Br, R_5 = 3,5-dinitrobenzyl
4a $R_{1,4}$ = H, $R_{2,3}$ = Br, R_5 = 3-nitrobenzyl
4b $R_{1,4}$ = H, $R_{2,3}$ = Br, R_5 = 3,5-dinitrobenzyl
4c $R_{1,4}$ = H, $R_{2,3}$ = Br, R_5 = 2,4-dinitrobenzyl
5a $R_{1,2,4}$ = H, R_3 = Br, R_5 = 3-nitrobenzyl
5b $R_{1,2,4}$ = H, R_3 = Br, R_5 = 3,5-dinitrobenzyl
6a $R_{1,4}$ = H, $R_{2,3}$ = Cl, R_5 = 2-nitrobenzyl
6b $R_{1,4}$ = H, $R_{2,3}$ = Cl, R_5 = 3-nitrobenzyl
6c $R_{1,4}$ = H, $R_{2,3}$ = Cl, R_5 = 4-nitrobenzyl
6d $R_{1,4}$ = H, $R_{2,3}$ = Cl, R_5 = 3,5-dinitrobenzyl
6e $R_{1,4}$ = H, $R_{2,3}$ = Cl, R_5 = 2,4-dinitrobenzyl
7a R_{1-4} = Cl, R_5 = 2-nitrobenzyl
7b R_{1-4} = Cl, R_5 = 3-nitrobenzyl
7c R_{1-4} = Cl, R_5 = 4-nitrobenzyl
7d R_{1-4} = Cl, R_5 = 3,5-dinitrobenzyl
7e R_{1-4} = Cl, R_5 = 2,4-dinitrobenzyl

Scheme 1.

respectively), whereas this signal was shifted upfield by 0.17 ppm for their 2-nitro isomers.

The results of antimycobacterial activity testing of the investigated compounds are shown in Table 2. The tests revealed the importance of nitro-substitution in the benzyloxy part of the molecule as well as of the halogen position in benzotriazole for the biological activity of these derivatives. Interestingly, the 5,6-dibromo- and 5,6-dichloro-substituted benzotriazoles (**4a** and **6d**, respectively) carrying 3,5-dinitrobenzyloxy groups in position N^1 were considerably active against the reference strain $H_{37}R_v$, their MIC values being similar to that of isoniazid on molecular weight basis. It is to note that the other nitro-substituted isomers studied showed markedly lower antimycobacterial activity. As for the other mycobacterial strains tested, only *M. kansasii* and *M. tuberculosis 1753* (isoniazide-resistant) were moderately sensitive to the 5,6-dichloro isomer (**6b**), whereas the corresponding 5,6-dibromo-derivative (**4b**) showed much weaker activity against these strains. 4,5,6,7-Tetrachloro-1-(3,5-dinitrobenzyloxy)-benzotriazole (**7d**) also showed noteworthy activity against *M. kansasii* and *M. tuberculosis 1753* strains, whereas none of the compounds described above

Table 1. Physicochemical properties of substituted 1-benzyloxybenzotriazoles.

Comp. No.	Formula (MW)[b]	M.p. (°C)	Yield [%] Method of synthesis	[a]R_f	[1]H-NMR δ (ppm)
2a	$C_{13}H_5N_5Br_4O_5$ (630.83)	177-179	71, B	0.25	5.93 (s, 2H, CH_2), 8.86 and 8.89 (3H, arom. H)
4a	$C_{13}H_8N_4Br_2O_3$ (428.04)	168	70, A	0.40	5.76 (s, 2H, CH_2), 7.70-8.50 (m, 6H, arom. H)
4b	$C_{13}H_7N_5Br_2O_5$ (473.04)	190-192	74, B	0.13	5.89 (s, 2H, CH_2), 8.50-8.90 (m, 5H, arom. H)
4c	$C_{13}H_7N_5Br_2O_5$ (473.04)	141	68, B	0.15	6.08 (s, 2H, CH_2), 8.25-8.55 (m, 5H, arom H)
5a	$C_{13}H_9N_4BrO_3$ (349.15)	147	60, A	0.22	5.76 (s, 2H, 2H, CH_2), 7.55-8.45 (m, 7H, arom. H)
5b	$C_{13}H_8N_5BrO_5$ (394.14)	167-168	68, B	0.12	5.89 (s, 2H, CH_2), 7.55-8.45 (m, 6H, arom. H)
6a	$C_{13}H_8N_4Cl_2O_3$ (339.14)	140-142	55, B	0.31	5.94 (s, 2H, CH_2), 7.70-8.50 (m, 6H, arom. H)
6b	$C_{13}H_8N_4Cl_2O_3$ (339.14)	151-153	71, B	0.38	5.77 (s, 2H, CH_2), 7.70-8.50 (m, 6H, arom. H)
6c	$C_{13}H_8N_4Cl_2O_3$ (339.14)	201-203	64, A	0.28	5.77 (s, 2H, CH_2), 7.80-8.50 (m, 6H, arom. H)
6d	$C_{13}H_7N_5Cl_2O_5$ (384.14)	191-192	56, A	0.19	5.89 (S, 2H, CH_2), 8.51-8.90 (m, 5H, arom. H)
6e	$C_{13}H_7N_5Cl_2O_5$ (384.14)	149-151	48, B	0.25	6.01 (s, 2H, CH_2), 8.25-8.85 (m, 5H, arom. H)
7a	$C_{13}H_6N_4Cl_4O_3$ (408.03)	188	74, B	0.56	5.99 (s, 2H, CH_2), 7.70-8.30 (m, 4H, arom. H)
7b	$C_{13}H_6N_4Cl_4O_3$ (408.03)	166	62, B	0.52	5.82 (s, 2H, CH_2), 7.70-8.70 (m, 4H, arom. H)
7c	$C_{13}H_6N_4Cl_4O_3$ (408.03)	212	70, B	0.50	5.81 (s, 2H, CH_2), 7.84 and 8.30 (2d, 4H, arom. H)
7d	$C_{13}H_5N_5Cl_4O_5$ (453.03)	186	66, B	0.35	5.96 (s, 2H, CH_2), 8.86 and 8.89 (d and t, 3H, arom. H)
7e	$C_{13}H_5N_5Cl_4O_5$ (453.03)	183-186	50, B	0.49	6.15 (s, 2H, CH_2), 8.20-8.80 (m, 3H, arom. H)

[a] $CHCl_3$ was used as the developing solvent
[b] for comparison MW of isoniazide (INH) is 137.14 g/mol.

was appreciably active against *M. avium* intercellular complex.

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Table 2. *In vitro* antimycobacterial activity of nitrobenzyloxylated benzotriazoles (minimum inhibitory concentration, μg/mL).

Compound tested	*M. tuberculosis* $H_{37}R_v$	*M. kansasii* (MOTT)	*M. avium*	*M. tuberculosis 1753* (Isoniazide-resistant)
2a	25	50	100	25
4a	25	100	> 100	50
4b	6.2	50	100	50
4c	100	100	100	50
5a	50	50	100	50
5b	25	50	> 100	25
6a	50	50	100	50
6b	50	50	> 100	50
6c	50	100	100	50
6d	6.2	12.5	100	12.5
6e	100	50	100	100
7a	50	100	> 100	50
7b	100	100	100	100
7c	50	> 100	100	100
7d	12.5	25	100	12.5
7e	50	100	100	25
INH[a]	3.1	100	100	6.2

[a] Reference compound; isoniazide

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