Synthesis and Antimycobacterial and Antiprotozoal Activities of Some Novel Nitrobenzylated Heterocycles

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A series of N-, S-, and O-mononitro- and dinitrobenzyl derivatives of heterocycles was synthesized by alkylation of heterocyclic bases with the respective nitrobenzyl chlorides. Of the newly synthesized compounds, dinitrobenzylsulfanyl derivatives of 1-methyl-2-mercaptoimidazole (**2c**) and of 5-nitro- and 5,6-dichloro-2-mercaptobenzimidazole (**8b** and **8c**, and **8e** and **8f**, respectively) showed considerable antimycobacterial activity. On a molar basis, nine of the novel compounds showed also a considerably higher antiprotozoal efficacy than metronidazole that reduced *T. hominis* viability to 73.5% at 8 μg/ml.

Key words: Nitrobenzyl Derivatives, Antimycobacterial Activity, Antiprotozoal Activity, *Trichomonas hominis*

Introduction

Tuberculosis (TB) is a growing global health problem in terms of both disease burden and resistance to conventional chemotherapy. Nearly one–third of the world population is infected with *Mycobacterium tuberculosis*. This concerns both the developing and well-developed countries. The World Health Organization estimated that over 8 million new cases appeared in 2002, and the global incidence rate of TB was growing by about 1.1% per year. An important aspect of the epidemic is also the rise in the occurrence of multidrug-resistant strains of *M. tuberculosis*. Infections due to mycobacteria other than tuberculosis (MOTT), 'synergy' of mycobacterial and HIV infections, and mycobacterial infections in immunocompromised patients add to the complexity of the issue.

The standard treatment for TB as recommended by WHO is a multidrug regimen that includes four antibiotics: rifampicin, isoniazid (INH), pyrazinamid, and either streptomycin or ethambutol. This treatment scheme is usually effective against *M. tuberculosis*. However, it may fail in settings with high frequency of drug resistance, resulting in markedly lowered cure rate [1]. For instance, if an *M. tuberculosis* strain is resistant to rifampicin and INH, the effectiveness of the standard treatment decreases by 15 to 77% [2]. Despite enormous work done in genetics and biology of this bacterium, practically no new clinically useful drug against this disease was developed over the last 40 years. Therefore, there is an urgent need for designing, synthesis, and testing of new potential anti-TB agents.

Most recent studies of novel compounds of benzimidazole 'ancestry' revealed that the nitrobenzylsul-

fanyl substituent in position 2 of the benzimidazole core especially enhanced antimycobacterial activity in 5-methylbenzimidazole and in benzimidazoles carrying no substituent in the benzene ring [3 – 5]. It also has been found that 4,6-dichloro- and 4,6-dibromo-2-(*p*-nitrobenzylsulfanyl)benzimidazoles showed high efficacy against some Gram-positive bacteria [6]. Having this in mind we synthesized a number of heterocycles carrying the most promising S-nitrobenzylated substituents.

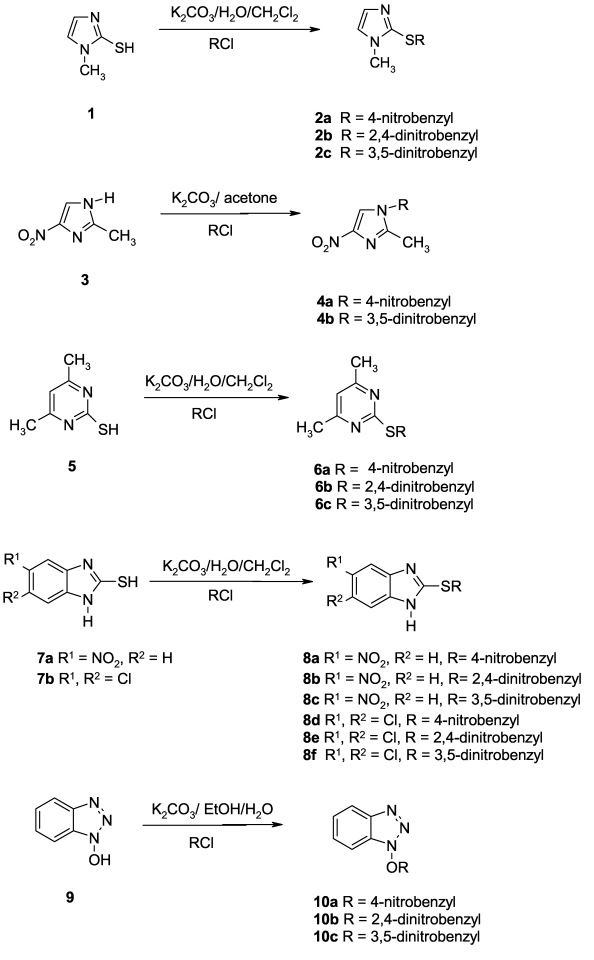
We decided to check as well the activity *in vitro* of the newly synthesized nitrobenzyl derivatives against the protozoan species *Trichomonas hominis* (also called *Pentatrichomonas hominis*). The flagellate resides as a trophozoite in the distal part of small intestine and in large intestine in humans; no cyst stage is known. While the parasite is cosmopolitan by nature, it is more common in the subtropical and tropical zones. Infections with *T. hominis* were reported in persons of both sexes and all ages. However, because of prevailingly fecal-oral transmission route, the flagellate is found more often in children than in adults. *T. hominis* is often identified in human diarrheic stools. Severe *T. hominis*-associated diarrhea cases have been reported in newborns and children up to 5 years of age, some of which were caused by mixed infections with this and other protozoa, including *Entamoeba histolytica*, *Giardia intestinalis* and *Blastocystis hominis* [7 – 12]. A rare case was also described of a mixed infection with *T. hominis*, oral bacteria, and an oral protozoan *Trichomonas tenax* in pus from a subhepatic abscess in a patient with perforated penetrating ventricular ulcer [13].

Whereas infections with *T. hominis* are even more common than those with *Giardia intestinalis* in some world regions, an optimal treatment for the former has not been defined yet. The drug used widely for many protozoan anaerobic parasites is metronidazole (chemical name: 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole), which is also recommended to fight intestinal trichomonosis. Due to increased use of the agent, many metronidazole-resistant strains of *Clostridium*, *Helicobacter pylori*, *Entamoeba histolytica*, *Trichomonas vaginalis* and *Giardia intestinalis* emerge, which are reported more and more frequently (see [14 – 18]). Therefore, there is a growing need for new antiprotozoal agents.

In this study we tested numerous nitrobenzyl derivatives of heterocyclic compounds to find a heterocyclic core structure that would be the most promising candidate for the synthesis of modified derivatives as prospective drugs against *M. tuberculosis* and *T. hominis*. The results of the present study offer some hints for the search of novel candidate drugs among congeners of the heterocyclic systems presented.

Results and Discussion

The S-substituted heterocyclic compounds studied were obtained by the alkylation of compounds **1**, **3**, **5**, **7** and **9** with the appropriate nitrobenzyl chlorides (Scheme 1). While alkylation of **3** and **9** were performed in a water-acetone or water-ethanol mixture, in the presence of K_2CO_3 as base, to give **4a** – **b** and **10a** – **c**, respectively, "phase transfer" conditions were employed to prepare compounds **2a** – **c**, **6a** – **c** and **8a** – **f**. The products were obtained in good or satisfactory yields; however, flash chromatography was needed to



Scheme 1.

Table 1. Some physicochemical data of nitrobenzylated heterocycles.

Compound	Formula (m. w.)	Yield (%)	M. p. (°C)	R_f	^{1}H NMR [D$_6$]-DMSO δ [ppm]	UV solvent (v/v), λ_{max} [nm], (ε)
2a	$C_{11}H_{11}N_3O_2S$ (249.29)	60	83 – 84	(A) 0.45	3.50 (s, Me), 4.60 (s, CH_2), 7.20 – 8.20 (3 m, H-arom. and H-imid.)	H_2O/MeOH (1:1): 270, (2900); 0.1M HCl/MeOH (1:1): 265 (5000)
2b	$C_{11}H_{10}N_4O_4S$ (294.28)	68	207 – 208[a]	(A) 0.41	3.40 (s, Me), 4.60 (s, CH_2), 6.90 – 8.60 (4 m, H-arom. and H-imid.)	H_2O/MeOH (1:1): 248 (6300); 0.1M HCl/MeOH (1:1): 243 (11500)
2c	$C_{11}H_{10}N_4O_4S$ (294.28)	76	98 – 100	(A) 0.45	3.40 (s, Me), 4.50 (s, CH_2), 7.00 – 8.70 (4 m, H-arom. and H-imid.)	H_2O/MeOH (1:1): 249 (5700); 0.1M HCl/MeOH (1:1): 247 (9000)
4a	$C_{11}H_{10}N_4O_4$ (262.22)	65	180 – 181	(B) 0.55	2.30 (s, Me), 5.50 (s, CH_2), 7.30 and 8.20 (2 d, H-arom.) 8.50 (s, H-imid.)	H_2O/MeOH (1:1): 270 (6000); 0.1M HCl/MeOH (1:1): 257 (9000)
4b	$C_{11}H_9N_5O_6$ (243.22)	50	178 – 179[b]	(B) 0.39	2.30 (s, Me), 5.50 (s, CH_2), 8.50 (s, H-imid.), 8.60 and 8.80 (2 m, H-arom.)	H_2O/MeOH (1:1): 252 (7900), 307 (6500); 0.1M HCl/MeOH (1:1): 307 (9100), 343 (16500)
6a	$C_{13}H_{13}N_3O_2S$ (275.33)	39	105 – 108	(C) 0.76	2.40 (s, 2 × Me), 4.50 (s, CH_2), 7.00 (s, H-pir.), 7.70 and 8.20 (2 d, H-arom.)	H_2O/MeOH (1:1): 250 (5200), 276 (5100); 0,1M HCl/MeOH (1:1): 252 (7400), 282 (6900)
6b	$C_{13}H_{12}N_4O_4S$ (320.32)	90	148 – 149	(C) 0.59	2.40 (s, 2 × Me), 4.80 (s, CH_2), 7.00 (s, H-pir.), 8.10 – 8.70 (3 m, H-arom.)	H_2O/MeOH (1:1): 247 (7800); 0.1M HCl/MeOH (1:1): 249 (12200)
6c	$C_{13}H_{12}N_4O_4S$ (320.22)	73	142 – 145	(C) 0.62	2.40 (s, 2 × Me), 4.60 (s, CH_2), 7.00 (s, H-pir.), 8.60 and 8.70 (d, m, H-arom.)	H_2O/MeOH (1:1): 254 (5900); 0.1 M HCl/MeOH (1:1): 250 (10000)
8a	$C_{14}H_{10}N_4O_4S$ (330.32)	29	124 – 126	(A) 0.38	4.70 (s, CH_2), 7.80 – 8.50 (5 m, H-benz. and H-arom.), 13.30 (s, H-N)	H_2O/MeOH (1:1): 264 (7 100), 319 (5 000); 0.1 M HCl/MeOH (1:1): 262 (21 800), 304 (10 300); 0.1 M NaOH/MeOH (1:1): 276 (13 600), 396 (10 500)
8b	$C_{14}H_9N_5O_6S$ (375.32)	28	176 – 179	(A) 0.38	5.00 (s, CH_2), 7.50 – 8.90 (5 m, H-benz. and H-arom.), 13.30 (s, H-N)	H_2O/MeOH (1:1): 244 (5 600), 336 (4 800); 0.1 M HCl/MeOH (1:1): 246 (14 800), 317 (5 600); 0.1 M NaOH/MeOH (1:1): 278 (5 800), 406 (9 500)
8c	$C_{14}H_9N_5O_6S$ (375.32)	21	105 – 108	(A) 0.29	4.90 (s, CH_2), 7.60 – 8.80 (5 m, H-benz. and H-arom.), 13.30 (s, H-N)	H_2O/MeOH (1:1): 250 (3 900), 331 (3 300); 0.1 M HCl/MeOH (1:1): 245 (11 800), 321 (5 600); 0.1 M NaOH/MeOH (1:1): 275 (5 100), 398 (8 900)
8d	$C_{14}H_9N_3O_2SCl_2$ (345.21)	77	197 – 200	(A) 0.29	4.60 (s, CH_2), 7.80 – 8.10 (2 m, H-benz. and H-arom.), 12.80 (s, H-N)	H_2O/MeOH (1:1): 308 (5000); 0.1 M HCl/MeOH (1:1): 308 (11800); 0.1 M NaOH/MeOH (1:1): 307 (17000)
8e	$C_{14}H_8N_4O_4SCl_2$ (399.21)	67	165 – 167	(A) 0.29	4.90 (s, CH_2), 7.70 – 8.80 (bs, 2 m, H-benz. and H-arom.), 12.80 (s, H-N)	H_2O/MeOH (1:1): 256 (7800); 0.1 M HCl/MeOH (1:1): 255 (9000), 307 (8800); 0.1 M NaOH/MeOH (1:1): 311 (16200)
8f	$C_{14}H_8N_4O_4SCl_2$ (399.21)	39	201 – 203	(A) 0.19	4.60 (s, CH_2), 7.80 – 9.00 (bs, 2 m, H-benz. and H-arom.), 13.00 (s, H-N)	H_2O/MeOH (1:1): 243 (16900), 306 (13600); 0.1 M HCl/MeOH (1:1): 234 (26800); 307 (18000) 0.1 M NaOH/MeOH (1:1): 233 (38000); 311 (14500)
10a	$C_{13}H_{10}N_4O_3$ (270.25)	65	165 – 167	(C) 0.15	5.80 (CH_2), 7.40 – 8.30 (6 m, H-arom.)	H_2O/MeOH (1:1): 267 (7400); 0.1 M HCl/MeOH (1:1): 256 (13000)
10b	$C_{13}H_9N_5O_5$ (315.24)	70	191 – 194	(C) 0.40	4.80 (CH_2), 8.60 – 9.00 (2 d, 3 m, H-arom.)	H_2O/MeOH (1:1): 256 (15600); 0.1 M HCl/MeOH (1:1): 251 (23900)
10c	$C_{13}H_9N_5O_5$ (315.24)	71	182 – 184	(C) 0.10	5.90 (CH_2), 7.40 – 8.90 (s, 4 m, H-arom.)	H_2O/MeOH (1:1): 249 (8700); 0.1 M HCl/MeOH (1:1): 241 (17700)

[a] Isolated as hydrochloride; [b] m. p. 177 – 179 °C [20]. (A) $CHCl_3$/MeOH (95:5); (B) $CHCl_3$/MeOH (9:1); (C) $CHCl_3$.

Table 2. *In vitro* antimycobacterial activity of nitrobenzylated heterocycles expressed as the minimum inhibitory concentration (μg/ml).

Compound tested	*Mycobacterium* strain used											
	M. tuberculosis $H_{37}R_v$		*M. tuberculosis* INH-resistant strain		*M. bovis*		MOTT *M. kansasii*		MOTT *M. xenopii*		*M. avium-intercellulare* complex (MAIC)	
	Incubation time (days)											
	14	21	14	21	14	21	14	21	14	21	14	21
2a	> 16	> 16	> 16	> 16	> 16	> 16	> 100	> 100	> 100	> 100	> 16	> 16
2b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
2c	**8**	**8**	> 16	> 16	**16**	**16**	**16**	**16**	**16**	**16**	> 100	> 100
4a	> 16	> 16	> 16	> 16	> 16	> 16	> 100	> 100	> 100	> 100	> 16	> 16
4b	> 16	> 16	**16**	**16**	> 16	> 16	> 100	> 100	> 16	> 16	> 16	> 16
6a	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
6b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
6c	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
8a	**16**	> 16	> 16	> 16	> 16	> 16	**16**	**16**	> 16	> 16	> 16	> 16
8b	**16**	**16**	**16**	**16**	> 16	> 16	> 16	> 16	> 16	> 16	> 16	> 16
8c	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	> 16	> 16	> 16	> 16
8d	> 16	> 16	> 16	> 16	> 16	> 16	> 100	> 100	> 16	> 16	> 16	> 16
8e	**16**	**16**	> 16	> 16	**16**	**16**	> 16	> 16	> 16	> 16	> 16	> 16
8f	**16**	**16**	**16**	**16**	**8**	**8**	> 16	> 16	> 16	> 16	> 16	> 16
10a	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
10c	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
10c	> 16	> 16	**16**	**16**	> 16	> 16	> 16	> 16	**16**	**16**	> 100	> 100
INH	1	1	> 100	> 100	10	10	> 100	> 100	10	10	10	10

INH – isoniazid used as a reference compound.

remove some minor byproducts. One of the reported compounds (**4a**) was described earlier [20]. Yet, the reaction condictions used were different and the compound was not fully characterized in that report; therefore it was included in the present study.

As mentioned above, some (nitrobenzylthio)benzimidazoles, of which the 3,5-dinitrobenzyl derivatives showed the highest activity *in vitro*, were found earlier to be effective antimycobacterial agents [3–5]. In the present study, we attempted to assess the importance of the structure of the heterocyclic portion of the nitrobenzylated derivatives. Such experiments may allow finding another leading structure to expand the series of the most promising heterocyclic derivatives. 4,6-Dimethylpyridine derivatives **6a**–**c** were totally inactive against all mycobacteria strains utilized. Of the imidazoles substituted at the exocyclic sulfur (**2a**–**c**) or N^1-nitrogen (**4a**, **4b**), only 3,5-dinitrobenzyl derivatives exhibited a considerable antimycobacterial activity; interestingly, **2c** showed a wider activity than the N^1-substituted compound **4b** that was only effective against the INH-resistant *M. tuberculosis* strain. The 5-nitro- and 5,6-dichlorobenzimidazole derivatives **8a**–**f**, including both 2,4-dinitro- (**8b** and **8e**) and 3,5-dinitrobenzylsulfanyl compounds (**8c** and **8f**) were toxic to four out of six mycobacterial strains tested. Of the N^1-O-substituted benzotriazole derivatives **10a**–**c** only the 3,5-dinitrobenzylsulfanyl compound was considerably toxic to the INH-resistant *M. tuberculosis* strain and to *M. xenopii*. None of the compounds reported here was appreciably active against the *M. avium intercellulare* complex.

Results of the trichomonacidal activity of the newly synthesized compounds are presented in Table 3. *T. hominis* trophozoites showed great variation in susceptibility to the tested chemicals. Our previous studies on susceptibility *in vitro* of diverse protozoan species to selected chemicals also showed marked differences in antiprotozoal efficacy of currently used drugs or antiseptic agents [21–23].

In the present study, antiprotozoal activity manifested itself in higher concentration (8–9 μg/ml) of most compounds examined. Metronidazole at 8 μg/ml decreased the survival of *T. hominis* trophozoites by 26.5%. Strikingly, the lower tested concentration of this drug (4 μg/ml) increased the number of surviving trophozoites. This paradoxical effect has also been observed in our earlier studies [22].

Of the novel nitrobenzyl derivatives tested, **4a**, **4b** and **10a** (two N-imidazoles and a single N^1-O-hydroxybenzotriazole derivative) were the most effective in reducing the number of viable protozoa (by up

Compound	Concentration [μg/ml]	Survivors* [%]	Compound	Concentration [μg/ml]	Survivors* [%]
2a	4	76 ± 2.9	**8a**	4	81.5 ± 4.5
	8	95.5 ± 5.5		8	82.5 ± 2.5
2b	4.2	79.0 ± 1.0	**8b**	4	84.0 ± 2.0
	8.2	62.0 ± 1.0		8	66.5 ± 6.5
2c	4	70.5 ± 4.5	**8c**	4	105.5 ± 1.5
	8.2	74 ± 1.0		8	51.5 ± 3.5
4a	4	76 ± 1.0	**8d**	4	87.5 ± 3.5
	8	35.5 ± 1.5		8	51.5 ± 0.5
4b	4.4	89.0 ± 0.8	**8e**	4	81.0 ± 1.0
	8.8	38.0 ± 2.0		8	71.0 ± 1.0
6a	4.1	76.5 ± 3.5	**8f**	4	77.5 ± 1.5
	8.2	79.5 ± 1.5		8	98.5 ± 1.5
6b	4.1	79 ± 1.0	**10a**	4.3	90 ± 1.0
	8.2	73.5 ± 2.5		8.6	45.0 ± 1.0
6c	4.1	74 ± 1.0	**10b**	4.3	88.5 ± 1.5
	8.2	97 ± 2.0		8.6	59.7 ± 2.4
Metronidazole	4	186.2 ± 9.2	**10c**	4.5	76.0 ± 1.0
	8	73.5 ± 4.2		9	66.5 ± 3.5
Control**		100 ± 2.5			

Table 3. Percentage of surviving *Trichomonas hominis* trophozoites after 24 h incubaction with the compounds shown.

* Values shown are mean $\pm$ SD of four counts performed using a single 1 ml culture sample;
** the value is the mean for control culture and culture with only DMSO added.

to 64, 62 and 55%, respectively). A slightly weaker effect, comparable with that observed in our previous study at high chlorhexidine concentration [22], was observed for compounds **8c** and **8d**. Derivatives **2b**, **8b**, **10b** and **10c** also showed a higher anti-protozoan efficacy than metronidazole.

Metronidazole is favored in some countries for the treatment of a wide variety of infections caused by bacteria and protists living in low-aerobic environments, *e.g.* by *Helicobacter*, *Clostridium*, *Trichomonas*, *Giardia*, and *Entamoeba*. In most protozoans studied, metronidazole's cytotoxicity relies on the reduction of its nitro group by ferredoxin [15]. It is a common belief that, in *Trichomonas*, this drug undergoes activation to an active catabolite in specialized organelles called hydrogenosomes. Although the treatment with metronidazole is generally effective, resistance *in vitro* and *in vivo* has been described both in bacteria and protists [14 – 16, 24]. An increasing occurrence of metronidazole-resistant clinical cases shows that the problem will need more attention in the near future.

The search for antiprotozoal drugs that would be useful against *Giardia*, *Enthamoeba* or *Trichomonas vaginalis* was the subject of numerous studies. *T. hominis* was given much less attention, probably due to a doubtful opinion that it is a "mild" pathogen. The results of this study reveal that this intestinal parasite is clearly susceptible *in vitro* to many of the novel compounds examined, and particularly to **4a**, **4b** and **10a**. The mechanism of action of these nitrobenzyl derivatives may be similar to that of metronidazole; however, the biochemistry of *T. hominis* has not been investigated thoroughly. The results presented warrant further studies on the nitrosubstituted heterocycles as prospective agents against this protozoan.

Experimental Section

Instrumentation: 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. Ultraviolet absorption spectra were recorded in a Kontron Uvikon 940 spectrophotometer. ^{1}H NMR spectra (in ppm) were measured on a model Varian Gemini 200 MHz (or Varian UNITY plus 500 MHz) spectrometer at 298 K in [D_6]-DMSO using tetramethylsilane as internal standard. Flash chromatography was performed with Merck silica gel 60 (200 – 400 mesh). Analytical TLC was carried out on precoated silica gel F_{254} (Merck) plates (0.25 mm thickness). Analyses of the new compounds, indicated by the symbols of the elements, were within $\pm$0.4% of the respective theoretical values.

Synthesis: All the chemicals used were analytical grade commercial products and were used with no further purification.

Synthesis of 2-S-substituted heterocycles ***2a – c, 6a – c, 8a – f***

To a vigorously stirred suspension of the mercapto-substituted heterocycle **1**, **5**, or **7** (7 mmol) in a biphasic mixture of water (25 ml) and CH_2Cl_2 (25 ml), containing K_2CO_3 (1.5 g) and benzyltrimethylammonium chloride (0.1 g, 1 mmol), the respective nitrobenzyl chloride (6 mmol)

was added. The solution was stirred overnight at room temperature. The lower phase was separated, washed twice with water (50 ml), and adsorbed on silica gel that was placed on the top of a silica gel column (3×15 cm) and chromatographed with petroleum ether (200 ml) followed by petroleum ether/ethyl acetate (1:1, v/v). Product-containing fractions were evaporated to dryness, and the residue was crystallized from EtOH/water. The yields, melting points, R_f values, ^{1}H NMR and UV data are listed in Table 1.

*Synthesis of N-nitrobenzyl imidazoles **4a** and **4b***

To a solution of 2-methyl-5-nitrobenzimidazole (**3**, 0.22 g, 1.75 mmol) in acetone (35 ml), anh. K_2CO_3 (0.5 g) and 4-nitro- (0.275 g, 1.6 mmol) or 3,5-dinitro benzyl chloride (0.354 g, 1.6 mmol) were added. The mixture was stirred overnight at r. t., and the solids were separated by filtration. The filtrate was adsorbed on silica gel that was placed on the top of a silica gel column (3×15 cm) and chromatographed with $CHCl_3$ (150 ml) followed by $CHCl_3$/MeOH (95:5, v/v). The product-containing fractions were evaporated to dryness and the residue was crystallized from EtOH/water. The yields, melting points, R_f values, and ^{1}H NMR and UV data are listed in Table 1.

*Synthesis of 1-O-nitrobenzyloxybenzotriazoles **10a – c***

To the stirred solution of 1-hydroxybenzotriazole (**9**, 4.5 mmol) in a mixture of water (25 ml) and EtOH (15 ml), containing K_2CO_3 (900 mg), the respective nitrobenzyl chloride (4.5 mmol) was added portionwise over three hours. The stirring was continued overnight. The precipitate formed was filtered off and crystallized from EtOH/water. The yields, melting points, R_f values, ^{1}H NMR and UV data are listed in Table 1.

Antimycobacterial activity studies: 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. bovis*, and a few INH-resistant or -sensitive MOTT strains: *M. kansasii*, *M. xenopii* and *M. avium-intercellulare* complex.

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

Antiprotozoal activity studies: *Trichomonas hominis* trophozoites derived from diarrheic stool of an adult patient were cultured at 37 °C in 15 ml tubes containing the liquid Pahm medium [19], and were subcultured twice a week. One-ml samples of the cultures were used to test susceptibility to both the reference drug (metronidazole) and novel compounds. The addition of 10 μl of dimethyl sulfoxide (DMSO) to 1 ml of *T. hominis* cultures exerted no effect on the number and status of the protozoan. Therefore the same DMSO concentration was used for negative controls and when testing the compounds of interest. Two concentrations of each agent were used. After 24 h exposure at 37 °C to the tested compounds, the cultures were vortexed and 20 μl samples were taken for trophozoite counting; means of four counts were calculated. Bürker chamber was used to determine the quantity of the trichomonads; only motile protozoans were counted. For microscopic assessment of the status and number of the surviving flagellates, 100× and 400× magnifications were used. The percentage of surviving trophozoites was determined in relation to the respective negative control cultures. Because of specific reaction of this protozoan species to some of the tested compounds (see below), we decided to present surviving trophozoites' percentages at two concentrations rather than minimum inhibitory concentrations that we considered less representative.

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