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Antimicrobial activity of substituted azoles and their nucleosides

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Dedicated to the late Prof. Dr. Frank Seela, Osnabrück, on the occasion of his 60th birthday

Four new 2′-deoxynucleosides of benzimidazole derivatives were prepared. Antimicrobial activity of many indazole, benzotriazole, benzimidazole derivatives and their nucleosides were tested by the agar diffusion method. Among the investigated compounds, dinitro- and trifluoromethyl-substituted benzimidazoles and their nucleosides were the most potent.

1. Introduction

Benzimidazole nucleosides and other azole derivatives, such as benzotriazoles and indazoles, are of wide interest because of their biological activities and clinical applications [1]. For instance, those ring systems are present in antiparasitic, fungicidal, anthelmintic and antiinflamatory drugs [e.g., see 2–5]. Some of them, such as mebendazole, chlorimidazole, albendazole and thiabendazole, are clinically and agriculturally useful drugs. Of benzimidazole nucleosides, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) is of particular interest and has been studied extensively as a specific inhibitor of heterogeneous nuclear RNA synthesis [6, 7]. This nucleoside inhibits viral and cellular RNA synthesis, most likely as a consequence of inhibition of RNA polymerase II [8]. DRB is also an inhibitor of casein kinase and DNA topoisomerase II, and an interferon inducer [9–11]. 2-Substituted DRB derivatives show activity against human cytomegalovirus [12]. It is also known that 5,6-dinitrobenzimidazole can substitute 5,6-dimethylbenzimidazole in the vitamin B12 molecule in *Corynebacterium diphteriae* [13]. 2-Trifluoromethylbenzimidazoles are potent decouplers of oxidative phosphorylation in mitochondria. They are also known as inhibitors of photosynthesis, and some of them exhibit appreciable herbicidal and insecticidal activities [14, 15].

Antimicrobial activity of benzimidazole nucleosides was not extensively investigated. During our previous work [16–21] on the synthesis of azole nucleosides we have obtained a large number of compounds used for antimicrobial activity testing in the present study. In the present study we would like to present preliminary observations on antibacterial activity of a number of azole bases and their nucleosides. The intention of this paper is to build a list of candidates for further search of practically useful compounds, particularly nucleosides, whose activity could be potentiated with the use of appropriate substituents. So far, the results obtained indicate to nitro- and 2-trifluoromethyl derivatives as the most promising ones.

2. Investigations, results and discussion

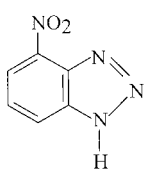
2.1. Synthesis of 2′-deoxyribofuranosides of benzimidazole derivatives

In addition to benzimidazole nucleosides obtained earlier [16–18, 20], we have made an attempt to enlarge our list of compounds for further studies by synthesizing several new 2′-deoxynucleosides of benzimidazole derivatives employing a stereoselective condensation of sodium salts of the respective azole bases with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-erythropentofuranosyl chloride [22]. The reaction products are sugar-protected 2′-deoxy-1-β-D-ribofuranosides of the respective benzimidazole. In the case symmetrical 5,6-dinitrobenzimidazole (**1a**) and 2-aminobenzimidazole (**1b**) only one expected anomer was formed (**1c** and **1d**, respectively). For 4,6-dibromobenzimidazole (**1e**) two regioisomers (**1f** and **1g**) were obtained and isolated. Removal of the sugar-protecting groups was achieved by the treatment of isolated *O′*-*p*-toluoyl derivatives with methanolic sodium methoxylate. In the case of **1b**, the synthesis of its 2-deoxyribofuranoside was performed without isolated of an intermediate product (Scheme). The newly obtained 2′-deoxyribofuranosides were characterized by UV, NMR and elemental analyses.

2.2. Antimicrobial activity of benzotriazole and indazole derivatives

Of many indazoles and benzotriazoles studies (mostly monosubstituted halogeno-, nitro- and aminoderivatives), some antibacterial activity was found for 4-nitrobenzotriazole (**2**) and 4-nitroindazole (**3**). Other compounds tested, e.g., 3-, 4-, 5- and 6-monochloro- (or monobromo-) indazoles (dihalogenated indazoles are practically insoluble in aqueous medium) and mono- or disubstituted benzotriazoles (e.g., 5-methyl-, 5,6-dichloro-, 4,6-dibromobenzotriazole and 5,6-dimethylbenzotriazole, or their 1-β-D- and 2-β-D-ribofuranosides [19]) showed no antibacterial activity. No antibacterial activity was also found for 3-carboxyindazole and monohalogenated indazole 1-β-D-ribofuranosides, e.g., 3-chloro-, 4-chloro-, 5-chloro-, 3-bromo- and 5-bromoindazole-1-β-D-ribofuranosides [21].

4-Nitroindazole was inactive against Gram-negative bacteria and the yeast tested, and showed only a limited activity against three out of 32 *Staphylococcus aureus* strains tested (not shown).



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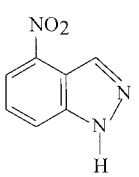

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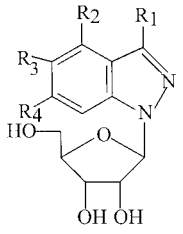


4a $R_1, R_2 = H,\ R_3, R_4 = Cl$
4b $R_1 = Br, R_2 = Cl, R_3, R_4 = H$
4c $R_1, R_2 = Br,\ R_3, R_4 = H$

Scheme

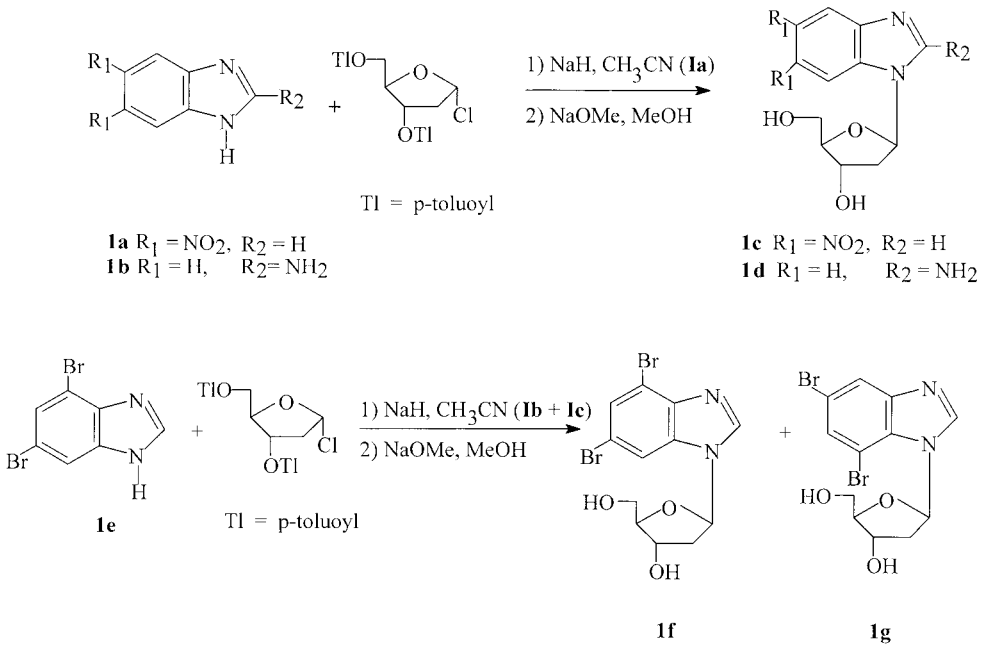


Table 1: Sensitivity of Gram-positive and Gram-negative bacteria to 4-nitrobenzotriazole (2), and 4-nitroindazole (3) and indazole 1-β-D-ribofuranosides 4a–c

Bacteria strain	Diameter of growth inhibition area (mm)[a]				
	Compound tested				
	2	**3**	**4a**	**4b**	**4c**
Escherichia coli ATCC 25922	19, 18	0, 0	12, 12	14, 14	13, 14
Escherichia coli NCTC 8196	19, 19	0, 0	13, 12	15, 15	14, 13
Proteus vulgaris NCTC 4635	14, 16	0, 0	12, 13	14, 14	14, 14
Bordetella bronchiseptica ATCC 4617	12, 13	0, 0	0, 0	0, 0	0, 0
Micrococcus flavus NCIB 8166	0, 0	0, 0	17, 17	traces	12, 12

[a] Values shown are results of two individual experiments
No activity was found against *Enterococcus faecium* ATCC 6057, *Pseudomonas aeruginosa* NCTC 6749, *Candida albicans* ATCC 10231 and *Sacharomyces cerevisiae RW 1–4D, D273, and N330*

4-Nitrobenzotriazole was found active against a number of *S. aureus* strains. Of 32 strains tested, six showed distinct sensitivity to this compound with a of growth inhibition area ranging from 13 to 16 mm in diameter. Fourteen more strains showed poor sensitivity (growth inhibition area diameter 11 mm or less), and 12 strains were totally insensitive (results for all compounds tested are shown in Table 1). Activity of varying degree was also observed against standard *S. aureus* strains and two multi-drug-resistant nosocomial strains (methicillin resistant *S. aureus*, MRSA), SW 310 and SW 362, whereas no activity was found against SW 180 (not MRSA multi-drug-resistant strain). These findings suggest the presence of a specific mechanism in certain *S. aureus* strains enhancing their resistance to 4-nitrobenzotriazole.

4-Nitrobenzotriazole activity against standard strains of Enterobacteriae was also clearly demonstrated (growth inhibition area diameter 14–19 mm). The drug was less active against *B. bronchiseptica*, and was completely inactive against *P. aeruginosa* and Gram-positive cocci *E. faecium* and *M. flavus*. Interestingly, 4-nitrobenzotriazole and 4-nitroindazole showed no activity against *S. aureus* strain 31 that was particularly sensitive to disinfectants and β-lactam antibiotics (J.Z.S. and B.J.S., unpublished observations).

Of indazole nucleosides tested, only three dihalogeno-substituted (in contrast to completely inactive monosubstituted) derivatives (**4a**, **4b**, **4c**) showed a moderate but distinct activity against *E. coli* and *P. vulgaris*, and were inactive against Gram-negative Enterobacteriae *P. aeruginosa* and *B. bronchiseptica*. They were also active against Gram-positive *M. flavus* NCIB 8166, but not the other Gram-positive coccus *E. faecium* (Table 2).

2.3. Antimicrobial activity of benzimidazole derivatives

Benzimidazole derivatives used in antimicrobial activity tests were modified in the nucleus and substituted on nitrogen with hydroxyethoxymethyl and 2,3-dihydroxypropyl substituents. These substituents mimicking a fragment of ribofuranose moiety (which substitution is typical of various antiviral drugs, e.g., acyclovir) did not influence antimicrobial activity of the tested compounds (compared to their respective parent compounds) except for enhancing it in 5,6-dichloro-1-hydroxyethoxymethylbenzimidazole (**5a**) [23]. Interestingly, the 2-trifluoromethyl congener of this latter drug was inactive against all bacteria and fungi strains tested. 4,6-Dibrominated as well as 5(6)mono- and 5,6-dihalogenated benzimidazoles, as well as amino- and methyl-substituted benzimidazoles also showed no antibacterial activity. However, adding a 2-trifluoromethyl group into the benzimidazole ring strongly potentiated their antimicrobial activity, e.g., 5,6-difluoro-2-trifluoromethylbenzimidazole (**5c**) showed distinct activity against a great majority of *S. aureus* strains tested, whereas 5,6-difluorobenzimidazole and 2-trifluoromethylbenzimidazole (**5b**) were inactive.

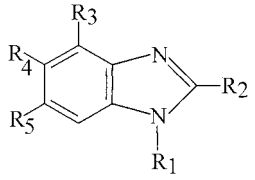
Another substitution that clearly influenced antibacterial activity of benzimidazoles was the introduction of a nitro

Table 2: Antimicrobial activity of azole derivatives and their nucleosides against *Staphylococcus aureus* strains

S. aureus strain	Diameter of growth inhibition area (mm)[a]											
	Compound tested											
	1a	**1c**	**5a**	**5c**	**5g**	**5f**	**6a**	**6b**	**6c**	**6d**	**2**	**Nf**
ATCC 25923	18, 17	0	traces	22, 23	13, 12	16, 16	24, 25	28, 26	0	12, 11	traces	23
NCTC 4163	22, 21	20, 18	0, 0	23, 23	12, 12	15, 14	19, 19	24, 23	0	15, 14	17, 16	21
ATCC 6538P	14, 14	13, 13	0, 0	20, 20	12, 13	13, 14	23, 25	26, 28	0	12, 13	14, 14	20
SW 180	15, 14	19, 18	traces	22, 21	12, 12	13, 13	25, 24	27, 26	0	14, 14	0	20
SW310	19, 18	15, 14	0, 0	21, 21	trace, 12	13, 13	23, 20	28, 29	0	14, 14	15, 15	20
SW 362	16, 15	13, 13	0, 0	21, 21	13, 12	14, 13	33, 31	28, 29	0	12, 12	traces, 11	20
NCTC 8325 (PS 47)	13, 14	20, 20	0, 0	20, 21	12, 12	16, 16	24, 24	22, 23	0	11, traces	traces	16
PCRS #3001	16, 15	15, 15	0, 0	22, 21	15, 15	14, 13	27, 27	27, 28	0	11, 11	13, 14	17
PCRS #3003	0, 0	0, 0	12, 12	24, 22	13, 14	traces	27, 29	26, 24	0	0, 0	0	17
8325-4	17, 16	14, 15	0, 0	20, 20	12, 13	11, trace	28, 29	25, 24	0	0, 0	0	16
A-15	14, 14	traces	0, 0	28, 27	traces	13, 13	25, 26	27, 27	0, 0	0, 0	11, 11	22
81	14, 14	15, 14	0, 0	24, 26	13, 14	16, 15	26, 26	26, 26	0	14, 13	0, 0	16
21	13, 14	14, 14	traces	23, 21	14, 12	trace, 11	24, 23	18, 19	traces	traces	0, 0	12
23	15, 14	18, 16	traces	20, 22	traces	13, 14	23, 23	18, 19	0	0, 0	0, 0	22
26	13, 14	13, 14	14, 13	24, 23	13, 14	11, 12	27, 26	22, 23	0, 0	11, 12	traces	20
27	15, 15	13, 14	traces	20, 21	trace, 12	14, 13	20, 21	19, 19	0, 0	0, 0	0, 0	16
29	18, 19	30, 28	traces	23, 25	12, 12	15, 15	23, 23	21, 22	0, 0	0, 0	0, 0	17
30	12, 11	18, 17	13, 14	22, 22	14, 13	11, 12	22, 20	27, 25	0, 0	traces, 11	0, 0	19
31	23, 24	19[b], 18[b]	traces	22, 23	traces	13, 14	20, 20	17, 16	0, 0	traces	0, 0	20
32	13, 14	16, 15	13, 12	22, 22	12, 12	14, 13	22, 21	19, 20	0, 0	traces	traces	21
33	15, 15	18, 17	12, 12	23, 22	12, 13	13, 13	20, 19	19, 19	0, 0	11, 11	15, 14	22
34	16, 16	17, 18	traces	20, 22	13, 12	12, 12	20, 20	19, 18	0, 0	traces	0	21
35	16, 15	18, 18	traces	21, 20	traces	traces	22, 21	18, 19	0, 0	traces	14, 13	19
K574	16, 16	16, 15	traces	20, 22	13, 14	12, 11	20, 19	18, 18	0, 0	0, 0	traces	18
K576	17, 17	18, 17	12, 12	21, 20	12, traces	13, 13	25, 23	20, 21	0, 0	0, 0	traces	18
K581	18, 17	17, 17	traces	22, 20	traces	12, 11	20, 20	21, 21	0, 0	traces	traces	17
K590	15, 16	13, 12	0, 0	24, 24	12, traces	14, 14	23, 23	20, 20	0, 0	traces	traces	22
6e	17, 17	17, 16	traces	traces	20, 20	traces	20, 21	20, 19	0, 0	traces	traces	18
7e	15, 15	16, 16	traces	20, 18	traces, 12	traces	20, 19	19, 20	traces	0, 0	0, 0	17
8e	17, 17	15, 16	traces	19, 20	12, 12	13, 13	20, 22	22, 20	0, 0	0, 0	0, 0	19
9e	18, 16	14, 13	0, 0	22, 22	traces	14, 14	26, 24	19, 20	0, 0	0, 0	0, 0	18
20e	17, 16	15, 16	11, traces	19, 19	traces	traces	21, 20	17, 18	0, 0	traces	traces	16

[a] Values shown are results of two individual experiments, if not otherwise specified
[b] Secondary growth was apparent
Nf-nitrofurantoine as control substance

group into the heterocyclic nucleus. 5-Nitrobenzimidazole (**5d**) and 4-nitrobenzimidazole (**5e**) were inactive in *S. aureus* tests. However, **5d** showed some activity against *B. bronchiseptica* and all *E. coli* strains tested. Compared with benzimidazole 4- and 5-mononitroderivatives, disubstituted nitrobenzimidazoles were more active in *S. aureus* tests. 5,6-Dinitrobenzimidazole (**1a**) was active against 31 out of 32 strains tested, whereas 4,6-dinitrobenzimidazole (**5f**) was less active and showed moderate activity against only seven out of these strains. In contrast



5a $R_1 = HOC_2H_4OCH_2$, $R_2, R_3 = H$, $R_4, R_5 = Cl$
5b $R_1, R_3, R_4, R_5 = H$, $R_2 = CF_3$
5c $R_1, R_3 = H$, $R_2 = CF_3$, $R_4, R_5 = F$
5d $R_1, R_2, R_3, R_5 = H$, $R_4 = NO_2$
5e $R_1, R_2, R_4, R_5 = H$, $R_3 = NO_2$
5f $R_1, R_2, R_3 = H$, $R_3, R_5 = NO_2$
5g $R_1, R_3 = H$, $R_4, R_5 = NO_2$, $R_2 = CF_3$

to the effect of 2-trifluoromethylation of 5,6-difluorobenzimidazole, introduction of the 2-trifluoromethyl group into 5,6-dinitrobenzimidazole (**1a**) rendered the resulting benzimidazole (**5g**) less active against *S. aureus* strains compared with its respective mother compound.

There were marked differences in benzimidazoles′ antimicrobial activity against standard *S. aureus* strains NCTC 8325 (PS 47), PCRS #3001 (i.e., NCTC 8325 with plasmid pI258) and PCRS #3003 (NCTC 8325 with plasmid pII147). Dinitrobenzimidazoles **1a** and **5f** were both toxic to *S. aureus* strains with plasmid pI258, and inactive against strains containing plasmid pII147, whereas **5a** and **5g** showed moderate activity against for those carrying plasmid pII147, and **5c** was highly active against all these three strains. These data indicate that *S. aureus* sensitivity to various benzimidazole derivatives depends also on its genetically determined resistance to antibiotics. In the case described here, plasmid pI258 carried the antierythromycin resistance gene localized in transposon Tn551 that is missing in NCTC 8325 and PCRS #3003 strains (Table 2).

The benzimidazole derivatives tested also showed some activity against other bacteria. 5-Nitrobenzimidazole (**5d**) (which was totally inactive against *S. aureus*) was toxic to Gram-negative bacilli *E. coli* and *B. bronchiseptica*, but not to *P. vulgaris*. Similarly, 2-trifluoromethylbenzimidazole (**5b**) (also inactive against *S. aureus*) showed moder-

ate activity against *E. coli* and *B. bronchiseptica* as well as weak activity against *P. vulgaris*. Antimicrobial activity was also observed for **5a**, **5c** and **5g** that, similarly to the other benzimidazoles tested, were not toxic to *P. aeruginosa* and *E. faecium*. Benzimidazole derivative **5c** that showed the widest spectrum of antimicrobial activity was also effective against *C. albicans* (fungus), *E. coli* and *P. vulgaris* (Enterobacteriacae), and was highly potent against *B. bronchiseptica*. Distinct activity against *M. flavus* was observed for **5a**, **5c** and **5g**, whereas **1a**, **5b** and **5d**–**f** were inactive against this bacteria (Table 3).

2.4. Antimicrobial activity of benzimidazole nucleosides

Benzimidazole nucleosides tested in the present study included a number of newly synthesized derivatives (**1c**, **1d**, **1f** and **1g**) as well as several previously described compounds. No activity was observed for, among others, 5(6)-mono- and 5,6- as well as 4,6-dibromobenzimidazole-1-α-D-arabinofuranosides, 5,6-difluororibofuranosides, and 5,6-dichloro-1-β-D-ribofuranoside and its 3′-*O*-methyl-, 5′-chloro- and 2′-deoxyderivatives. Ribo- and 2′-deoxynucleosides of **1d** as well as of 2-unsubstituted benzimidazoles showed no activity against any bacterial strain tested. Introducing 2-trifluoromethyl substituent dramatically enhanced the activity against *S. aureus* strains. Two of 2-trifluoromethyl-substituted 1-β-D-ribofuranosides of 5,6-disubstituted benzimidazoles (**6a** and **6b**) tested in this study were active against all *S. aureus* strains tested, whereas their 2-unsubstituted counterparts showed no activity. Diameter of the growth inhibition area was even greater for **6a** and **6b** then for nitrofuranotoine that was used as a reference compound. Interestingly, these two nucleosides also showed high activity against SW 362 strain (MRSA). Maybe the changes in penicillin binding proteins (PbPs) and the related increase in the permeability of bacterial membrane facilitate penetration of the cell. It is to note that 5,6-dimethyl-2-trifluoromethylbenzimidazole was inactive against *S. aureus* strains, whereas its 1-β-D-ribofuranoside (**6a**) was very potent. Less active then **6a** and **6b** were the dinitrobenzimidazole nucleosides. For instance, 4,6-dinitrobenzimidazole 1-β-D-ribofuranoside (**6c**) was practically inactive, and its 5,6-dinitroisomer (**6d**) showed only moderate activity against 9 out of 31 *S. aureus* strains tested. 2′-Deoxyderivative of **6d** (**1c**) was moderately active against a great majority of these strains and showed high activity against one of nosocomial strains employed (Table 2).

Table 3: Sensitivity of Gram-positive and Gram-negative bacteria against substituted benzimidazoles

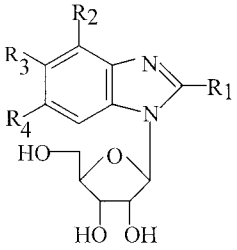
	Diameter of growth inhibition area (mm)[a]							
	Compound tested							
Bacteria strain	**1a**	**5a**	**5b**	**5c**	**5d**	**5f**	**5g**	**Nf**
Escherichia coli ATCC 25922	17, 16	13, 12	16, 16	24, 23	18, 18	13, 12	traces	27
Escherichia coli NCTC 8196	17, 18	15, 15	13, 13	21, 21	22, 22	14, 14	traces	22
Proteus vulgaris NCTC 4635	16, 18	12, traces	trace, 12	26, 27	0, 0	13, 15	traces	14
Bordetella bronchiseptica ATCC 4617	15, 15	0, 0	20, 20	32, 30	16, 19	traces	0, 0	traces
Candida albicans ATCC 10231	0, 0	0, 0	0, 0	19, 20	0, 0	0, 0	0, 0	0
Micrococcus flavus NCIB 8166	0, 0	13, 14	0, 0	27, 28	0, 0	0, 0	12, 13	traces

[a] results of two experiment of growth inhibition area (mm) were given, Nf-nitrofurantoine
No activity of investigated compounds were observed against *Enterococcus faecium* ATCC 6057, *Pseudomonas aeruginosa* NCTC 6749 and *Saccharomyces cerevisiae RW1–4, D273 and N330*

Table 4: Sensitivity of Gram-positive and Gram-negative bacteria against substituted benzimidazole nucleosides

	Diameter of growth inhibition area (mm)[a)]						
	Compound tested						
Bacteria strain	**1c**	**1f**	**1g**	**6b**	**6c**	**6d**	**Nf**
Escherichia coli ATCC 25922	16, 16	traces	traces	23, 22	19[b], 19[b]	17[b], 17[b]	27
Escherichia coli NCTC 8196	22, 23	15, 16	13, 12	21, 20	18, 18	25, 27	22
Proteus vulgaris NCTC 4635	0, 0	12, 12	12, trace	23, 24	0, 0	0, 0	14
Bordetella bronchiseptica ATCC 4617	0, 0	0, 0	0, 0	13, 13	0, 0	17, 18	traces
Micrococcus flavus NCIB 8166	0, 0	20*, 14 19*, 14	13, 12	20, 22	0, 0	0, 0	traces

[a] Results of two experiment of growth inhibition area (mm) were given, Nf-nitrofurantoine.
[b] Secondary growth was apparent.
No activity of investigated compounds were observed against *Enterococcus faecium* ATCC 6057, *Pseudomonas aeruginosa* NCTC 6749, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* RW1–4, D273 and N330



6a $R_1 = CF_3$, $R_2 = H$, $R_3, R_4 = Cl$
6b $R_1 = CF_3$, $R_2 = H$, $R_3, R_4 = CH_3$
6c $R_1, R_3 = H$, $R_2, R_4 = NO_2$
6d $R_1, R_2 = H$, $R_3, R_4 = NO_2$

Whereas it seems difficult to generalize the relationship between structure and activity against *S. aureus* of the compounds tested in the present study, we will attempt to offer here some conclusions that may help in deciding on the choice of candidate bases and their nucleosides for further studies. The 2-trifluoromethyl-substituted benzimidazoles appeared generally less active then their respective ribofuranosides. (Unfortunately, no 5,6-difluoro-2-trifluorobenzimidazole-1-β-D-ribofuranoside was available to compare with the highly active benzimidazole (**5c**)). On the other hand, dinitrobenzimidazoles **1a** and **5f** were more active then their corresponding 1-β-D-ribofuranosides **6c** and **6d**. Interestingly, introducing a 2-trifluoromethyl group into the dinitro-substituted benzimidazole did not enhance antimicrobial activity of the heterocyclic base (cf. **5g** and **1a** data, Table 2).

More antimicrobial activity data of the benzimidazole nucleosides tested are presented in Table 4. Benzimidazole nucleoside **6b** that was highly effective against *S. aureus* (vide supra) showed also moderate to high activity against Enterobacteriae (*E. coli, P. vulgaris, B. bronchiseptica* and *M. flavus*). Activity against *M. flavus* showed also dibrominated nucleosides **1g** and **1f**, whereas the dinitronucleosides **6c**, **6d** and **1a** were inactive against this species.

3. Experimental

^{1}H NMR spectra were measured with a Varian UNITY plus 500 MHz spectrometer. Chemical shifts were given in parts per million (ppm) relative to TMS that was used as an internal standard. UV spectra were recorded on a Kontron Uvikon 940 spectrometer. Melting points (uncorr.) were measured on a Boetius microscope hot stage. Analytical TLC was performed on precoated silica gel 60 F_{254} (Merck). Results of elemental analyses (C, H, N) were in an acceptable range.

3.1. Materials

All chemicals were analytical grade commercial products (Aldrich), and were used without further purification. Microorganisms used were as follows: 1a) *Staphylococcus aureus* strains ATCC 25923, NCTC 4163, ATCC 6538P, NCTC 8325 (PS 47), PCRS #3001 (NCTC 8325 with plasmid pI258 = Tn551 erm, bler, mer, cad, asi, i.e., with transposon Tn551 and carrying resistance genes to erythromycin, bleomycin, and mercury, cadmium and arsenium salts), PCRS #3003 (NCTC 8325 with plasmid pII147 bler, cad, mer, asi), 8325-4 (NCTC 8325 following delisogenisation of phages ∅11, ∅12 and ∅13); 1b) nosocomial multi-drug resistant strains SW180, SW310, SW363, and A-15 strain 81; 1c) ATCC 6538P, strains isolated from nosocomial infections (strains 21 through 35), outpatient-derived strains K 574, K 576, K 581, K 590, and natural environment-derived strains 6e, 7e, 8e, 9e and 20e; 1d) NCTC 4163. 2a) *Escherichia coli* ATCC 25922; 2b) *Escherichia coli* NCTC 8196. 3) *Proteus vulgaris* NCTC 4635; 4) *Bordetella bronchiseptica* ATCC 4617; 5) *Enterococcus faecium*, ATCC 6057; 6) *Pseudomonas aeruginosa* NCTC 6749; 7) *Micrococcus flavus* NCIB 8166; 8) *Candida albicans* ATCC 10231; 9) standard strains of infectious yeast used for antibiotic and disinfectant testing: strains RW1-4D, D273 and N330, the daughter strains of *S. cerevisiae* strain C52 [24]; RW1-4D strain carries mutations *sup*35 and *sup*45 that increase its sensitivity to antifungal drugs, e.g.; to methylbenzimidazole-2-yl carbamate, an active principle of the fungicide benomyl [25].

S. aureus strains shown under 1a were kindly donated by Drs. A. Młynarczyk and G. Młynarczyk, Medical University of Warsaw, and those shown under 1b were obtained from the National Reference Center for Bacteriophages *S. aureus* Typing. Microorganisms listed under 1d, 2b, 3, 5, 6, 8 were purchased from the National Institute of Hygiene, Warsaw, Poland. Bacteria listed under 2–6 were standard strains of Gram-negative bacilli used for antibiotic and disinfectant testing. *S. cerevisiae* was kindly donated by Dr. R. Wolinowska, Medial Academy of Warsaw. Strains listed under 1c, 2a, 4, 7 were from the authors' (J. Z. S., R. G. and B. J. S.) collection.

Benzimidazole, benzotriazole and indazole derivatives used in the present study were either commercial products (Aldrich), or were synthesized and published previously by the authors of the present report [16–21].

3.2. Synthesis of 1-(2′-deoxyribofuranosyl) benzimidazole derivatives

3.2.1. 1-[2′-Deoxy-3′,5′-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-5,6-dinitrobenzimidazole **(Ia)**

A solution of 5,6-dinitrobenzimidazole [20] (625 mg, 3 mmol) in acetonitrile (35 ml) was treated with NaH (160 mg, 3.2 mmol, 50% suspension in oil), and the mixture was stirred for 10 min. Then 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-erythropentofuranosyl chloride [26] (1.2 g, 3.07 mmol) was added and stirring was continued for 20 min. The reaction mixture was filtered through Cellite, the solvent was evaporated and the residue was chromatographed on a silica gel column (silica gel 60, Merck, 4 × 10 cm) with toluence (200 ml) and toluene-acetone (8 : 2, 500 ml). The nucleoside-containing fractions were evaporated to yield a yellow oil that crystallized after treatment with methanol (1.06 g, 63%, needles). M.p. 90–93 °C. TLC (silica gel, toluene-acetone, 8 : 2) Rf 0.35. ^{1}H NMR (D_6-DMSO): 2.34 and 2.40 (2s, 2 CH_3); 2.90 and 3.05 (2m, H-2′); 4.60 (m, H-4′ and H-5′); 5.73 (q, H-3′); 6.76 (t, H-1′); 7.2–8.0 (m, 8 arom. H); 8.54, 8.72 and 9.04 (3s, H-2, H-4 and H-7).
$C_{28}H_{24}N_4O_9$ (560.5)

3.2.2. 1-(2′-Deoxy-β-D-erythro-pentofuranosyl)-5,6-dinitrobenzimidazole (1-β-D-(2′-deoxyribofuranosyl)-5,6-dinitrobenzimidazole) **(1c)**

To a stirred suspension of **Ia** (840 mg, 1.5 mmol) in methanol (50 ml), a methanolic solution of sodium methanolate (1 M, 3 ml) was added. The stirring was continued overnight at room temperature. The solution was neutralized with acetic acid, evaporated to dryness and chromatographed on a silica gel column (silica gel 60, Merck, 3 × 10 cm) with chloroform (200 ml) and chloroform-methanol (8 : 2, 500 ml). The nucleoside-containing fractions were evaporated to dryness and the residue crystallized from methanol/water to give yellow plates (395 mg, 81%). M.p. 152–153 °C. TLC (silica gel, chloroform-methanol, 9 : 1) Rf 0.25. UV (pH 7): 240 nm (15000), 295 nm (5500). ^{1}H NMR (D_6-DMSO): 2.40 and 2.65 (2m, H-2′); 3.59 (m, H-5′ and H-5″); 3.92 (q, H-4′); 4.43 (m, H-3′); 5.12 (t, HO-5′); 5.40 (d, HO-3′), 8.57, 8.85 and 9.01 (3s, H-2, H-4 and H-7).
$C_{12}H_{12}N_4O_7$ (324.3)

3.2.3. 5,7-Dibromo-1-[2-deoxy-3′,5′-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-bezimidazole **(Ib)** *and 4,6-dibromo-1-[2-deoxy-3′,5′-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]benzimidazole* **(Ic)**

To a stirred solution of 4,6-dibromobenzimidazole (1.38 g, 5 mmol) in dry acetonitrile (50 ml) sodium hydride (270 mg, 50% in oil, 5.4 mmol) was added at room temperature. After 20 min stirring, 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-erythro-pentofuranosyl chloride [26] (2.35 g, 6 mmol) was added portionwise within 15 min. Stirring was continued for another 20 min. The mixture was filtered through Cellite and evaporated to dryness. The residue was chromatographed on a silica gel column (4 × 18 cm) with toluene acetone (9 : 1) as eluent. Two main zones were obtained. The 5,7-dibromoderivative was isolated from the fast-migrating zone as a colorless foam (520 mg, 17%). TLC (silica gel, toluene-acetone, 9 : 1) Rf 0.24. ^{1}H NMR (D_6-DMSO): 2.38 and 2.40 (2s, 2 CH_3), 2.95 and 3.10 (2m, H-2′), 4.60 (m, H-4′ and H-5′), 5.72 (m, H-3′), 7.14 (t, H-1′), 7.25–8.00 (m, 8 aromatic H), 8.74 (s, H-2).

The 4,6-dibromoderivative was obtained from the slow-migrating zone as needles (from methanol, 930 mg, 31%). M.p. 172–173 °C. TLC (silica gel, toluene-acetone, 9 : 1) Rf 0.35. ^{1}H NMR (D_6-DMSO): 2.37 and 2.42 (2s, 2 CH_3); 2.95 and 3.05 (2m, H-2′); 4.60 (m, H-4′ and H-5′); 5.72 (m, H-3′); 6.60 (t, H-1′); 7.30–8.05 (m, 8 aromatic H); 8.64 (s, H-2).
$C_{26}H_{24}Br_2N_2O_5$ (604.3)

3.2.4 5,7-Dibromo-1-(2′-deoxy)-β-D-erythro-pentofuranosyl)-benzimidazole (5,7-dibromo-1-β-D-(2′deoxyribofuranosyl)-benzimidazole **(1g)**

A stirred solution of **Ib** (450 mg, 0,75 mmol) was treated with methanolic sodium methanolate (1 M, 3 ml). The suspension dissolved within 15 min and stirring was continued for 1 h.

The reaction mixture was evaporated to dryness and chromatographed on a silica gel column (3 × 12 cm) with chloroform (100 ml) and chloroform-methanol (9 : 1, 300 ml). The nucleoside-containing fractions were evaporated and crystallized from methanol-water to give colorless needles (170 mg, 73%). M.p. 132–134 °C. TLC (silica gel, chloroform-methanol,

9 : 1) Rf 0.55 UV (pH 7): 254.5 nm (6100), 287 nm (3000), 295 nm (2300). ^{1}H NMR (D_6-DMSO): 2.40 and 2.60 (2m, H-2′); 3.60 (m, H-5′ and H-5″); 3.38 (q, H-4′); 4.41 (m, H-3′); 5.06 (t, HO-5′); 5.37 (d, HO-3′); 6.97 (t, H-1′); 7.68 and 7.94 (2d, H-4 and H-6); 8.76 (s, H-2).
$C_{12}H_{12}Br_2N_2O_3$ (312.2)

3.2.5 4,6-Dibromo-1-(2′-deoxy)-β-D-erythropentofuranosyl)benzimidazole (4,6-dibromo-1-β-D-(2′deoxyribofuranosyl)benzimidazole **(1f)**

Analogously as described above starting from **Ic**. Colorless from methanol-water. (195 mg, 83%). M.p. 168–170 °C. TLC (silica gel, chloroform-methanol, 9 : 1) Rf 0.50. UV (pH 7): 265 nm (7900), 281 nm (4800), 289.5 nm (3000). ^{1}H NMR (D_6-DMSO): 2.35 and 2.60 (2m, H-2′); 3.60 (m, H-5′ and H-5″); 3.88 (q, H-4′); 4.41 (m, H-3′); 5.06 (t, HO-5′); 5.37 (d, HO-3′); 6.38 (t, H-1′); 7.66 and 8.12 (2d, H-5 and H-7), 8.62 (s, H-2).
$C_{12}H_{12}Br_2N_2O_3$ (312.2)

3.2.6 2-Amino-1-(2′-deoxy-β-D-erythro-pentofuranosyl)-benzimidazole. (2-amino-1-β-D-(2′deoxyribofuranosyl)benzimidazole **(1d)**

To a stirred suspension of 2-aminobenzimidazole (266 mg, 2 mmol) in acetonitrile (15 ml) sodium hydride was added (120 mg, 50% in oil, 2.5 mmol). The stirring was continued for 10 min, then 2-deoxy-3,5-di-*O*-(*p*-toluoyol)-α-D-erythropentofuranosyl chloride [26] (860 mg, 2.2 mmol) was added portionswise over 10 min. After additional 1 h stirring the mixture was filtered through Cellite and the filtrate was evaporated to dryness. The residue was treated with chloroform (30 ml), and the chloroform phase was washed with 5% ammonium chloride (2 × 20 ml) 5% sodium bicarbonate (2 × 20 ml) and water (50 ml) to remove unchanged benzimidazole. Chloroform phase was next dried over sodium sulfate and evaporated to dryness. The dried residue was dissolved in 10 ml of methanol and treated with methanolic sodium methanolate (1 M, 4 ml). After 1 h the solution was evaporated to dryness and chromatographed on a silica gel column (3 × 12 cm) with chloroform (100 ml) and chloroform-methanol 9 : 1, 300 ml). The nucleoside-containing fractions were evaporated to dryness and the residue was crystallized from methanol-water to give pale yellow prisms (170 mg, 34%). M.p. 185–187 °C. TLC (silica gel, chloroform-methanol, 9 : 1) Rf 0.12. UV (pH 1): 274 nm (7400), 280 nm (6800); (pH 7) 243 nm (5300), 280 nm (6600). ^{1}H NMR (D_6-DMSO): 2.04 and 2.49 (2m, H-2′); 3.67 (m, H-5 and H-5″); 3.82 (q, H-4′); 4.39 (m, H-3′); 5.30 (m, HO-3′ and HO-5′); 6.22 (pt, H-1′); 6.51 (bs, NH_2); 6.85–7.28 (aromatic H).
$C_{12}H_{15}N_3O_3$ (249.3)

3.3. Antimicrobial activity testing

Antimicrobial activity was tested by disc-diffusion method under standard conditions using Mueller-Hinton agar medium as described by NCCLS [27]. Sterile filter discs (Whatman No. 2 chromatography paper, 9 mm diameter) were soaked in test compound solutions and finally contained 400 μg of compound per disc. In yeast experiments Saboraud medium was used. The results were read following 24 h incubation at 35 °C for bacteria, and 30 °C for *M. flavus* and fungi. Control discs contained nitrofurantoine (200 μg per disc), thioconazole (10 μg per disc) for yeast testing and metronidazole (400 μg per disc).

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