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Synthesis and activity of 1*H*-benzimidazole and 1*H*-benzotriazole derivatives as inhibitors of *Acanthamoeba castellanii*

Katarzyna Kopańska (née Zastąpiło),[a] Andżelika Najda,[a] Justyna Żebrowska,[b] Lidia Chomicz,[b] Janusz Piekarczyk,[c] Przemysław Myjak[d] and Maria Bretner[a,*]

[a]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 5a Pawińskiego, 02-106 Warsaw, Poland*
[b]*Department of Medical Biology, Medical University of Warsaw, 73 Nowogrodzka, 02-018 Warsaw, Poland*
[c]*2nd Department of Maxillofacial Surgery, Medical University of Warsaw, 4 Lindleya, 02-005 Warsaw, Poland*
[d]*Department of Tropical Parasitology, Medical University of Gdańsk, 9b Powstania Styczniowego, 081-516 Gdynia, Poland*

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Abstract—Chloro-, bromo- and methyl- analogues of 1*H*-benzimidazole and 1*H*-benzotriazole and their *N*-alkyl derivatives have been synthesized and tested in vitro against the protozoa *Acanthamoeba castellanii*. The results indicate that 5,6-dimethyl-1*H*-benzotriazole (**11**) and 5,6-dibromo-1*H*-benzotriazole (**14**) have higher efficacy than the antiprotozoal agent chlorohexidine.

1. Introduction

During the last 10 years it has become apparent that several species of free-living amoebae belonging to the genus *Acanthamoeba* present a serious risk to human health. They act as causative agents of serious infections involving the human brain, lung, skin and eyes.[1–3] When the amoebae invade the human brain through the nasal cavity by penetrating the ethmoid bone (e.g., during swimming), primary granulomatous encephalitis (GAE) develops, unlike during infection with *Entamoeba histolytica*, where brain involvement is usually secondary. GAE as well as the pneumonitis and dermatitis caused by *Acanthamoeba* sp. are serious progressive diseases occurring predominantly in patients undergoing immunosuppressive therapy for organ transplants and in immunocompromized patients with AIDS.[4–6] For this reason, the amphizoic amoebae are now considered opportunistic pathogens.[2–5,7–9]

Infection with *Acanthamoeba* sp. can result in eye disease—acanthamoeba keratitis, with 85% of cases affecting contact lens wearers.[2,9,10] In our comparative studies[11–13] on microorganisms colonizing the oral cavity of 77 patients with or without systemic diseases, we found mixed amoebic infection with *Entamoeba gingivalis* trophozoites and *Acanthamoeba* sp. trophozoites and cysts in the mouths of four patients with deterioration of periodontium and gingiva.

The above data, together with the fact that *Acanthamoeba* may serve as a protective host for pathogenic microorganisms, such as *Legionella*, *Escherichia* or *Pseudomonas*,[14] indicate the importance of the *Acanthamoeba* for human health. The amoebae are ubiquitous in nature: they are found in water (fresh, sea, chlorinated, bottled mineral and domestic tap water), soil and air in many parts of the world.[1,15,16] *Acanthamoeba castellanii* occurs in two developmental stages: as amoeboid trophozoites—with filamentous pseudopods (acanthopodia) and as cysts. Cysts are highly resistant to changes of temperature, pH and to extreme dryness; they are found in dust and may be dispersed by wind. Generally, recent studies[9] indicate that these protozoans are more resistant to environmental factors as well as to antimicrobial and antiparasitic drugs than other amphizoic amoebae.

Treatment of diseases caused by *Acanthamoeba* sp. is difficult and the results are disappointing. Various

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* Corresponding author. Tel.: +48-22-658-44-99; fax: +48-22-658-46-36; e-mail: majka@ibb.waw.pl

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agents such as clotrimazole, diminazine, paromomycin and ketoconazole were cysticidal in vitro, but only at high concentrations. Neomycin was ineffective against *Acanthamoeba* cysts in vivo. Introduction of chlorohexidine has improved the treatment of acanthamoeba keratitis, but relapses occur with continued culture-positive isolation of *Acanthamoeba*.[17] The long duration of therapy, its toxicity and development of resistance make the search for more effective treatment necessary.

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications. Some of them, such as mebendazol, chlorimidazol and albendazol, are clinically and agriculturally useful drugs. Most recently, antiprotozoal activity of substituted nitro-, halogenobenzimidazoles and 2-trifluorobenzimidazoles has been reported[18,19] while earlier studies showed antigiardial activity of various benzimidazole derivatives.[20,21]

We synthesized chloro-, bromo- and methyl- derivatives of 1*H*-benzimidazole and 1*H*-benzotriazole, and their N-alkylated analogues and tested them in vitro against the protozoa *A. castellanii*.

2. Materials and methods

2.1. Chemistry

Compounds (**1**–**5**, **11**–**13**, **15**–**17**) shown in Figure 1 have been reported previously but some of them were prepared by different methods. Analogues, which have not been synthesized previously (**6**–**10**, **14**) were prepared by adapting the known methods.

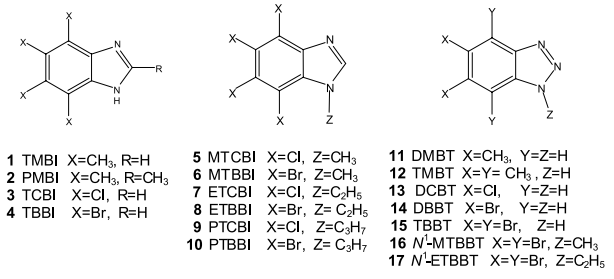
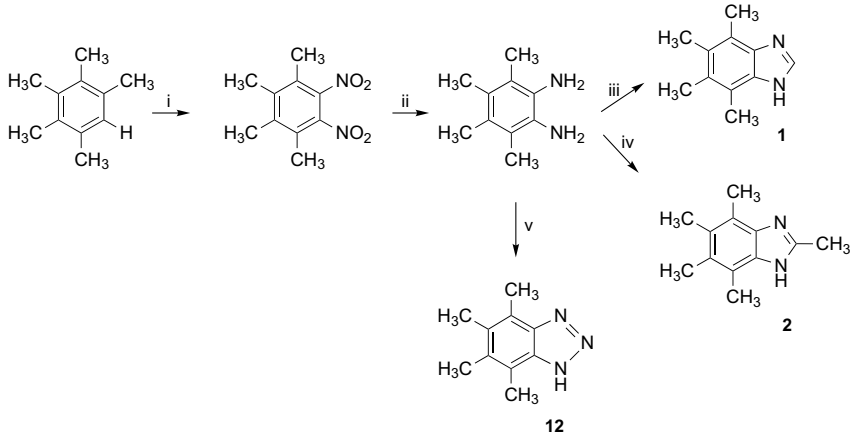


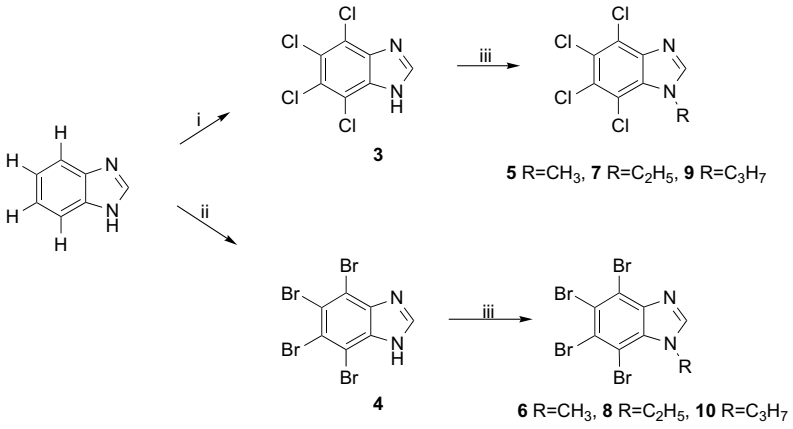
Figure 1. Benzimidazole and benzotriazole analogues synthesized in this work.

Methyl derivatives **1**, **2** and **12** were synthesized from pentamethylbenzene (Scheme 1), which was nitrated[22] to obtain 1,2-dinitro-3,4,5,6-tetramethylbenzene. After reduction with $SnCl_2$ in solution of concentrated HCl, 1,2-diamino-3,4,5,6-tetramethylbenzene was prepared as starting material. The reaction with formic acid[23] gave 4,5,6,7-tetramethyl-1*H*-benzimidazole. The method of Smith and Harris[22] was followed for synthesis of 2,4,5,6,7-pentamethyl-1*H*-benzimidazole (PMBI, **2**). Diazotization with $NaNO_2$ yielded 4,5,6,7-tetramethyl-1*H*-benzotriazole (TMBT, **12**), as was previously reported by us.[24]

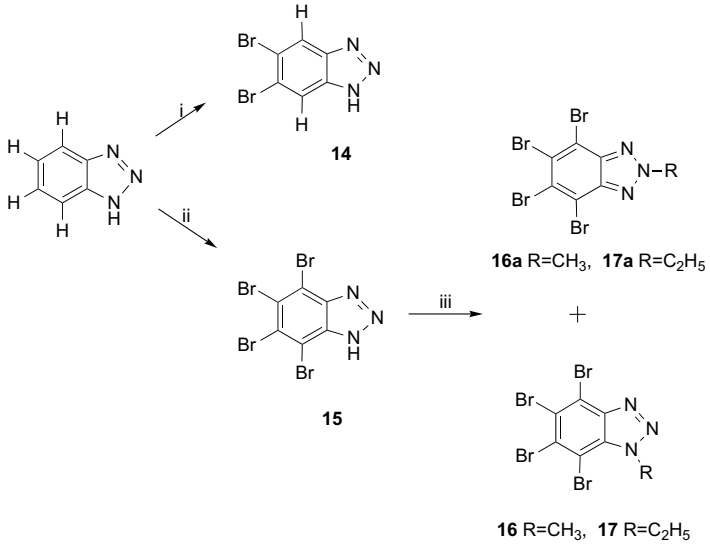
Chlorination of 1*H*-benzimidazole, with the use of the procedure reported for chlorination of 1*H*-benzotriazole[25] and published recently,[26] gave the 4,5,6,7-tetrachloro-1*H*-benzimidazole (TCBI, **3**) (Scheme 2). The 4,5,6,7-tetrachloro derivative was N-alkylated using appropriate alkyl iodides in acetonitrile with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base, to give *N*-methyl- (MTCBI, **5**), ethyl- (ETCBI, **7**) and propyl- (PTCBI, **9**) derivatives (Scheme 2). Of the N-alkylated 4,5,6,7-tetrachloro-1*H*-benzimidazoles only 4,5,6,7-tetrachloro-1-methyl-1*H*-benzimidazole had previously been synthesized[27,28] using a different method. The 4,5,6,7-tetrabromo derivatives (TBBI, **4**; TBBT, **15**) were synthesized by bromination of 1*H*-benzimidazole or 1*H*-benzotriazole according to published procedures.[24,29] The 4,5,6,7-tetrabromo derivatives **6**, **8**, **10** (Scheme 2) and **16**–**17** (Scheme 3) were obtained by alkylation as mentioned above. N^1 and N^2-alkyltetrabromo-1*H*-benzotriazoles **16**, **16a**, **17** and **17a** were synthesized,[25] but reaction in acetonitrile and with DBU improved the yield and simplified the purification. The 5,6-dimethyl-1*H*-benzotriazole (DMBT, **11**) was synthesized from 4,5-dimethylphenylenediamine, and the 5,6-dichloro-1*H*-benzotriazole (DCBT, **13**) was synthesized from 4,5-dichlorophenylenediamine, by closing the triazole ring with the use of $NaNO_2$. Bromination of 1*H*-benzotriazole (Scheme 3) with the use of a procedure



Scheme 1. Reagents and conditions: (i) fuming HNO_3, concentrated H_2SO_4, <10 °C; (ii) $SnCl_2$, HCl, reflux; (iii) HCOOH; (iv) CH_3COOH; (v) $NaNO_2$, HCl.



Scheme 2. Reagents and conditions: (i) HCl, HNO_3, reflux; (ii) HNO_3, Br_2, reflux; (iii) CH_3I (or C_2H_5I or C_3H_7I), CH_3CN, DBU.



Scheme 3. Reagents and conditions: (i) H_2SO_4, Ag_2SO_4, Br_2, room temperature; (ii) HNO_3, Br_2, reflux; (iii) CH_3I (or C_2H_5I), CH_3CN, DBU.

published by Cheeseman[30] for bromination of 2,3-dihydroxyquinoxaline yielded the 5,6-dibromo-1*H*-benzotriazole (DBBT, **14**).

2.2. Biological assays

The *A. castellanii* Neff strain from the reference laboratory of the Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine (Gdynia, Poland) was used in these studies. All protozoans were extensively grown in bacteria-free (axenic) condition in sterile 15 mL tubes containing BSC culture medium[31] enriched with calf serum, at 24–26 °C. The amoebae were subcultured twice a month. All amoebae used both in the control and in the drug susceptibility assays were grown for 4 days following regular subculturing. For the control dimethyl sulfoxide (C_{DMSO}) assays, a concentration of 20 μL dimethyl sulfoxide (DMSO) added to 1 mL of culture medium containing *Acanthamoeba* has been determined as having no effect on the number and status of the protozoans. A similar dilution of DMSO was used for the drug susceptibility assays. 7 benzotriazole and 10 benzimidazole derivatives at two different concentrations were examined (see Table 1) for their antiprotozoal activity. For each compound and control assay, 1 mL of vortexed culture containing *A. castellanii* was transferred to individual 1.5 mL Eppendorf tubes to which 20 μL dilution of the proper compound in DMSO or DMSO without the analogue were added. Each compound concentration was tested in three replicates including control assays without drugs and DMSO. After 24 h, the tube cultures of amoebae with tested compound as well as the controls were intensively vortexed, and subsequently 20 μL samples were withdrawn from each of them for preparation of wet slides covered with 24 × 24 mm glass slips. The number of amoebae as well as the trophozoites and cysts were counted under the microscope at 100-fold magnification. Percentage content of particular stage of the *Acanthamoeba* was assessed and compared in the tested compound versus the control.

Table 1. The reduction in viability of *A. castellanii* trophozoites and cysts after 24 h of incubation with synthesized compounds or with chlorohexidine

Compounds	Concentrations [μmol/L]	Percentage of survivors			Percentage content of particular stages	
		Trophozoites	Cysts	Total	Trophozoites	Cysts
1 TMBI	4.5	32.3 ± 1.8	16.4 ± 3.2	30.1 ± 1.9	94.8 ± 5.2	5.2 ± 1.0
	9.0	16.3 ± 1.3	93.0 ± 11.4	23.8 ± 2.3	61.7 ± 4.8	38.3 ± 4.7
2 PMBI	5.4	30.0 ± 3.5	130.0 ± 10.2	39.2 ± 4.2	69.3 ± 8.2	30.7 ± 2.4
	10.8	35.5 ± 2.6	51.0 ± 7.6	37.0 ± 3.1	86.6 ± 6.3	13.4 ± 2.0
3 TCBI	4.5	31.2 ± 3.1	332.0 ± 23.2	59.8 ± 5.1	48.6 ± 4.9	51.5 ± 3.6
	9.0	18.0 ± 1.5	25.0 ± 3.8	18.7 ± 1.7	87.7 ± 7.1	12.4 ± 1.9
4 TBBI	5.0	40.0 ± 4.4	79.0 ± 13.5	44.0 ± 5.3	82.5 ± 9.1	17.5 ± 3.0
	7.6	47.9 ± 5.3	165.0 ± 27.3	59.4 ± 7.4	72.8 ± 8.0	27.2 ± 4.5
5 MTCBI	3.8	58.0 ± 5.1	173.0 ± 27.5	69.0 ± 7.3	75.5 ± 6.7	24.5 ± 3.9
	7.6	36.0 ± 3.8	60.0 ± 9.0	38.4 ± 4.3	84.6 ± 9.0	15.4 ± 2.3
6 MTBBI	4.0	52.4 ± 4.1	30.0 ± 5.1	50.0 ± 4.2	94.1 ± 7.4	5.9 ± 1.0
	8.0	41.5 ± 1.5	52.0 ± 11.3	42.5 ± 2.4	88.0 ± 3.1	12.0 ± 2.6
7 ETCBI	5.2	26.5 ± 2.3	19.0 ± 3.4	25.8 ± 2.4	92.7 ± 7.9	7.3 ± 1.3
	7.9	22.0 ± 1.8	121.0 ± 12.6	31.6 ± 2.9	62.5 ± 5.2	37.5 ± 3.9
8 ETBBI	3.5	36.4 ± 1.7	12.3 ± 2.1	34.0 ± 1.7	96.5 ± 4.4	3.6 ± 0.6
	7.0	53.8 ± 6.5	110.0 ± 19.9	59.4 ± 7.8	81.8 ± 9.9	18.2 ± 3.3
9 PTCBI	5.5	23.3 ± 2.0	15.0 ± 2.3	22.5 ± 2.0	93.4 ± 8.0	6.6 ± 1.0
	11.1	41.2 ± 2.8	76.0 ± 9.7	44.5 ± 3.5	83.6 ± 5.7	16.4 ± 2.1
10 PTBBI	3.6	53.0 ± 5.4	61.6 ± 11.0	53.9 ± 5.9	88.8 ± 9.0	11.2 ± 2.0
	10.9	5.7 ± 1.0	66.6 ± 5.0	11.7 ± 1.4	44.2 ± 7.7	55.8 ± 4.2
11 DMBT	3.7	35.8 ± 3.5	8.0 ± 1.5	33.2 ± 3.3	97.8 ± 9.5	2.2 ± 0.4
	7.5	39.0 ± 3.7	11.8 ± 1.9	36.5 ± 3.6	96.9 ± 9.3	3.1 ± 0.5
12 TMBT	3.9	63.2 ± 5.1	162.0 ± 26.6	71.8 ± 7.0	79.9 ± 6.4	20.1 ± 3.3
	7.8	24.0 ± 3.1	162.0 ± 25.1	37.5 ± 5.3	60.0 ± 7.8	40.0 ± 6.2
13 DCBT	4.1	44.8 ± 4.4	17.3 ± 2.8	42.0 ± 4.3	96.0 ± 9.5	4.0 ± 0.7
	8.3	48.2 ± 1.7	51.5 ± 9.9	48.5 ± 2.5	89.6 ± 3.2	10.4 ± 2.0
14 DBBT	4.0	18.7 ± 0.7	12.5 ± 1.6	18.0 ± 0.8	93.6 ± 3.7	6.4 ± 0.8
	8.2	26.0 ± 1.1	14.5 ± 2.8	24.8 ± 1.3	94.3 ± 4.1	5.7 ± 1.1
15 TBBT	5.2	32.0 ± 3.4	62.5 ± 10.8	35.0 ± 4.2	82.6 ± 8.9	17.4 ± 3.0
	7.8	23.0 ± 1.5	16.4 ± 2.8	23.0 ± 1.7	93.0 ± 6.0	7.0 ± 1.2
16 N^1-MTBBT	5.4	35.0 ± 2.1	6.3 ± 1.0	32.0 ± 2.0	98.1 ± 5.9	1.9 ± 0.3
	10.8	10.7 ± 1.2	38.4 ± 6.9	13.5 ± 1.8	72.1 ± 8.1	27.9 ± 5.0
17 N^1-ETBBT	3.7	51.6 ± 4.3	54.2 ± 10.6	52.0 ± 4.9	89.8 ± 7.4	10.2 ± 2.0
	7.5	36.6 ± 1.7	17.3 ± 2.8	34.7 ± 1.8	95.2 ± 4.3	4.8 ± 0.8
Chlorohexidine	4.4	23.4 ± 0.7	11.0 ± 1.6	22.3 ± 0.8	95.3 ± 2.9	4.7 ± 0.7
	11.0	24.2 ± 1.1	31.0 ± 4.8	24.8 ± 2.6	88.4 ± 3.9	11.6 ± 1.8

All assays were performed in triplicate and repeated on at least twice to check the results. Values are means of two experiments ±SD.

3. Results and discussion

Biological assays were performed as described in Materials and methods. Comparison was made among new compounds and the previously used drug, the chlorohexidine. The number of trophozoites and cysts surviving the assay was calculated, compared with the controls and the results are presented in Table 1. In general all the tested compounds showed activity against *A. castellanii*. Compounds with a methyl group at position 4,5,6,7- of the benzene ring did not reduce the viability of the cysts, but reduced the viability of the trophozoites. Among the 4,5,6,7-tetrachloro-1*H*-benzimidazole derivatives at low concentration (Table 1), the most effective in reducing the number of trophozoites and cysts were 4,5,6,7-tetrachloro-1-propyl-1*H*-benzimidazole (**9**) (23.3% T and 15% C), and 4,5,6,7-tetrachloro-1-ethyl-1*H*-benzimidazole (**7**) (26.5% T and 19% C). The 4,5,6,7-tetrabromo-1*H*-benzimidazole derivatives appeared to be less effective. Among the 1*H*-benzotriazole derivatives the most effective in reducing the number of trophozoites was 5,6-dibromo-1*H*-benzotriazole (**14**), whereas in reduction of cysts it was 5,6-dimethyl-1*H*-benzotriazole (**11**) and 4,5,6,7-tetrabromo-1-methyl-1*H*-benzotriazole (**16**). The reduction of the number of surviving cysts was more effective than with the use of the known drug, chlorohexidine. When a higher concentration of compounds was used, compounds **3** and **14**–**16** showed higher efficacy than chlorohexidine.

The mode of action of therapeutic agents against *Acanthamoeba* is not known. In bacteria, chlorohexidine causes cytoplasmic membrane damage, resulting in an irreversible loss of essential cellular components.[32] The different chemical structure and low molecular weight of benzimidazole and benzotriazole analogues (molecular weight 200–500, compared to 898 for chlorohexidine digluconate) might permit better penetration through the membrane to achieve the therapeutic effect. It was previously reported that 4,5,6,7-tetrabromo-1*H*-benzotriazole is a highly selective inhibitor of protein kinase 2 (CK2),[33,34] and of the NTPase/helicase from hepatitis C virus and West Nile virus.[26] A subsequent study with

Table 2. Calculated ClogP for 1*H*-benzimidazole and 1*H*-benzotriazole derivatives

Compound	ClogP
1 TMBI	3.41
2 PMBI	3.68
3 TCBI	4.17
4 TBBI	4.53
5 MTCBI	4.11
6 MTBBI	4.47
7 ETCBI	4.64
8 ETBBI	5.00
9 PTCBI	5.17
10 PTBBI	5.53
11 DMBT	2.35
12 TMBT	3.23
13 DCBT	2.71
14 DBBT	2.93
15 TBBT	4.26
16 N^1-MTBBT	4.07
17 N^1-ETBBT	4.60
Chlorohexidine	4.81

other analogues of benzotriazole and benzimidazole demonstrated that 4,5,6,7-tetrabromo-1*H*-benzimidazole is more effective versus CK2 than TBBT,[24] while TMBT is inactive. Appropriate physicochemical properties (e.g., molecular weight, ClogP and solubility) together with pharmacokinetic properties and toxicity are the major indicators for progressing from a good lead to a good drug. The compounds synthesized in this work are hydrophobic (their ClogP are shown in Table 2), and can reach hydrophobically protected regions.

4. Conclusions

We have synthesized bromo-, chloro- and methyl- analogues of benzimidazole and benzotriazole and their alkyl derivatives, and screened them for their in vitro antiprotozoal activity. The obtained results are very promising since many of the compounds showed activity comparable with the currently used drug chlorohexidine, whereas compounds **11** and **14** exhibited even higher activity, especially towards *Acanthamoeba* cysts. It is known[35] that *Acanthamoeba* cysts may maintain their viability and virulence for as long as 25 years. Further optimization and pharmacokinetic characterization of this series of compounds are in progress.

5. Experimental

Melting points were determined in open capillary tubes using Büchi apparatus B504 and are uncorrected. UV absorption spectra were recorded using Cary 300 instrument. All compounds were checked by thin-layer chromatography (TLC) on 0.2 mm Merck silica gel 60 F_{254} plates. Preparative separations were carried out by column chromatography using Merck silica gel (230–400 mesh), or on preparative glass plates (2 mm, Merck silica gel 60 F_{254}). Mass spectrometry was recorded on Micromass ESI Q-TOF spectrometer at IBB PAS. ^{13}C NMR spectra were obtained with a Varian 500 or Varian 200 spectrometer. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (Me_4Si, $\delta = 0$). Starting materials: 1*H*-benzimidazole, 1*H*-benzotriazole, 4,5-dichloro-1,2-phenylenediamine, pentamethylbenzene and 4,5-dimethyl-1,2-phenylenediamine were purchased from Aldrich Co. 4,5,6,7-Tetrachloro-1*H*-benzimidazole (TCBI, **3**), 4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBI, **4**) and 4,5,6,7-tetramethyl-1*H*-benzotriazole (TMBT, **12**) were synthesized by adapting the published methods,[24] whereas 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBBT, **15**) as published.[26] The ClogP values were obtained using ChemDraw 8 Pro software.

5.1. 4,5,6,7-Tetramethyl-1*H*-benzimidazol (TMBI, 1)

To a stirred, cooled (2–5 °C) nitrating mixture consisting of 5 mL of fuming nitric acid, 18 mL of concentrated H_2SO_4 and 18 mL of chloroform, the solution of pentamethylbenzene (2.5 g, 16.9 mmol) in 5 mL of $CHCl_3$ was added dropwise. The whole mixture was stirred vigorously with temperature never exceeding 10 °C. The chloroform layer was separated, washed with water and sodium bicarbonate and dried with anhydrous sodium sulfate. After evaporation the residue was crystallized from ethanol to give 3,4,5,6-tetramethyl-1,2-dinitrobenzene (2.5 g, 11.16 mmol, 66% yield) as light yellow needles, mp 178 °C, (lit.[22] 176–177 °C).

To the solution of the above nitro compound (268 mg, 1.2 mmol) in 1.6 mL of concentrated HCOOH, $SnCl_2$ (2.68 g, 14 mmol) dissolved in 2.4 mL of concentrated HCl was added dropwise during 5 h with heating and stirring. The mixture was cooled, and the solid filtered off and washed with water and ether. The solid was dissolved in MeOH and 1 N NaOH was added to obtain pH 8. The solution was evaporated and the residue was crystallized from ethanol and water (1:1) to give 120 mg of product (0.68 mmol, 57% yield), mp 243–244 °C (lit.[22] 187–188 °C); R_f 0.32 ($CHCl_3/CH_3OH$, 9:1). UV λ_{max} (ε) pH 2: 275 (6500), 301 (3930); pH 7: 253 (6900), 276 (4200), 285 (3500); pH 12: 253 (7000), 276 (4000), 285 (3300); MeOH: 253 (7200), 278 (3900), 288 (3500); ^{13}C NMR (DMSO-d_6) δ 139.970; 128.102; 15.632; 14.013; MS $[M+H]^+$: found 175.3882, calcd 175.2556.

5.2. 2,4,5,6,7-Pentamethyl-1*H*-benzimidazole (PMBI, 2)

To the solution of 3,4,5,6-tetramethyl-1,2-dinitrobenzene (1.1 g, 5 mmol) in 5 mL concentrated HCl, the solution of $SnCl_2$ (5.7 g, 30 mmol) in 20 mL of concentrated HCl was added dropwise, followed by addition of 2.5 mL CH_3COOH. The reaction mixture was stirred and heated for 5 h. The resulting solid was filtered off and crystallized from CH_3OH. The white solid of 3,4,5,6-tetramethyl-1,2-phenylenediamine was filtered off, and the brown filtrate was evaporated and crystallized from EtOH to obtain 1.2 g of hydrochloride of **2**. The solid was dissolved in MeOH and 1 N NaOH was added to obtain pH 8. The solution was evaporated and the residue was crystallized from ethanol and water (1:1) to give 2,4,5,6,7-pentamethyl-1*H*-benzimidazole

(715 mg, 3.8 mmol, 76% yield), mp 265 °C (lit.[22] 264 °C), R_f 0.24 ($CHCl_3/CH_3OH$, 9:1). UV λ_{max} (ε) pH 2: 266 (5400), 275 (6700), 284 (6500); pH 7: 251 (6900), 276 (5100), 285 (4800); pH 12: 251 (7600), 276 (4300), 286 (4100); MeOH: 251 (7900), 278 (4400), 287 (4300); ^{13}C NMR (DMSO-d_6) δ 149.546; 135.830; 127.363; 118.416; 15.583; 14.147; 13.989; MS $[M+H]^+$: found 189.1083; calcd 189.274.

5.3. General procedure for alkylation of halogeno 1*H*-benzimidazoles 5–10 and halogeno-1*H*-benzotriazoles 16–17

To the solution of 1 mmol of appropriate halogeno 1*H*-benzimidazole (**3** or **4**) or 4,5,6,7-tetrabromo-1*H*-benzotriazole (**15**) in 10 mL of acetonitrile with 0.5 mL of DBU, the alkyl iodide was added (5 mM) and the solution was heated and mixed for 12–20 h. The reaction was checked by TLC, and DBU and alkyl iodide was added to complete the reaction. The solution was concentrated and products were purified with column chromatography or preparative silica gel plates chromatography.

5.4. 4,5,6,7-Tetrachloro-1-methyl 1*H*-benzimidazole (MTCBI, 5)

After column chromatography with chloroform, 195 mg of 4,5,6,7-tetrachloro-1-methyl-1*H*-benzimidazole (MTCBI) was obtained (yield 72%), mp 258 °C (lit.[27] 258–261 °C), R_f 0.40 ($CHCl_3$/MeOH, 100:1) 0.13 ($CHCl_3$/hexane, 75:25). UV λ_{max} (ε) pH 2: 272 (5600), 278 (5660), 289 (4520), 305 (2760); pH 12: 271 (5500), 278 (5600), 290 (4080), 304 (2400); MeOH: 264 (7900), 272 (9250), 289 (4400), 300 (3370); ^{13}C NMR (DMSO-d_6) δ 149.052; 140.958; 130.358; 125.208; 124.131; 122.338; 115.284; 34.138–34.120. MS for $C_8H_5Cl_4N_2$ $[M+H]^+$: obtained 270.9234; calcd 270.942.

5.5. 4,5,6,7-Tetrabromo-1-methyl-1*H*-benzimidazole (MTBBI, 6)

After column chromatography with chloroform 277 mg of 4,5,6,7-tetrabromo-1-methyl-1*H*-benzimidazole was obtained (yield 62%), mp 242–243 °C, R_f 0.44 ($CHCl_3$/MeOH, 100:1), 0.14 ($CHCl_3$/hexane, 75:25). UV λ_{max} (ε) pH 2: 275 (7750), 283 (7700), 307 (4100); pH 7: 275 (7400), 282 (7300), 307 (3800); pH 12: 274 (7400), 281 (7200), 307 (4000); MeOH: 268 (11,000), 274 (11,000), 302 (4260); ^{13}C NMR (DMSO-d_6) δ 149.084; 143.257; 132.039; 121.865; 120.115; 116.299; 106.725; 34.880. MS for $C_8H_5Br_4N_2$ $[M+H]^+$ obtained 448.7366; calcd 448.747.

5.6. 4,5,6,7-Tetrachloro-1-ethyl-1*H*-benzimidazole (ETCBI, 7)

After silica gel column chromatography with chloroform and crystallization 184 mg of 4,5,6,7-tetrachloro-1-ethyl-1*H*-benzimidazole (**7**) was obtained (yield 65%), mp 140 °C, R_f 0.54 ($CHCl_3$/MeOH, 100:1), 0.19 ($CHCl_3$/hexane, 75:25). UV λ_{max} (ε) pH 2: 264 (8080), 272 (8800), 287 (5100), 298 (3600), 305 (2760); pH 12: 264 (9180), 272 (10,400), 289 (5780), 300 (4300); MeOH: 265 (7710), 272 (9000), 289 (4200), 300 (3300). ^{13}C NMR (DMSO-d_6) δ 148.288; 141.202; 129.478; 125.433; 124.247; 122.443; 114.797; 41.577; 17.136. MS for $C_9H_7Cl_4N_2$ $[M+H]^+$ obtained 284.9821; calcd 284.976.

5.7. 4,5,6,7-Tetrabromo-1-ethyl-1*H*-benzimidazole (ETBBI, 8)

After silica gel column chromatography with chloroform and crystallization 305 mg of 4,5,6,7-tetrabromo-1-ethyl-1*H*-benzimidazole was obtained (yield 66%), mp 151–152 °C, R_f 0.63 ($CHCl_3$/MeOH, 100:1); 0.21 ($CHCl_3$/hexane, 75:25). UV λ_{max} (ε) pH 2: 269 (9240), 274 (9500), 302 (5300), pH 12: 268 (12,300), 274 (12,000), 304 (6400); MeOH: 269 (10700), 274 (10800), 302 (4100). ^{13}C NMR (DMSO-d_6) δ 148.372; 143.588; 131.166; 122.159; 120.239; 116.423; 106.343; 41.646; 17.326. MS for $C_9H_7Br_4N_2$ $[M+H]^+$ obtained 462.8498; calcd 462.7812.

5.8. 4,5,6,7-Tetrachloro-1-propyl-1*H*-benzimidazole (PTCBI, 9)

After chromatography on silica gel plates ($CHCl_3$/MeOH, 100:2) 155 mg of 4,5,6,7-tetrachloro-1-propyl-1*H*-benzimidazole was obtained (yield 67%), mp 103–104 °C, R_f 0.62 ($CHCl_3$/MeOH, 100:1); 0.24 ($CHCl_3$/hexane, 75:25). UV λ_{max} (ε) pH 2: 265 (10,900), 272 (11,700), 287 (8070), 298 (5960); pH 12: 265 (12,980), 273 (14,000), 289 (10,300), 300 (7700); MeOH: 264 (8080), 272 (9500), 289 (4500), 300 (3500). ^{13}C NMR (DMSO-d_6) δ 148.779; 141.180; 129.511; 125.495; 124.258; 122.450; 114.852; 47.699; 24.550; 10.392. MS for $C_{10}H_9Cl_4N_2$ $[M+H]^+$ obtained 298.9992; calcd 299.0032.

5.9. 4,5,6,7-Tetrabromo-1-propyl-1*H*-benzimidazole (PTBBI, 10)

After column chromatography with chloroform 304 mg of 4,5,6,7-tetrabromo-1-propyl-1*H*-benzimidazole was obtained (yield 64%), mp 128–129 °C, R_f 0.70 ($CHCl_3$/MeOH, 100:1); 0.28 ($CHCl_3$/hexane, 75:25). UV λ_{max} (ε) pH 2: 277 (10,000), 308 (6200); pH 12: 278 (10,500), 308 (6000); MeOH: 269 (10,500), 274 (10,600), 302 (4000). ^{13}C NMR (DMSO-d_6) δ 148.837; 143.585; 131.210; 122.283; 120.293; 116.445; 106.496; 47.724; 24.739; 10.305. MS for $C_{10}H_9Br_4N_2$ $[M+H]^+$ obtained 476.7985; calcd 476.808.

5.10. 5,6-Dimethyl-1*H*-benzotriazole (DMBT, 11)

This compound was synthesized from 4,5-dimethyl-1,2-phenylenediamine according to the method of Plaut.[36]

Yield 78%; mp 156.6–156.8 °C after crystallization from MeOH (lit.[36] 156–157 °C), R_f 0.59 ($CHCl_3$/MeOH, 9:1). UV λ_{max} (ε) pH 2: 268 (6300), 284 (6000); pH 7: 267 (6400), 282 (5900); pH 12: 281 (9100), 289 (7500); MeOH: 263 (5400), 283 (4800); (lit.[37] H_2O: 266 (5623), 280 (5248)). ^{13}C NMR (DMSO-d_6) δ 143.308; 137.066; 133.120; 131.897; 117.511; 109.894; 19.999. MS for $C_8H_{10}N_3$ $[M+H]^+$ obtained 148.2683; calcd 148.281.

5.11. 5,6-Dichloro-1*H*-benzotriazole (DCBT, 13)

This compound was synthesized from 4,5-dichloro-1,2-phenylenediamine according to Wiley and Hussung.[29] Yield 60%, mp 264 °C, (lit.[29] 264–266 °C); R_f 0.58 ($CHCl_3$/MeOH, 9:1). UV λ_{max} (ε) pH 2: 264 (5400), 270 (5440), 292 (5750); pH 7: 290 (7280), pH 12: 290 (9100), 298 (7350); CH_3OH: 262 (4850), 269 (5000), 292 (5800). ^{13}C NMR (DMSO-d_6) δ 137.467; 128.472; 116.525. MS for $C_6H_4Cl_2N_3$ $[M+H]^+$ obtained 189.0765; calcd 189.0269.

5.12. 5,6-Dibromo-1*H*-benzotriazole (DBBT, 14)

To the solution of 1*H*-benzotriazole (600 mg, 5 mmol) and 1.56 g (5 mmol) of Ag_2SO_4 in 20 mL of concentrated H_2SO_4, 2 mL of Br_2 was added dropwise during 4 h. The reaction was stirred at room temperature for 12 h. The precipitate was filtered off, and ethanol with ice was added to the filtrate. The new precipitate was crystallized from EtOAc and water, to give 858 mg (62% yield) of product, mp 248–250 °C, R_f 0.56 ($CHCl_3$/MeOH, 9:1). UV λ_{max} (ε) pH 2: 266 (11,600), 273 (12,500), 291 (11,260); pH 6: 285 (12,600), 294 (11,800), 305 (6560); pH 12: 284 (16,890), 294 (15,100), 305 (7790); MeOH: 265 (7300), 272 (8600), 289 (8700), 305 (4700). ^{13}C NMR (DMSO-d_6) δ 139.138; 129.465; 106.725. MS for $C_6H_4Br_2N_3$ $[M+H]^+$ obtained 277.9385; calcd 277.924.

5.13. 4,5,6,7-Tetrabromo-1-methyl-1*H*-benzotriazole (*N*[1]-MTBBT, 16)

To a solution of TBBT (435 mg, 1 mmol) in 8 mL of acetonitrile with 0.48 mL of DBU, methyl iodide (300 μL) was added. The mixture was heated to 50–60 °C. After 24 h the reaction mixture was evaporated, the residue dissolved in methanol with chloroform and separated with the use of preparative plate chromatography (hexane/$CHCl_3$, 1:1). After elution and evaporation 180 mg of *N*[1]-methyl-TBBT was obtained (40% yield), mp 226 °C (lit.[29] 225–226 °C), R_f 0.41 ($CHCl_3$/hexane, 1:1). ^{13}C NMR ($CDCl_3$) δ 144.515, 132.080, 127.862, 123.177, 115.395, 106.911, 29.704. UV λ_{max} (ε) pH 2: 288 (10,350), 296 (10,500), 310 (9800); pH 6: 285 (9400), 296 (9300), 313 (8000); pH 12: 295 (8560), 313 (7000), MeOH: 278 (9400), 288 (9200), 308 (6600); (lit.[29] MeOH: 278 (9120), 289 (8910), 308 (6310)). MS for $C_7H_4Br_4N_3$ $[M+H]^+$ found 449.7857; calcd 449.752.

And 134 mg of *N*[2]-methyl-TBBT (yield 30%), mp 252 °C (lit.[29] 250–253 °C), R_f 0.63 ($CHCl_3$/hexane, 1:1). ^{13}C NMR ($CDCl_3$) δ 143.352; 126.460; 113.615; 44.076. UV λ_{max} (ε) MeOH: 296 (12,700), 306 (13,100); (lit.[29] MeOH: 294 (12,590), 299 (12,590), 305 (13,180)). MS for $C_7H_4Br_4N_3$ $[M+H]^+$ found 449.7848; calcd 449.752.

5.14. 4,5,6,7-Tetrabromo-1-ethyl-1*H*-benzotriazole (*N*[1]-ETBBT, 17)

To a solution of TBBT (215 mg, 0.5 mmol) in 4 mL of acetonitrile with 0.24 mL of DBU, 150 μL of ethyl iodide was added. The mixture was heated to 50–60 °C. After 24 h the reaction mixture was evaporated, residue was dissolved in the mixture of methanol/chloroform and the products were separated with the use of preparative plate chromatography ($CHCl_3$/hexane, 1:1). After elution and crystallization 81 mg of *N*[1]-ethyl TBBT (yield 35%) was obtained, mp 151 °C (lit.[29] 150–152 °C), R_f 0.81 (MeOH/$CHCl_3$, 100:1); 0.48 ($CHCl_3$/hexane, 1:1). UV λ_{max} (ε) MeOH: 277 (8800), 289 (8500), 308 (6000); (lit.[29] MeOH: 278 (8710), 290 (8710), 308 (6166)). ^{13}C NMR ($CDCl_3$) δ 145.704; 131.515; 129.067; 124.266; 116.702; 105.646; 45.744; 16.832. MS for $C_8H_6Br_4N_3$ $[M+H]^+$ found 463.7757; calcd 463.7652.

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