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Synthesis, antiprotozoal and antibacterial activity of nitro- and halogeno-substituted benzimidazole derivatives★✪

Zygmunt Kazimierczuk[1] ✉, Jacqueline A. Upcroft[2], Peter Upcroft[2], Agata Górska[1], Bohdan Starościak[3] and Agnieszka Laudy[3]

[1]*Institute of Chemistry, Agricultural University, Warszawa, Poland;* [2]*The Queensland Institute of Medical Research, Brisbane, Australia;* [3]*Medical University, Department of Pharmaceutical Microbiology, Warszawa, Poland*

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Two series of benzimidazole derivatives were sythesised. The first one was based on 5,6-dinitrobenzimidazole, the second one comprises 2-thioalkyl- and thioaryl-substituted modified benzimidazoles. Antibacterial and antiprotozoal activity of the newly obtained compounds was studied. Some thioalkyl derivatives showed remarkable activity against nosocomial strains of *Stenotrophomonas malthophilia*, and an activity comparable to that of metronidazole against Gram-positive and Gram-negative bacteria. Of the tested compounds, 5,6-dichloro-2-(4-nitrobenzylthio)-benzimidazole showed the most distinct antiprotozoal activity.

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications [1]. This ring system is present in numerous antiparasitic, fungicidal, anithelemintic and anti-inflammatory drugs [2–5]. Also, some benzimidazole nucleosides, particularly 5,6-dichlorobenzimidazole-1-β-D-ribofuranoside (DRB) and its 2-substituted derivatives show activity against human cytomegalovirus [6]. It is also known that 5,6-dinitrobenzimidazole can substitute 5,6-dimethylbenzimidazole in the vitamin B_{12} molecule in *Corynebacterium diphteriae* [7] and 2-trifluorobenzimidazoles are

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✉Corresponding author: Zygmunt Kazimierczuk, Agricultural University, Institute of Chemistry, Nowoursynowska 159C, 02-787 Warszawa, Poland; e-mail: kazimierczuk@delta.sggw.waw.pl

Abbreviations: CFU, colony-forming unit; DBU, 1,8-diazabicyclo[5,4,0]undecen-7-en; DRB, 5,6-dichlorobenzimidazole-1-β-D-ribofuranoside; Me_2SO, dimethylsulfoxide; MIC, minimal inhibitory concentration.

potent decouplers of oxidative phosphorylation in mitochondria. They are also inhibitors of photosynthesis, and some exhibit appreciable herbicidal activity [8]. Most recently, antiprotozoal activity of substituted 2-trifluorobenzimidazoles has been reported [9], consistent with several earlier studies on the anti-giardial activity of various benzimidazole derivatives [10, 11]. However, the general antimicrobial activity of benzimidazole derivatives has not been extensively investigated. The earliest report of their antibacterial activity appeared in 1964 [12], and more recently we have found two groups of substituted benzimidazoles, namely the 5,6-dinitro and 2-trifluoromethyl derivatives, to be promising candidates for antimicrobial drugs [13]. In this paper we present new data on the antimicrobial and antiprotozoal activities of 5,6-dinitro and 2-dialkylaminosubstituted benzimidazoles.

MATERIALS AND METHODS

General methods. 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 with a Varian Gemini (200 MHz) and a Varian UNITY plus (500 MHz) spectrometer at 298 K in $D_6(Me_2SO)$ 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 were within ±0.4% of the theoretical values.

Bacterial strains and drug susceptibility assays. Bacteria were used as follows: *Staphylococcus aureus* strains ATCC 6538P, ATCC 25923 and NCTC 4163; *Bacillus subtilis* ATCC 6633, *Bacillus stearothermophilus* ATCC 7953 and *Bacillus cereus* ML 98; *Escherichia coli* NCTC 8196; *Proteus vulgaris* NCTC 4635; *Micrococcus flavus* NCIB 8166.

Bacterial strains from *Pseudomonadaceae* family: *Pseudomonas aeruginosa* ATCC 27863, ATCC 15442 and NCTC 6749; *Burkholderia cepacia* ATCC 25416; *Stenotrophomonas malthophilia* ATCC 13637 and clinical isolates – 67 strains of *S. malthophilia* were used. Bacterial strains were obtained from the State Institute of Hygiene (Warszawa), the Medical University, Warszawa and the Children's Memorial Health Institute.

Antimicrobial activity was tested by the disc-diffusion method under standard conditions using Mueller-Hinton agar medium as described by NCCLS [14]. Sterile filter paper discs (9 mm diameter, Whatman No. 3 chromatography paper) were soaked in test compound solutions prepared in EtOH/Me_2SO mixture (1:1, v/v) and finally contained 400 μg of the compound per disc. The results were read following 18–20 h incubation at 35°C. MIC – the minimum inhibitory concentration – was performed by the agar dilution method according to guidelines established by the NCCLS [15]. MIC was described as the lowest concentration of the drug that visibly inhibited growth. A single colony or faint haze was regarded as no growth. MICs were interpreted after 18 and 42 h of incubation at 35°C. Concentrations of agents in Muller-Hinton II Agar medium (Becton Dickinson) ranged from 12.5 to 400 $\mu g \times cm^{-3}$. Agar plates were inoculated using a replicator with 2 μl volumes. The final inoculum of bacteria was 10^{-4} CFU $\times$ cm^{-3}. Solutions of the agents tested were prepared in 0.125 M HCl, for compounds **4d**, **4f** and **4n**, and in a 0.125 M HCl/MeOH mixture (1:0.6, v/v) for compound **4e**.

Protozoan parasite isolates, culture and drug susceptibility assays. *Giardia duodenalis*, *Trichomonas vaginalis* and *Entamoeba histolytica* were grown in TYI-S-33 which was supplemented with bile for *Giardia* [16]. Parasites were subcultured three times a week ex-

cept for *Entamoeba* which was subcultured twice a week [16]. *T. vaginalis* strain BRIS/92/STDL/F1623 and the metronidazole resistant line derived from it BRIS/92/STDL/F1623-M1 (F1623-M1), *E. histolytica* strain HTH-56:MUTM (MUTM) and *G. duodenalis* strains BRIS/83/HEPU/ 106 (referred to as 106), the metronidazole-resistant line BRIS/83/HEPU/106-2ID$_{10}$ derived from 106 (106-2ID$_{10}$), WB1B, the metronidazole-resistant line WB-M3 and the albendazole resistant line WB-M3-Alb (WB/Alb) are described in [16]. Drug susceptibility assays were detailed previously [16].

Syntheses

1-Substituted 5,6-dinitrobenzimidazoles. *5,6-Dinitro-1-(dimethylaminoeth-1-yl)benzimidazole* (**2a**): 1.05 g (5 mmol) of 5,6-dinitrobenzimidazole (**1**) was dissolved in acetonitrile (40 ml) containing 2.4 ml (16 mmol) 1,8-diazabicyclo[5,4,0]undecen-7-en (DBU) followed by addition of 0.864 g (6 mmol) of 2-dimethylaminoethylchloride hydrochloride. The solution was stirred overnight at room temperature and then refluxed for 1 h. The reaction mixture was evaporated to dryness and the residue was chromatographed on a silica gel column (3×15 cm) with $CHCl_3$ (200 ml), followed by $CHCl_3$/MeOH (95:5). The product-containing fractions were evaporated and the residue crystallized from ethyl acetate/MeOH to give **2a** (0.90 g, 64%); m.p. 173–177°C; ^{1}H NMR (D_6-Me_2SO) δ (ppm): 2.16 (s, 2 × CH_3), 2.65 and 4.49 (2 × t, -CH_2CH_2-), 8.53, 8.72 and 8.75 (3s, H-2, H-4 and H-7). UV (H_2O/MeOH, 1:1): 246 nm (16 900), 300 (sh, 6000). Elemental analysis: calculated for $C_{11}H_{13}N_5O_4$: C, 47.30; H, 4.70; N, 25.08. Found: C, 47.47; H, 4.61; N, 25.07.

5,6-Dinitro-1-(diethylaminoeth-1-yl)benzimidazole (**2b**): Compound **2b** was similarly synthesised to the procedure described above for **2a,** from **1** and 2-diethylaminoethylchloride hydrochloride: (m.p. 123–127°C, 49%); ^{1}H NMR (D_6-Me_2SO): δ (ppm): 0.71 and 2.42 (t and q, N-CH_2CH_3), 2.71 and 4.44 (2 × t, -CH_2CH_2-), 8.52, 8.70 and 8.73 (3s, H-2, H-4, H-7). UV (H_2O/MeOH, 1:1): 246 nm (18 200), 300 (sh, 6000). Elemental analysis: calculated for $C_{13}H_{17}N_5O_4$: C, 50.80; H, 5.59; N, 22.79. Found: C, 50.92; H, 6.05; N, 22.62.

5,6-Dinitro-1-(dimethylaminoprop-1-yl)benzimidazole (**2c**): The synthesis was similar to the procedure for **2a,** from **1** and 3-dimethylaminopropylchloride hydrochloride: (m.p. 232–235°C, 38%); ^{1}H NMR (D_6-Me_2SO): δ (ppm): 2.70 (s, 2 × CH_3), 2.24, 3.03 and 4.53 (2 × t and m, -$CH_2CH_2CH_2$-), 8.59, 8.79 and 8.87 (3s, H-2, H-4, H-7). UV (H_2O/MeOH, 1:1): 246 nm (18 200), 300 (sh, 6000). Elemental analysis: calculated for $C_{12}H_{15}N_5O_4$: C, 49.13; H, 5.17; N, 23.88. Found: C, 49.30; H, 5.19; N, 22.62.

5,6-Dinitro-1-(morpholineth-1-yl)benzimidazole (**2d**): Similar to **2a**, from **1** and *N*-(2-chloroeth-1-yl)morpholine hydrochloride: (m.p. 166–169°C, 42%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 2.69 and 4.54 (2 × t, -CH_2CH_2-), 2.42 and 3.48 (2 × t, CH_2-morpholine), 8.51, 8.71 and 8.75 (3s, H-2, H-4 and H-7). UV (H_2O/MeOH, 1:1): 247 nm (18 500), 310 (sh, 6000). Elemental analysis: calculated for $C_{13}H_{15}N_5O_5$: C, 48.59; H, 4.71; N, 21.80. Found: C, 48.69; H, 4.86; N, 21.60.

5,6-Dinitro-1-(piperidineth-1-yl)benzimidazole (**2e**): Similar to **2a**, from **1** and *N*-(2-chloroeth-1-yl)piperidine hydrochloride: (m.p. 206–208°C, 26%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 1.38 (m, CH_2-piperidine), 2.63 and4.49 (2 × t, -CH_2CH_2-), 8.53, 8.71 and 8.73 (3s, H-2, H-4 and H-7). UV (H_2O/MeOH, 1:1): 246 nm (17 200), 300 (sh, 6000). Elemental analysis: calculated for $C_{14}H_{17}N_5O_4$: C, 52.65; H, 5.38; N, 21.93. Found: C, 52.83; H, 5.52; N, 21.77.

5,6-Dinitro-1-(2-hydroxyeth-1-yl)benzimidazole (**2f**): Similar to **2a**, from **1** and 2-bromoethanol: (m.p. 235–238°C, 56%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 3.75 and 4.46 (q and t, -CH_2CH_2-), 8.53, 8.66 and 8.72 (3s, H-2, H-4 and H-7). UV (H_2O/MeOH, 1:1): 247 nm

(16 500), 300 (sh, 6000). Elemental analysis: calculated for $C_9H_8N_5O_5$: C, 42.86; H, 3.20; N, 22.22. Found: C, 42.99; H, 3.34; N, 22.01.

5,6-Dinitro-1-(2,3-dihydroxyprop-1-yl)benzimidazole (**2 g**): Similar to **2a**, from **1** and glycitole: (m.p. 198–201°C, 47%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 3.32 (m, CH_2-3′), 4.35 (m, CH-2′), 4.53 (dd, CH_2-1′), 4.91 (t, HO-3′), 5.19 (d, HO-2′), 8.53, 8.61 and 8.62 (3s, H-2, H-4 and H-7); UV (H_2O/MeOH, 1:1): 249 nm (17 200), 300 (sh, 7000). Elemental analysis: calculated for $C_{10}H_{10}N_4O_6$: C, 42.55; H, 3.58; N, 19.86. Found: C, 42.33; H, 3.45; N, 19,80.

S-alkylated and -arylalkylated derivatives of C-substituted benzimidazoles **(**4a–m**).** *2-(Dimethylaminoeth-1-ylthio)benzimidazole* (**4a**): 0.6 g (4 mmol) of 2-thiobenzimidazole was dissolved in acetonitrile (20 ml) containing 2 ml (14 mmol) DBU followed by the addition of 0.72 g (5 mmol) of 2-dimethylaminoethylchloride hydrochloride. The solution was stirred overnight at room temperature and the solvent evaporated. Water (5 ml) was added to the residue and the mixture was brought to pH 7 with CH_3COOH. The precipitate formed was filtered and crystallized from H_2O to give **4a** (0.424 g, 48%); m.p. 137–140°C; ^{1}H NMR (D_6-Me_2SO) δ (ppm): 2.41 (s, 2 × CH_3), 2.88 and 3.47 (2t, -CH_2CH_2-), 7.10 and 7.44 (2m, H-benzimidazole). UV (H_2O, pH 7.0): 282 (10 500), 289 (10 300). Elemental analysis: calculated for $C_{11}H_{15}N_3S$: C, 59.70; H, 6.85; N, 18.99. Found: C, 59.55; H, 6.66: N, 18.83.

5,6-Dimethyl-2-(dimethylaminoeth-1-ylthio)benzimidazole (**4b**): Similar to **4a,** from **3b** and 2-dimethylaminoethylchloride hydrochloride. The crude product was purified by silica gel column chromatography (3 × 15 cm) using $CHCl_3$/MeOH (85:15) as an eluent. Crystallization from diethyl ether/petroleum ether yielded **4b** (340 mg, 39%); m.p. 135–137°C; ^{1}H NMR (D_6-Me_2SO) δ (ppm): 2.18 and 2.26 (2s, 2 × CH_3-benzimidazole and 2 × N-CH_3), 2.57 and 3.35 (2t, -CH_2CH_2-), 7.19 (s, H-4 and H-7). UV, (H_2O, pH 7.0): 291 (13 500), 297 (13 300). Elemental analysis: calculated for $C_{13}H_{19}N_3S$: C, 62.61; H, 7.70; N, 16.86. Found: C, 62.86; H, 7.55; N, 16.88.

5-Chloro-2-(dimethylaminoeth-1-ylthio)benzimidazole hydrochloride (**4c**): Similar to **4b,** from **3c** and 2-dimethylaminoethylchloride hydrochloride. Pure product was treated with MeOH saturated with dry HCl to give **4c** (m.p. 239–242°C 42%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 2.83 (s, 2 × CH_3), 3.48 and 3.74 (2t, -CH_2CH_2-), 7.26 and 7.54 (2dd, H-6 and H-7), 7.61 (d, H-4). UV (H_2O, pH 7.0): 251 (5600), 290 (12 500), 298 (12 300). Elemental analysis: calculated for $C_{11}H_{14}N_3SCl \cdot HCl$: C, 45.21; H, 5.18; N, 14.38. Found: C, 45.12; H, 5.27; N, 14.50.

5,6-Dichloro-2-(dimethylaminoeth1-ylthio)benzimidazole hydrochloride (**4d**): Similar to **4a,** from **3d** and 2-dimethylaminoethylchloride hydrochloride and transformed to hydrochloride analogously to the method given for **4c** (m.p. 193–195°C, 44%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 2.82 (s, 2 × CH_3), 3.43 and 3.66 (2t, -CH_2CH_2-), 7.73 (s, H-4 and H-7). UV (H_2O, pH 7.0): 256 (6 300), 297 (12 900), 306 (13 300). Elemental analysis: calculated for $C_{11}H_{13}N_3Cl_2S \cdot HCl$: C, 40.44; H, 4.33; N, 12.86. Found: C, 40.31; H, 4.52; N, 12,73.

5,6-Dichloro-2-(diethylaminoeth-1-ylthio)benzimidazole (**4e**): Similar to **4a**, from **3d** and 2-diethylaminoethylchloride hydrochloride: (m.p. 266–269°C, from H_2O/EtOH, 81%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 1.26 and 3.18 (t and q, 2 × C_2H_5), 3.35 and 3.61 (2t, -CH_2CH_2-), 7.73 (s, H-4 and H-7). UV (H_2O, pH 7.0): 256 (5 800), 291 (13 000), 297 (13 300). Elemental analysis: calculated for $C_{13}H_{17}N_3Cl_2S$: C, 49.06; H, 5.40; N, 13.21. Found: C, 49.30; H, 5.54; N, 13.43.

5,6-Dichloro-2-(dimethylaminoprop-1-ylthio)-benzimidazole hydrochloride (**4f**): Similar to **4a,** from **3d** and 3-dimethylaminopropylchloride hydrochloride. The crude product was purified by column chromatography (silica gel, 3 × 15 cm) using $CHCl_3$/MeOH (80 : 20) as an eluent. The product-containing fractions were evaporated and the product was transformed to the hydrochloride as de-

scribed for **4c:** (m.p. 219–222°C, 56%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 2.51 (s, 2 × CH_3), 2.15, 3.16 and 3.44 (2t and q, -$CH_2CH_2CH_2$-), 7.76 (s, H-4 and H-7). UV (H_2O, pH 7.0): 256 (6000), 297 (13100), 306 (13400). Elemental analysis: calculated for $C_{12}H_{15}N_3Cl_2S \cdot HCl$: C, 42.31; H, 4.74; N, 12.34. Found: C, 42.44; H, 4.91; N, 12.48.

5,6-Dichloro-2-(morpholineth-1-yl)benzimidazole hydrochloride (**4g**): Similar to **4a**, from **3d** and morpholineethyl-chloride hydrochloride. The crude product was purified by column chromatography as described for **4f** and transformed into the hydrochloride as described for **4c**: (m.p. 147–150°C, 55%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 3.20 and 3.73 (bs and t, -CH_2CH_2-), 3.52 and 3.92 (t and bs, CH_2-morpholine), 7.73 (s, H-4 and H-7). UV (H_2O, pH 7.0): 256 (5900), 297 (13200), 306 (13500). Elemental analysis: calculated for $C_{13}H_{15}N_3Cl_2S \cdot HCl$: C, 42.35; H, 4.38; N, 11.40. Found: C, 42.46; H, 4.52; N, 11.27.

5,6-Dichloro-2-(4-nitrobenzylthio)benzimidazole (**4h**): Similar to **4a**, from **3d** and 4-nitrobenzyl chloride: (m.p.134–138°C, from EtOH/H_2O, 59%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 4.70 (s, CH_2), 7.60–8.10 (2m, H-arom. and H-benzimidazole). UV (H_2O/MeOH, 1:1): 265 (13400), 302 (17700), 308 (17100). Elemental analysis: calculated for $C_{14}H_9N_3O_2Cl_2S$: C, 47.48; H, 2.57; N, 11.87. Found: C, 47.40; H, 2.39; N, 11.70.

5,6-Dichloro-2-(3,4-dichlorobenzylthio)benzimidazole (**4i**): Similar to **4a**, from **3d** and 3,4-dichlorobenzyl chloride: (m.p.157–159°C, from EtOH/H_2O, 39%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 4.56 (s, CH_2), 7.40–7.80 (m, H-arom. and H-benzimidazole). UV (H_2O/MeOH, 1:1): 263 (7300), 303 (16800), 310 (17000). Elemental analysis: calculated for $C_{14}H_8N_2Cl_4S$: C, 44.48; H, 2.14; N, 7.41. Found: C, 44.63; H, 2.26; N, 7.30.

5-Carboxy-2-allylthiobenzimidazole (**4j**): Similar to **4a**, from **3j** and allyl chloride using NaOH instead of DBU: (from MeOH, 72%, m.p. 112–115°C); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 5.13 and 5.34 (2d, =CH_2), 6.00 (m, HC=), 7.52 and 7.78 (2dd, H-6 and H-7), 8.02 (s, H-4). UV (H_2O/MeOH, 9:1): 225 (24500), 299 (14400). Elemental analysis: calculated for $C_{14}H_8N_2Cl_4S \cdot H_2O$: C, 52.37; H, 4.80; N, 11.11. Found: C, 52.29; H, 4.58; N, 11.14.

5-Carboxy-2-benzylthiobenzimidazole (**4k**): Similar to **4j**, from **3j** and benzyl chloride: (m.p. 131–134°C, from MeOH, 64%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 4.62 (s, CH_2), 7.30–7.60 (m, H-arom.), 7.48 and 7.82 (2dd, H-6 and H-7), 8.03 (s, H-4). UV (H_2O /MeOH, 9:1): 224 (29800), 300 (14300). Elemental analysis: calculated for $C_{14}H_8N_2Cl_4S \cdot H_2O$: C, 63.37; H, 4.26; N, 9.86. Found: C, 63.30; H, 4.40; N, 9.81.

5-Carboxy-2-(4-nitrobenzylthio)benzimidazole (**4l**): Similar to **4j**, from **3j** and 4-nitrobenzyl chloride: (m.p. 283–286°C, from MeOH, 68%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 4.69 (s, CH_2), 7.20–8.20 (m, H-arom. and H-benzimidazole). UV (H_2O/MeOH, 1:1): 294 (18900). Elemental analysis: calculated for $C_{15}H_{11}N_3SO_4$: C, 54.71; H, 3.37; N, 12.76. Found: C, 54.89; H, 3.44; N, 12.62.

5-Carboxy-2-(3,4-dichlorobenzylthio)benzimidazole (**4m**): Similar to **4j**, from **3j** and 3,4-dichlorobenzylchloride: (m.p. 284–288°C, from MeOH, 73%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 4.64 (s, CH_2), 7.40–7.85 (m, H-arom., H-6 and H-7), 8.05 (s, H-4). UV (H_2O/MeOH, 1:1): 229 (36600), 302 (15400). Elemental analysis: calculated for $C_{15}H_{10}N_2SCl_2O_2 \cdot H_2O$: C, 48.53; H, 3.26; N, 7.55. Found: C, 47.41; H, 3.08; N, 7.31.

4,6-Dichloro-2-thiobenzimidazole (**6**): 1.168 g (6.6 mmol) of 1,3-dichloro-4,5-diaminobenzene (**5**) was dissolved in a mixture of EtOH (13 ml) and water (2 ml), followed by the addition of 0.8 ml (13.2 mmol) of carbon disulfide and 1.48 g (26.4 mmol) of KOH. The solution was stirred under reflux for 2 h, decolorized with charcoal and brought to pH 5 with acetic acid. The yellowish chromatographic pure precipitate was removed by filtration. Yield 0.9 g (62%). A small amount for analysis was crystallized from water: (m.p. > 300°C). ^{1}H NMR (D_6-Me_2SO) δ (ppm): 7.12 and 7.32 (2dd,

H-5 and H-7). UV (H_2O, pH 7): 230 (12 100), 251 (16 300), 314 (17 900). Elemental analysis: calculated for $C_7H_4N_2Cl_2S$: C, 38.38; H, 1.84; N, 12.79. Found: C, 38.28; H, 1.95; N, 12.62.

4,6-Dichloro-2-(dimethylaminoeth-1-yl)benzimidazole hydrochloride (**4n**): Similar to **4a**, from **6** and 2-dimethylaminoethyl chloride hydrochloride. The crude product was purified by column chromatography as described for **4f,** and transformed into the hydrochloride as described for **4c**: (m.p. 230–233°C, 38%); ^{1}H NMR (D_6-Me_2SO) δ (ppm): 2.84 (s, 2 × CH_3), 3.46 and 3.69 (2t, -CH_2CH_2-), 7.77 and 7.51 (2d, H-5 and H-7). UV (H_2O, pH 7): 257 (6700), 291 (12 300), 299 (12 600). Elemental analysis: calculated for $C_{11}H_{13}N_3SCl_2 \cdot 2H_2O \cdot HCl$: C, 36.43; H, 5.01; N, 11.58. Found: C, 36.40; H, 4.81; N, 11.45.

4,6-Dichloro-2-(4-nitrobenzylthio)benzimidazole (**4o**): Similar to **4a**, from **6** and 4-nitrobenzylchloride (m.p. 132–134°C from EtOH/ H_2O, 41%),^{1}H NMR (D_6-Me_2SO) δ (ppm): 4.71 (s, CH_2), 7.20–8.20 (m, H-arom. and H-benzimidazole). UV (H_2O/MeOH, 1:1): 260 (16 100), 303 (17 800). Elemental analysis: calculated for $C_{14}H_9N_3O_2Cl_2S$: C, 47.48; H, 2.57; N, 11.87. Found: C, 47.41; H, 2.39; N, 11.69.

RESULTS AND DISCUSSION

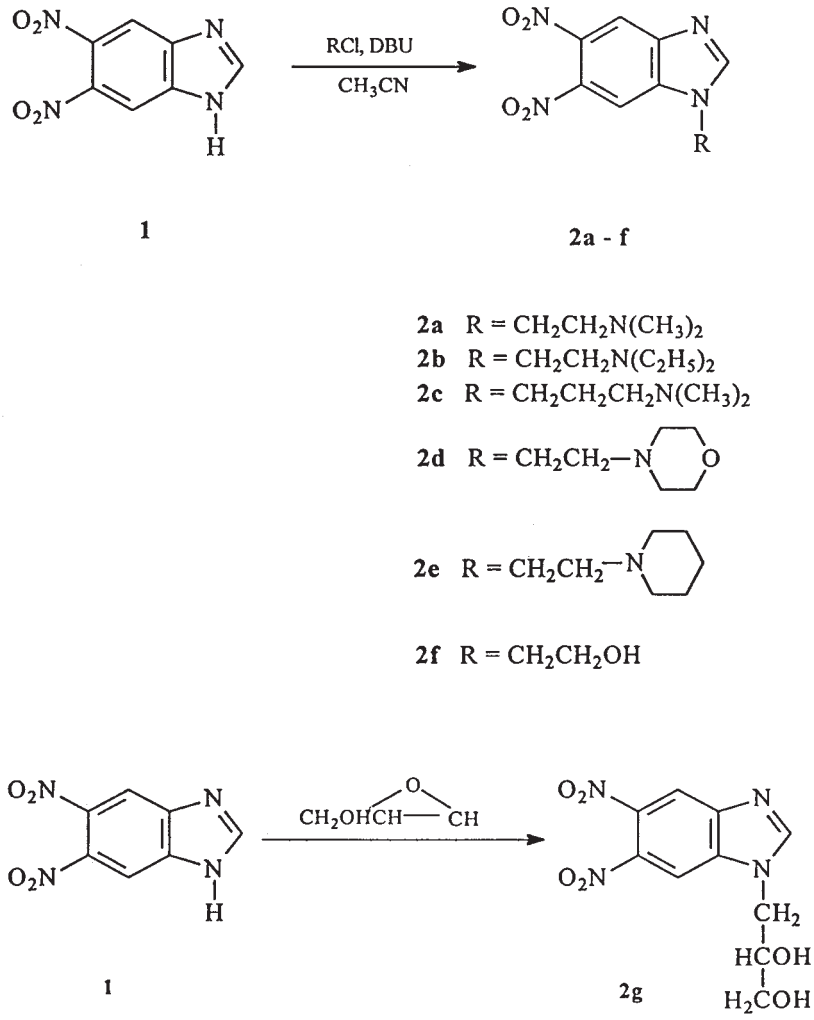
Chemical synthesis

In order to expand the group of benzimidazole derivatives, we synthesised several new benzimidazole ring-containing compounds. In addition to the earlier reported 5,6-dinitro- and 4,6-dinitrobenzimidazoles, their nucleosides and 5,6-dinitro-2-trifluoromethylbenzimidazole [17, 18], we have undertaken the synthesis of a series of 1-substituted 5,6-dinitrobenzimidazoles (**2a–f)** by alkylation of 5,6-dinitrobenzimidazole (**1)** with appropriate halogenoalkylamine in acetonitrile using 1,8-diazabicyclo[5,4,0]undec-7-en (DBU) as a base. Additionally, 5,6-dinitro-1-(2,3-dihydroxyprop-1-yl)benzimidazole (**2g)** was obtained in the reaction of **1** with glycitole under the same reaction conditions (Scheme 1). The choice of 1-substituents was motivated by their presence in numerous drugs showing a variety of antimicrobial, psychostimulant and other activities.

The second series were sulfur-substituted derivatives of a modified benzimidazole nucleus. The methyl-, chlorine- and carboxy-substituted derivatives of 2-thiobenzimidazoles (**3a–m**) were S-alkylated with halogenoalkylamines, allyl chloride, or benzyl chloride and its *p*-nitro and 3,4-dichlorosubstituted derivatives to provide the corresponding derivatives (**4a–o)**. The substrates **3a–m** were previously synthesised by the reaction of carbon disulfide with the corresponding phenylenediamine [6]. The novel 4,6-dichloro-2-thiobenzimidazole (**6)** was similarly prepared by treating 1,3-dichloro-4,5-diaminobenzene (**5)** with carbon disulfide (Scheme 2). The resulting derivatives were purified by crystallization or flash column chromatography. In a few cases it was necessary to transform the products into their respective hydrochlorides to obtain a crystalline material. Of the N- and S-substituted derivatives shown in Schemes 1 and 2, only **3c** has been previously mentioned in the patent literature [19].

Antibacterial activity

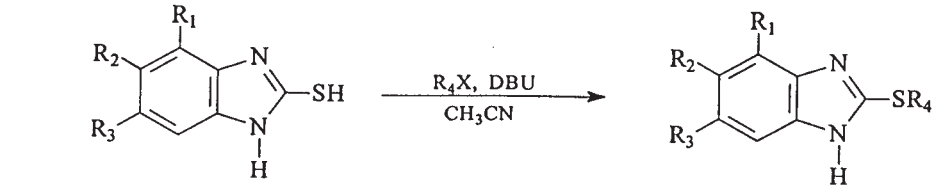
The antibacterial activity of the benzimidazole derivatives was first tested by the agar disc-diffusion method against Gram-positive and Gram-negative bacteria. The results of these studies are summarized in Table 1. The compounds not shown in the table had no antibacterial activity. The new benzimidazole derivatives reported here, particulary **4d–4n,** are approximately as active as metronidazole used as a control substance. In the case of enterobacteria, such as *E. coli* and *P. vulgaris,* the bezimidazoles **4d–4n** exhibited moderate activity, whereas metronidazole was inactive.



Scheme 1

On the other hand, metronidazole was more active against bacilli then **2g** and **4a–f** and **4n**.

More attention was devoted to the investigation of antibacterial activity against *Stenotrophomonas maltophilia* strains. *S. maltophilia* (previously called *Pseudomonas maltophilia* and *Xanthomonas maltophilia*) is an increasingly recognized nosocomial pathogen, particularily for immunocompromised patients. Risk factors for *S. maltophilia* are colonization and infection due to mechanical ventilation, previous exposure to broad spectrum antibiotics, fungal infections, catheterization, prolonged hospitalization and the use of equipment contacting with the respiratory tract, such as nebulizers. *S. maltophilia* is found in a wide variety of ageratic, soil, and rhizosphere environments and on various contaminated materials and fomites, including faucets, showers, ice-making machines etc. Several of these have been implicated as sources of nosocomial infections with *S. maltophilia*. With the exception of trimetoprim-sulfamethoxazole (Biseptol, Cotrimorazole), many post-therapy isolates of *S. maltophilia* quickly become resistant to antimicrobial agents [20]. The tests presented here (Table 2) show that only compounds **4d**, **4e**, **4f** and **4n**, which are inactive against standard and nosocomial strains of *B. cepacia* and *P. aeruginosa*, were active against nosocomial strains of *S. maltophilia*. Compounds **4f** and **4n** displayed antibacterial activity also



3a-m **4a-m**

a $R_1, R_2, R_3 = H, R_4 = CH_2CH_2N(CH_3)_2$

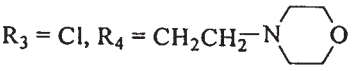
b $R_1 = H, R_2, R_3 = CH_3, R_4 = CH_2CH_2N(CH_3)_2$

c $R_1, R_3 = H, R_2 = Cl, R_4 = CH_2CH_2N(CH_3)_2$ [19]

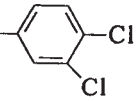
d $R_1 = H, R_2, R_3 = Cl, R_4 = CH_2CH_2N(CH_3)_2$

e $R_1 = H, R_2, R_3 = Cl, R_4 = CH_2CH_2N(C_2H_5)_2$

f $R_1 = H, R_2, R_3 = Cl, R_4 = CH_2CH_2CH_2N(CH_3)_2$

g $R_1 = H, R_2, R_3 = Cl, R_4 =$ 

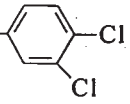
h $R_1 = H, R_2, R_3 = Cl, R_4 =$ *p*-nitrobenzyl

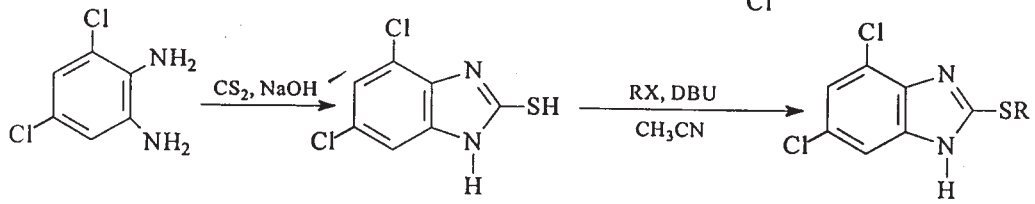
i $R_1 = H, R_2, R_3 = Cl, R_4 =$ 

j $R_1, R_3 = H, R_2 = COOH, R_4 = CH_2CH{=}CH_2$

k $R_1, R_3 = H, R_2 = COOH, R_4 =$ benzyl

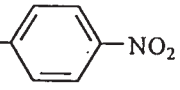
l $R_1, R_3 = H, R_2 = COOH, R_4 =$ *p*-nitrobenzyl

m $R_1, R_3 = H, R_2 = COOH, R_4 =$ 



5 **6**

4n $R = CH_2CH_2N(CH_3)_2$

4o $R =$ 

Scheme 2

against the standard strain of *S. maltophilia* NCTC 13637 (MIC 400 and 200 μg/ml, respectively). Derivatives **4d** and **4e** – inactive against standard strains of *Pseudomonas* family – are active against all of nosocomial strains of *S. maltophilia* (MIC 50–400 μg/ml) tested here. In contrast, compounds **4f** and **4n** moderately active against standard strains, were inactive against 49 and 16 nosocomial strains.

The results obtained indicate that the compounds studied here or their possible structural analogues could be potential chemotherapeutics in the case of *S. maltophilia* infection. It is noteworthy that the nosocomial *S. maltophilia* strains examined here were resistant to β-lactam antibiotics, including penems, azeotronams and cephalosporins of the 3rd generation and showed considerable resistance to aminoglycosides and quinolones.

Table 1. Antimicrobial activity of substituted benzimidazoles against Gram-positive and Gram-negative bacteria strains

Bacteria strain	Diameter of growth inhibition area (mm)								
	Compound								
	2g	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4n**	**Metronidazole**
S. aureus ATCC 6538P	0	trace	trace	0	12	12	14	15	11
S. aureus ATCC 25923	trace	11	trace	trace	12	14	12	14	12
S. aureus NCTC 4163	0	trace	0	0	11	12	12	11	11
M. flavus NCIB 8166	12	11	trace	trace	trace	0	10	10	12
B. subtilis ATCC 6633	17	11	0	11	11	11	12	14	32
B. stenothermophilus ATCC 7953	14	11	0	12	11	13	13	12	30
B. cereus ML 98	14	trace	trace	11	12	13	12	14	15
E. coli NCTC 8196	0	0	0	0	11	11	12	12	0
P. vulgaris NCTC 4635	0	trace	trace	trace	13	11	12	12	0

Anti-protozoal activity

Drugs (0.1 M) dissolved in dimethyl formamide (DMF) were used in microtitre plate susceptibility assays as previously described [16]. In a few cases (**2c**, **4f**, **4c**, **4n**) the compounds required mild heat for dissolution. **4e** was soluble at 10 mM and **4h** crystallized in the assays at > 100 μM. MIC values for **1**, **2a–2g** and **4a–o** were estimated as previously described and only compounds with MICs < 100 μM in any assay are presented in Table 2. The MIC values for metronidazole (Mz) and albendazole (Alb) are also presented. While neither of the compounds was uniformly as effective as metronidazole, or as effective as albendazole against *Giardia*, several compounds had MIC values which should encourage further development as lead compounds in a search for new anti-anaerobic

Table 2. Drug susceptibility assay of *Pseudomonas* family bacteria

Bacteria strain	MIC values (mg/ml)			
	Compound			
	4d	**4e**	**4f**	**4n**
S. maltophilia ATCC 13637	>400	>400	400	200
B. cepacia ATCC 25416	>400	>400	>400	>400
P. aeruginosa ATCC 27863	>400	>400	>400	>400
P. aeruginosa ATCC 15442	>400	>400	>400	>400
P. aeruginosa NCTC 6749	>400	>400	>400	>400
S. maltophilia 67 clinical strains	100 — 3 strains	50 –2 strains	100 — 2 strains	100 — 2 strains
	100–200 — 35 strains	100–200 — 28 strains	200 — 4 strains	200 –5 strains
	300–400 — 29 strains	300–400 — 37 strains	400 — 12 strains	400 — 44 strains
			>400 — 49 strains	>400 — 16 strains

Metronidazole used as control substance; MIC > 400 (μg/ml); replica plates method was employed.

Table 3. Drug susceptibility assays of *G. duodenalis*, *T. vaginalis* and *E. histolytica* tested against metronidazole (Mz), albendazole (Alb) and new benzimidazole derivatives.

Values are presented as MIC (μM).

Drug	*G. duodenalis*					*T. vaginalis*		*E. histolytica*	
	WB	WB/Alb	WB-M1	106	$2ID_{10}$	F1623	F1623-M1	MU	TM
Mz	6.3	6.3	50	6.3	50	1.6	25	25	
Alb	1.6	100	3.2	1.6	ND	>200	>200	>200	
1	200	ND	ND	200	ND	50	>200	200	
2a	>200	ND	ND	>200	ND	25	>200	100	
2b	100	200	ND	200	ND	25	>200	200	
2c	>200	ND	ND	>200	ND	50	>200	>200	
2d	200	ND	ND	200	ND	25	>100	>200	
2e	>200	ND	ND	>200	ND	50	>200	200	
2f	200	ND	ND	200	ND	100	>200	>200	
4l	100	ND	100	100	100	100	100	ND	
4h	25	25	25	25	25	100	100	25	
4o	50	50	50	50	50	50	>200	50	

*ND, not determined.

protozoal clinical agents. Compounds **2a**, **2b**, **2c**, and to a lesser extent **2e**, were the most effective against *T. vaginalis*. These 5,6-dinitro-1-(aminoethyl)benzimidazoles may act *via* reduction of the nitro group *via* ferredoxin in the same way metronidazole acts, but not as inhibitors of tubulin polymerization as albendazole does, since the metronidazole-resistant line F1623-M1 was not susceptible to these compounds. This is what is expected of drugs with a similar mechanism of action to metronidazole.

The compound **4h** and to a lesser extent **4o** were the most effective against *Giardia* and *Entamoeba*. While 5,6-dichloro and 4,6-dichloro benzimidazoles carry a nitrobenzyl group, the mechanism of action may not be *via* the nitro group, since the metronidazole-resistant *Giardia* lines and the parent isolates were similarly susceptible. In addition, the MIC of **4h** against *Entamoeba* was the same as that of metronidazole. Compound **4l** which also carries the nitrobenzyl group but is a 5-carboxybenzimidazole, rather than a dichloro-benzimidazole, was ineffective in all assays suggesting that the nitrobenzyl group was not the all-important functional moiety in **4h** and **4o**. Since benzimidazole compounds have not previously been shown to be effective against *Entamoeba* and in our hands albendazole is ineffective against *Trichomonas* (Table 3), our results, particularly those for **4h,** are worth pursuing in the search for new effective anti-protozoal drugs.

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