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Synthesis of Novel 4-(1*H*-benzimidazol-2-yl)benzene-1,3-diols and their Cytotoxic Activity against Human Cancer Cell Lines

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One-pot synthesis of new biologically active 4-(1*H*-benzimidazol-2-yl)benzene-l,3-diols has been developed. The compounds were prepared by the reaction of aryl-modified sulfinylbis[(2,4-dihydroxyphenyl)methanethione]s with benzene-l,2-diamines. Their structures were identified using elemental, IR, ^{1}H-NMR, and mass spectra analyses. The developed method offers short reaction times, relatively large-scale synthesis, easy and quick isolation of the products, and good yields. The cytotoxicity *in vitro* against the 4 human cancer cell lines: SW707 (rectal), HCV29T (bladder), A549 (lung), and T47D (breast) was determined. The antiproliferative properties of some compounds studies were stronger than those of cisplatin, which was used as a comparator drug.

Key words: 4-(l*H*-Benzimidazol-2-yl)benzene-l,3-diols, Synthesis, Antiproliferative activity, Cytotoxic activity

INTRODUCTION

Compounds exhibiting the functionality of benzimidazole have been extensively employed in the area of pharmaceuticals (Singh et al., 2010). They can be found in many commercial drugs such as omeprazole (Prilosec), pantoprazole (Protonix), Vermox, fenbendazole, Atacand and mibefradil (Posicor) (Fig. 1), as well as in numerous experimental drug candidates in a wide range of therapeutic areas (Biron, 2006; Pescovitz, 2008). The anticancer properties of the benzimidazole based compounds are also very valuable (Thomas et al., 2007).

A decade ago, a class of benzimidazole-4-carboxamides was identified as poly(ADP-ribose) polymerase (PARP) inhibitors and used for the design of potential anticancer drugs (White et al., 2000); Among them, the clinical candidates, A-620223 and ABT-888 were developed (Penning et al., 2009). Next, (*S*)-2-(2-fluoro-4-(pyrrolidin-2-yl)phenyl)-l*H*-benzimidazole-4-carboxamide (A-966492) was identified as a compound with high potency against the PARP-1 enzyme, showing activity at a concentration of 1 nM. In addition, it was orally bioavailable and possessed good *in vivo* efficacy in a murine melanoma model (Penning et al., 2010). Other benzimidazole-4-carboxamide analogues as PARP-1 inhibitors with high cellular activity were also presented (Tong et al., 2009).

Benzimidazoles are described as inhibitors of the kinesin spindle protein (KSP) (Lahue et al., 2009), hypoxia-inducible factor (HIF-1) (Won et al., 2009), the enzyme 17α-hydroxylase/17,20 (Bruno et al., 2008), and others (Bhattacharya et al., 2010), which are important targets for the development of new anticancer drugs. The derivatives of bis-benzimidazoles (Hoechst-33342, Hoechst-33258) are inhibitors of DNA topoisomerase I (Kazimierczuk and Shugar, 1989; Kraut et al., 1991).

There are numerous studies on preliminary cytotoxic screening of compounds with a benzimidazole core. For example, Vitale and co-workers described 2-naphthyl and 2-biphenyl derivatives acting as cytotoxic agents against human cell lines derived from hematological and solid tumors (Vitale et al., 2008, 2009). 2-Heterocyclic benzimidazoles exhibit cytotoxic effects against a panel of human cancer cell lines (Refaat, 2010). Other derivatives are described as precursors of antileuke-

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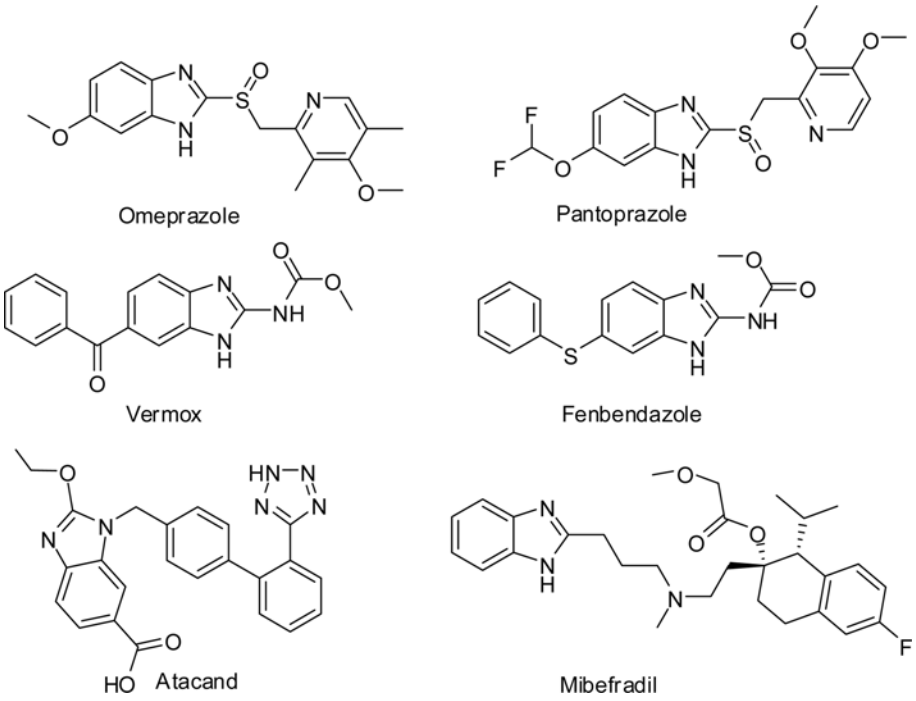

Fig. 1. Structures of 1*H*-benzimidazole-based commercial drugs

mic agents (Ranganatha et al., 2009). Benzimidazole-4,7-diones show cytotoxicity against colon, breast and lung cancer cell lines (Gellis et al., 2008). The broad profile of therapeutic applications of compounds with a benzimidazole core and their valuable biological properties has prompted extensive studies of their synthesis (Perry and Wilson, 1993; Brain and Brunton, 2002; Yang et al., 2005; Zhang et al., 2009; Bahrami et al., 2010; Peng et al., 2010).

We previously reported the synthesis and antiproliferative activity of 1,3,4-thiadiazoles and 4*H*-3,1-benzothiazines with a 2,4-dihydroxyphenyl residue against human cancer cell lines (Matysiak, 2006; Matysiak and Opolski, 2006). Taking into account their valuable biological properties and the frequent incidence of a benzimidazole core in the anticancer compounds, we developed an efficient synthesis of novel differently substituted 4-(l*H*-benzimidazol-2-yl)benzene-1,3-diols. We first obtained a series of compounds modified at positions 5, 6 or 7 of the benzimidazole core at the constant C-2 substitution. Next, we tried to find the best modification of a resorcinol moiety to produce a more active structure.

MATERIALS AND METHODS

Chemistry

Melting points (m.p.) were determined using a BÜCHI B-540 (Flawil) melting point apparatus. Elemental analyses were performed on a Perkin-Elmer 2400 instrument; the determined values (C, H, and N) were within ± 0.4% of the theoretical values. IR spectra were measured with a Perkin-Elmer FT-IR 1725X spectrophotometer (in KBr) in the range of 600-4000 cm^{-1}. ^{1}H-NMR spectra were recorded in DMSO-d_6 using a Bruker DRX 500 instrument. Chemical shifts (δ, ppm) were described in relation to tetramethylsilane (TMS). MS (EI, 70 eV) were recorded with an AMD-604 instrument.

The purity of the compounds was examined by a liquid chromatograph (Knauer) with a dual pump, a 20 μL simple injection valve, and a UV-visible detector at 280 nm. A Hypersil Gold C18 (1.9 μm, 100 × 2.1 mm) column was used as the stationary phase. The mobile phase included different contents of MeOH and acetate buffer (pH 4, 20 nM) as the aqueous phase. The flow rate was 0.5 mL/min at room temperature. The retention time of an unretained solute (t_0) was determined by the injection of a small amount of acetone dissolved in water. Log k values for 70% of methanol (v/v) in the mobile phase are presented.

4-(7-Methyl-1*H*-benzimidazol-2-yl)benzene-1,3-diol (1)

A mixture of 3-methylbenzene-1,2-diamine (0.002 mol) and STB (0.002 mol) in MeOH (10 mL) was heated to ref1ux for 3 h, and was left at room temperature (24 h). The formed solid was filtered via a Büchner funnel

and recrystallized from MeOH (6 mL).
Yield 87%; m.p. 158-160°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3175 (OH), 1624 (C=N), 1596 (C=C), 1517 (C=C), 1470, 1379, 1331, 1302, 1248, 1214 (C-OH), 1178, 1131, 1080, 1025, 1002, 978, 950, 846, 823, 734; ^{1}H-NMR (DMSO-d_6): δ 13.21 (s, 1H, HN), 13.02 (s, 1 H, HO-3), 10.52 (s, 1H, HO-1), 8.24 (m, 1H, H-5), 7.33 (m, 2H), 7.26 (m, 1H), 6.49 (m, 1H, H-2), 6.31 (m, 1H, H-6), 2.39 (s, 3H, CH_3); EI-MS (*m/z*, %): 240 (M^+, 100), 211 (6), 183 (22), 169 (3), 154 (3), 106 (9), 91 (8), 77 (5), 51 (2); log k = –0.331. Anal. calcd. for $C_{14}H_{12}N_2O_2$ (240.26): C, 69.99; H, 5.03; N, 11.66. Found: C, 69.87; H, 4.92; N, 11.73%.

4-(5,6-Dimethyl-1*H*-benzimidazol-2-yl)benzene-1,3-diol (2)

A mixture of 4,5-dimethylbenzene-1,2-diamine (0.0018 mol) and STB (0.0018 mol) in MeOH (9 mL) was heated to reflux for 2.5 h, and was filtered via a Büchner funnel. The filtrate was concentrated. The formed solid was recrystallized from MeOH (6 mL).
Yield 81%; m.p. 212-215°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3445 (OH), 3251 (OH), 3148 (OH), 2920 (CH), 2856 (CH), 1621 (C=N), 1598 (C=C), 1502 (C=C), 1474, 1453, 1383, 1325, 1297, 1263, 1240, 1208 (C-OH), 1178, 1139, 1082, 1030, 1004, 980, 953, 851, 817, 739; ^{1}H-NMR (DMSO-d_6): δ 14.00 (s, 1H, HN), 11.87 (s, 1H, HO-3), 10.69 (s, 1H, HO-1), 8.06 (d, *J* = 8.8 Hz, 1H, H-5), 7.55 (s, 2H, H-4',7'), 6.77 (d, *J* = 2.2 Hz, 1H, H-2), 6.55 (dd, *J* = 8.8, 2.2 Hz, 1H, H-6), 2.37 (s, 6H, CH_3); EI-MS (*m/z*, %): 254 (M^+, 100), 239 (16), 225 (7), 197 (4), 169 (3), 127 (4), 123 (5), 91 (7), 77 (2), 65 (3), 38 (24), 36 (68); log k = –0.144. Anal. calcd. for $C_{15}H_{14}N_2O_2$ (254.28): C, 70.85; H, 5.55; N, 11.02. Found: C, 71.02; H, 5.58; N, 10.98%.

4-(6,7-Dimethyl-1*H*-benzimidazol-2-yl)benzene-1,3-diol (3)

A mixture of 3,4-dimethylbenzene-1,2-diamine (0.0018 mol) and STB (0.0018 mol) in MeOH (9 mL) was heated to ref1ux for 2.5 h. The hot mixture was filtered via a Büchner funnel and the filtrate was concentrated. The formed solid was recrystallized from MeOH (6 mL).
Yield 78%; m.p. 236-238°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3395 (OH), 3212 (OH), 2985 (CH), 2860 (CH), 1617 (C=N), 1578 (C=C), 1507 (C=C), 1491, 1458, 1398, 1326, 1240 (C-OH), 1197, 1132, 1088, 1072, 1021, 983, 952, 915, 897, 840, 809, 772, 758; ^{1}H-NMR (DMSO-d_6): δ 13.80 (s, 1H, HN), 11.70 (s, 1H, HO-3), 10.62 (s, 1H, HO-1), 8.12 (d, *J* = 8.6 Hz, 1H, H-5), 7.52 (d, *J* = 8.2 Hz, 1H, H-4'), 7.28 (d, *J* = 8.2 Hz, 1H, H-5'), 6.75 (s, 1H, H-2), 6.55 (dd, *J* = 8.7, 2.3 Hz, 1H, H-6), 2.54 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); EI-MS (*m/z*, %): 254 (M^+, 100), 239 (22), 225 (5), 187 (8), 113 (5), 36 (26); log k = –0.002. Anal. calcd. for $C_{15}H_{14}N_2O_2$ (254.28): C, 70.85; H, 5.55; N, 11.02. Found: C, 71.02; H, 5.52; N, 10.97%.

4-(5,6-Dichloro-1*H*-benzimidazol-2-yl)benzene-1,3-diol (4)

A mixture of 4,5-dichlorobenzene-1,2-diamine (0.0015 mol) and STB (0.0015 mol) in MeOH (8 mL) was heated to reflux for 3 h and was left at room temperature (24 h). The formed solid was filtered via a Büchner funnel and combined with that removed after concentration of the filtrate. The compound was recrystallized from MeOH (4 mL).
Yield 80%; m.p. 279-281°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3313 (OH), 3256 (OH), 2861 (CH), 1622 (C=N), 1504 (C=C), 1498, 1450, 1389, 1331, 1251 (C-OH), 1198, 1136, 1084, 1069, 1027, 984, 950, 917, 892, 846, 812, 779, 764; ^{1}H-NMR (DMSO-d_6): δ 10.72 (s, 1H, HO-1), 8.07 (d, *J* = 8.8 Hz, 1H, H-5), 7.96 (s, 2H, H-4',7'), 6.71 (d, *J* = 2.0 Hz, 1H, H-2), 6.55 (dd, *J* = 8.8, 2.0 Hz, 1H, H-6); EI-MS (*m/z*, %): 295 (M^+, 63), 294 (100), 265 (4), 239 (11), 237 (12), 231 (3), 202 (4); log k = 0.470. Anal. calcd. for $C_{13}H_8Cl_2N_2O_2$ (295.12): C, 52.91; H, 2.73; N, 9.49. Found: C, 53.01; H, 2.75; N, 9.44%.

Methyl 2-(2,4-dihydroxyphenyl)-1*H*-benzimidazole-5-carboxylate (5)

A mixture of methyl 3,4-diaminobenzoate (0.0015 mol) and STB (0.0015 mol) in MeOH (7.5 mL) was heated to reflux for 2.5 h and was left at room temperature (24 h). The formed solid was filtered via a Büchner funnel and combined with that removed after concentration of the filtrate. The compound was recrystallized from MeOH (4 mL).
Yield 88%; m.p. 233-236°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3187 (OH), 3038 (CH), 1686 (C=O), 1619 (C=N), 1578 (C=C), 1505 (C=C), 1485, 1461, 1440, 1420, 1400, 1359, 1334, 1296, 1244, 1235 (C-OH), 1180, 1140, 1098, 979, 950, 900, 862, 840, 809, 766; ^{1}H-NMR (DMSO-d_6): δ 14.03 (s, 1H, HN), 11.51 (s, 1H, HO-2), 10.82 (s, 1H, HO-4), 8.35 (s, 1H, H-4), 8.16 (d, *J* = 8.8 Hz, 1H, H-6), 8.03 (dd, *J* = 8.5, 1.4 Hz, 1H, H-6'), 7.86 (d, *J* = 8.5 Hz, 1H, H-7'), 6.76 (d, *J* = 2.1 Hz, 1H, H-3), 6.56 (dd, *J* = 8.8, 2.2 Hz, 1H, H-5), 3.90 (s, 3H, CH_3); EI-MS (*m/z*, %): 284 (M^+, 100), 270 (12), 253 (63), 225 (24), 198 (3), 142 (4), 126 (12), 112 (5), 98 (3), 90 (2), 63 (5); log k = –0.137. Anal. calcd. for $C_{15}H_{12}N_2O_4$ (284.27): C, 63.38; H, 4.25; N, 9.85. Found: C, 63.60; H, 4.27; N, 9.80%.

Ethyl 2-(2,4-dihydroxyphenyl)-1*H*-benzimidazole-5-carboxylate (6)

A mixture of ethyl 3,4-diaminobenzoate (0.0014 mol) and STB (0.0014 mol) in MeOH (7 mL) was heated to reflux for 3.5 h. The hot mixture was filtered via a

Büchner funnel and the filtrate was concentrated. The formed solid was recrystallized from MeOH (4 mL).
Yield 89%; m.p. 231-233°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3192 (OH), 3051 (CH), 1690 (C=O), 1616 (C=N), 1579 (C=C), 1508 (C=C), 1487, 1462, 1438, 1423, 1399, 1362, 1329, 1289, 1250 (C-OH), 1184, 1148, 1087, 980, 947, 896, 860, 838, 811, 762, 744; ^{1}H-NMR (DMSO-d_6): δ 14.20 (s, 1H, HN), 11.20 (s, 1H, HO-2), 10.68 (s, 1H, HO-4), 8.35 (d, J = 1.0 Hz, 1H, H-4'), 8.12 (d, J = 8.8 Hz, 1H, H-6), 8.04 (dd, J = 8.5, 1.4 Hz, 1H, H-6'), 7.86 (d, J = 8.5 Hz, 1H, H-7'), 6.73 (d, J = 2.2 Hz, 1H, H-3), 6.58 (dd, J = 8.8, 2.2 Hz, 1H, H-5), 4.37 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.37 (t, 3H, J = 7.1 Hz, CH_2CH_3); EI-MS (*m/z*, %): 298 (M^+, 100), 270 (35), 253 (33), 225 (13), 213 (6), 169 (2), 126 (3), 90 (2), 36 (16); log k = –0.082. Anal. calcd. for $C_{16}H_{14}N_2O_4$ (298.29): C, 64.42; H, 4.73; N, 9.39. Found: C, 64.58; H, 4.70; N, 9.36%.

4-(5,6-Dimethyl-1*H*-benzimidazol-2-yl)-2-methylbenzene-1,3-diol (7)
A mixture of 4,5-dimethylbenzene-1,2-diamine (0.0018 mol) and S3MTB (0.0018 mol) in MeOH (9 mL) was heated to reflux for 2.5 h. The hot mixture was filtered via a Büchner funnel. The obtained solid was combined with that removed after concentration of the filtrate. The compound was recrystallized from MeOH-H_2O (3:1, 8 mL).
Yield 83%; m.p. 287-288°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3143 (OH), 1608 (C=N), 1568 (C=C), 1503 (C=C), 1471, 1382, 1311, 1269, 1205 (C-OH), 1027, 1039, 1002, 937, 914, 859, 809, 757, 736, 703; ^{1}H-NMR (DMSO-d_6): δ 13.95 (s, 1H, HN), 11.11 (s, 1H, HO-3), 10.44 (s, 1H, HO-1), 7.82 (d, J = 8.8 Hz, 1H, H-5), 7.52 (s, 2H, H-4',7'), 6.67 (d, J = 8.7 Hz, 1H, H-6), 2.37 (s, 6H, CH_3); EI-MS (*m/z*, %): 268 (M^+, 100), 254 (10), 239 (27), 223 (10), 197 (6), 169 (2), 134 (4), 103 (3), 91 (6), 65 (3), 36 (26); log k = 0.220. Anal. calcd. for $C_{16}H_{16}N_2O_2$ (268.31): C, 71.62; H, 6.01; N, 10.44. Found: C, 71.80; H, 5.88; N, 10.40%.

4-(5,6-Dimethyl-1*H*-benzimidazol-2-yl)-6-ethylbenzene-1,3-diol (8)
A mixture of 4,5-dimethylbenzene-1,2-diamine (0.0018 mol) and SETB (0.0018 mol) in MeOH (9 mL) was heated to reflux for 2.5 h. The hot mixture was filtered via a Büchner funnel and the filtrate was concentrated. The formed solid was recrystallized from MeOH (6 mL).
Yield 84%; m.p. 212-214°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3248 (OH), 2967 (CH), 1621 (C=N), 1567 (C=C), 1521 (C=C), 1469, 1404, 1313, 1272, 1239 (C-OH), 1165, 1074, 1023, 1002, 950, 893, 853, 788, 731; ^{1}H-NMR (DMSO-d_6): δ 13.89 (s, 1H, HN), 10.68 (s, 1H, HO-3), 7.95 (s, 1H, H-5), 7.54 (s, 2H, H-4',7'), 6.83 (s, 1H, H-2), 2.53 (q, 2H, J = 7.5 Hz, CH_2CH_3), 2.37 (s, 6H, CH_3), 1.20 (t, 3H, J = 7.5 Hz, CH_2CH_3); EI-MS (*m/z*, %): 282 (M^+, 49), 267 (M^+-CH_3, 100), 239 (5), 225 (2), 210 (2), 197 (4), 182 (2), 160 (4), 141 (3), 126 (4), 91 (4), 77 (3), 38 (4), 36 (11); log k = 0.040. Anal. calcd. for $C_{17}H_{18}N_2O_2$ (282.34): C, 72.32; H, 6.43; N, 9.92. Found: C, 72.48; H, 6.46; N, 9.83%.

4-Chloro-6-(5,6-dimethyl-1*H*-benzimidazol-2-yl)benzene-1,3-diol (9)
A mixture of 4,5-dimethylbenzene-1,2-diamine (0.0018 mol) and SClTB (0.0018 mol) in MeOH (9 mL) was heated to reflux for 2.5 h. The hot mixture was filtered via a Büchner funnel and the filtrate was concentrated. The formed solid was recrystallized from MeOH (6 mL).
Yield 89%; m.p. 241-243°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3156 (OH), 2927 (CH), 1622 (C=N), 1577 (C=C), 1512 (C=C), 1473, 1408, 1320, 1283, 1246 (C-OH), 1171, 1083, 1019, 1006, 949, 897, 849, 784, 726; ^{1}H-NMR (DMSO-d_6): δ 13.98 (s, 1H, HN), 11.51 (s, 1H, HO-1), 11.40 (s, 1H, HO-3), 8.23 (s, 1H, H-5), 7.55 (s, 2H, H-4',7'), 7.02 (s, 1H, H-2), 2.55 (s, 6H, CH_3); EI-MS (*m/z*, %): 288 (M^+, 100), 258 (12), 243 (5), 231 (3), 225 (6), 207 (3), 197 (10), 192 (15), 187 (8), 169 (6), 140 (7), 130 (5), 118 (5), 105 (4), 91 (4), 80 (9), 77 (9), 51 (7), 45 (28), 39 (7); log k = 0.179. Anal. calcd. for $C_{l5}H_{13}ClN_2O_2$ (288.73): C, 62.40; H, 4.54; N, 9.70. Found: C, 62.52; H, 4.56; N, 9.65%.

4-(5,6-Dimethyl-1*H*-benzimidazol-2-yl)benzene-1,2,3-triol (10)
A mixture of 4,5-dimethylbenzene-1,2-diamine (0.0018 mol) and S3TTB (0.0018 mol) in MeOH (9 mL) was heated to reflux for 2.5 h. The hot mixture was filtered via a Büchner funnel and the filtrate was concentrated. The formed solid was recrystallized from MeOH (6 mL).
Yield 64%; m.p. 223-225°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3216 (OH), 1623 (C=N), 1577 (C=C), 1511 (C=C), 1451, 1260 (C-OH), 1178, 1082, 954, 878, 844, 785, 759, 717; ^{1}H-NMR (DMSO-d_6): δ 14.09 (s, 1H, HN), 10.39 (s, 1H, HO-3), 9.35 (s, 1H, HO-2), 7.62 (d, J = 8.8 Hz, 1H, H-5), 7.55 (s, 2H, H-4',7'), 6.66 (d, J = 8.8 Hz, 1H, H-6), 2.37 (s, 6H, CH_3); EI-MS (*m/z*, %): 270 (M^+, 100), 255 (6), 241 (8), 236 (4), 197 (3), 171 (6), 159 (3), 135 (4), 121 (4), 91 (5), 77 (7), 44 (4); log k = –0.781. Anal. calcd. for $C_{15}H_{14}N_2O_3$ (270.28): C, 66.66; H, 5.22; N, 10.36. Found: C, 66.49; H, 5.25; N, 10.32%.

2-(5,6-Dimethyl-1*H*-benzimidazol-2-yl)benzene-1, 3,5-triol (11)

A mixture of 4,5-dimethylbenzene-1,2-diamine (0.0018 mol) and S6TTB (0.0018 mol) in MeOH (10 mL) was heated to reflux for 3 h. The hot mixture was filtered via a Büchner funnel and the filtrate was concentrated. The formed solid was recrystallized from MeOH-H_2O (3:1, 8 mL).

Yield 62%; m.p. 259-261°C; IR (KBr) $\bar{\nu}$ [cm^{-1}]: 3260 (OH), 1627 (C=N), 1578 (C=C), 1515 (C=C), 1453, 1268 (C-OH), 1190, 1076, 998, 870, 846, 782, 743; ^{1}H-NMR (DMSO-d_6): δ 11.67 (s, 2H, HO-1,3), 10.09 (s, 1H, HO-5), 7.21 (s, 2H, H-4',7'), 5.86 (s, 2H, H-4,6), 2.40 (s, 6H, CH_3); EI-MS (*m/z*, %): 270 (M^+, 56), 248 (5), 240 (4), 229 (5), 285 (6), 200 (13), 186 (49), 171 (12), 159 (45), 145 (43), 126 (46), 110 (23), 98 (12), 91 (19), 83 (25), 77 (15), 69 (25), 55 (38), 43 (30), 39 (100), 36 (6); log k = −0.838. Anal. calcd. for $C_{15}H_{14}N_2O_3$ (270.28): C, 66.66; H, 5.22; N, 10.36. Found: C, 66.74; H, 5.19; N, 10.40%.

Antiproliferative assay *in vitro*

The following established *in vitro* human cell lines were used in this study: T47D (breast cancer), SW707 (rectal adenocarcinoma), A549 (non-small cell lung carcinoma) from the American Type Culture Collection and HCV29T (bladder cancer) from the Fibiger Institute, Copenhagen, Denmark. Twenty-four h before the addition of the tested agent, the cells were plated in 96-well plates (Sarstedt Inc) at a density of 10^4 cells/well. All cell lines were maintained in the opti-MEM medium supplement with 2 mM glutamine (Gibco), streptomycin (50 μg/mL), penicillin (50 U/mL) (Polfa, Tarchomin), and 5% fetal calf serum (Gibco). The cells were incubated at 37°C in a humid atmosphere saturated with 5% CO_2. The solutions of compounds (1 mg/mL) were prepared *ex tempore* by dissolving the substance in 100 μL of DMSO followed by addition of 900 μL of tissue culture medium. Afterwards, the compounds were diluted in the culture medium to final concentrations ranging from 0.1 to 100 μg/mL. The solvent (DMSO) used at the highest concentration in the test did not reveal any cytotoxic activity. Cisplatin was used as a test reference agent. The cytotoxicity assay was performed after 72 h exposure of the cultured cells at concentrations of tested agents ranging from 0.1 to 100 μg/mL. The SRB test was used to measure inhibition of cell proliferation *in vitro* (Skehan et al., 1990). The cells attached to the plastic were fixed with cold 50% TCA (trichloroacetic acid, Sigma-Aldrich Chemie GmbH) added on the top of the culture medium in each well. The plates were incubated at 4°C for 1 h and then washed 5 times with tap water. The background optical density was measured in the wells filled with the medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B (SRB, Sigma-Aldrich Chemie GmbH) dissolved in 1% acetic acid (POCh) for 30 min. The unbound dye was removed by rinsing (4 times) with 1% acetic acid, and the protein-bound dye was extracted with 10 mM unbuffered Tris base (tris(hydroxymethyl)aminomethane, POCh) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Uniskan II (Labsystems). The compounds were tested in triplicate for each experiment, and the experiments were repeated at least 3 times.

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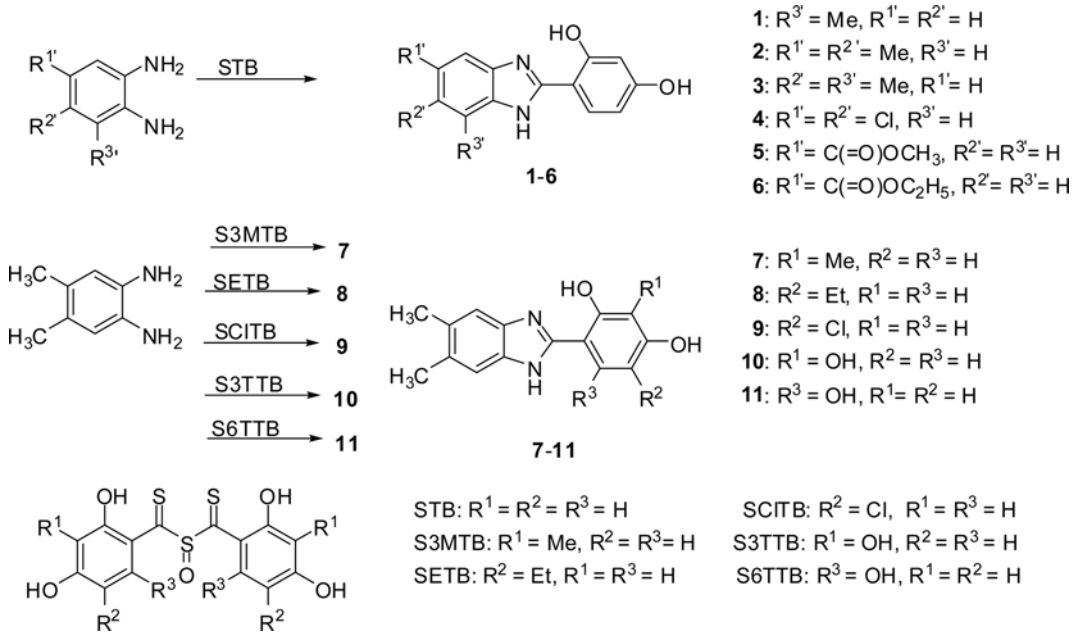
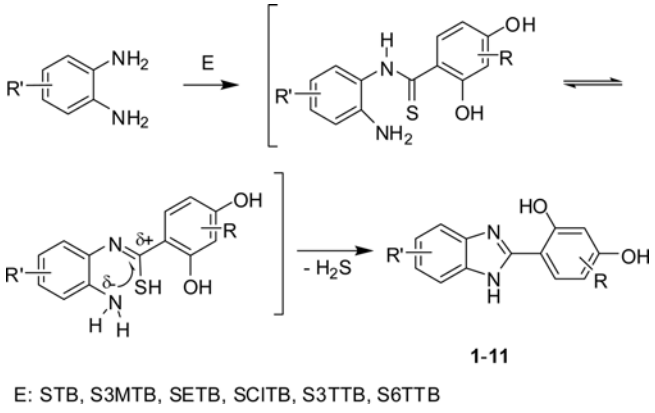
RESULTS AND DISCUSSION

4-(1*H*-Benzimidazol-2-yl)benzene-1,3-diols were produced by the reaction of benzene-1,2-diamines with a series of aryl-modified thioacylating reagents, i.e. sulfinylbis[(2,4-dihydroxyphenyl)methanethione]s: STB, S3MTB, SETB, SClTB, S3TTB, and S6TTB (Scheme 1). Their specifically substituted aromatic rings can produce electrophilic activation of a thiocarbonyl group. Favored thermodynamically released *in situ* carbocations transformed the substrates into monothioamides. In general, the reaction is carried out according to the electrophilic substitution (Scheme 2). The type and manner of the benzene ring substitutions influence the equilibrium states of the thion-thiole rearrangement and the ability to easily release outgoing groups. The properties of the equilibrium thiol-imine groups of the intermediate product were taken into account during development of the synthesis. They allow to reverse a typical acid-base reactivity. The interaction of the ring substituents of benzene-1,2-diamines is also decisive in the synthesis of 1*H*-benzimidazoles. It determines the basicity of amine groups and the structures of the products. Electrophilic reagents were obtained according to the method previously described, i.e. by treatment of the corresponding dithioic acids with $SOCl_2$ (Matysiak and Niewiadomy, 2006).

Purity of compounds was monitored by reversed-phase (RP-18) HPLC chromatography with methanol-water as a mobile phase. There were characteristic ν O-H and ν N-H vibration frequencies between 3450 and 3150 cm^{-1} in the IR spectra of the compounds. The ν C-H and δ C-H bands of ring residues are observed in the wave region between 3060-3038 cm^{-1} and 850-700 cm^{-1}, respectively. C=N asymmetric stretching fre-



Scheme 1. Synthetic pathway for compounds **1-11**



Scheme 2. Reaction mechanism of the formation of 4-(1*H*-benzimidazol-2-yl)benzene-l,3-diols

quencies appear at 1627-1608 cm^{-1}. In the ^{1}H-NMR spectra, the -NH- proton is detected as a broad singlet in the range >13 ppm, similar to other analogues (Refaat, 2010). The resonance bands of protons of hydroxyl groups usually appear in the range of ~11.5 and 10.6 ppm (Tavman and Birteksoz, 2009). They are sometimes invisible in the background of the base line (compounds **4** and **8**). In the ^{1}H-NMR spectra of 5,6-dimethyl-1*H*-benzimidazoles (compounds **2** and **7-11**), there is the characteristic singlet in the range of ~7.55 ppm corresponding to two protons of H-4 and H-7. In the case of 5,6-dichloro-1*H*-benzimidazole, these protons are recorded at 8 ppm (**4**). The resonance signals of the 5-substituted resorcinol moiety appear as characteristic two singlets in the range of ~7.9 and 6.6 ppm, corresponding to H-3 and H-6 protons, respectively (**8** and **9**).

4-(1*H*-Benzimidazol-2-yl)benzene-1,3-diols presented in Scheme 1 were evaluated for their antiproliferative activity against human bladder cancer HCV29T cells. The cytotoxic activity *in vitro* was expressed as IC_{50} [μg/mL], which is the concentration of the compound that inhibited the proliferation rate of tumor cells by 50% as compared to the untreated control cells. Cisplatin was used as a reference drug. Previously described unsubstituted 4-(1*H*-benzimidazol-2-yl)benzene-1,3-diol (bib-1,3-diol) was obtained and used for comparison (Tavman and Birteksoz, 2009). The results of the screening are summarized in Table I. The activity of compounds varied and evidently depended on the type of substitution on both rings. From the group of compounds not containing a modified resorcinol moiety (**1-6**) it is evident that the highest effect against HCV29T cells was exhibited by compound **2**, with two Me substituents in positions 5 and 6 (Table I). Strong antiproliferative activity was also exhibited by compounds **3** and **7** (IC_{50} < 5 μg/mL).

The structure of compound **2** as the most active, was modified in the resorcinol moiety in positions 2, 4 or 6 by the hydrophobic and hydrophilic substituents of the contrary electronic effect (Scheme 1). In this way compounds **7-11** were obtained. Table I reveals that none of new compounds showed better activity than compound **2**.

The selected compounds with the highest activity against HCV29T were also tested against A549 (non-

Table I. Antiproliferative activity of 4-(1*H*-benzimidazol-2-yl)benzene-1,3-diols against human cancer cell line HCV29T expressed as IC_{50} [μg/mL][a]

No.	IC_{50} [μg/mL]
1	22.13 ± 3.28
2	0.53 ± 0.17
3	4.43 ± 0.67
4	6.54 ± 2.23
5	14.34 ± 2.16
6	14.33 ± 2.15
7	2.26 ± 1.29
8	5.92 ± 1.97
9	4.92 ± 0.67
10	23.13 ± 7.62
11	33.20 ± 2.22
bib-1,3-diol[b]	28.50 ± 0.04
cisplatin	2.66 ± 1.15

[a]IC_{50} [μg/mL] indicates the compound concentration that inhibits the proliferation rate of tumor cells by 50% as compared to the untreated control cells. The values are the means ± S.D. of 9 independent experiments.
[b]4-(1*H*-Benzimidazol-2-yl)benzene-1,3-diol previously described (Tavman and Birteksoz, 2009).

Table II. Antiproliferative activity of compounds against the human cancer lines: A549, T47D and SW707 expressed as IC_{50}

No.	IC_{50} [μg/mL]		
	A 549	T47D	SW 707
2	3.51 ± 037	0.65 ± 0.17	1.72 ± 0.67
3	7.64 ± 1.04	4.37 ± 1.84	8.47 ± 3.57
7	4.76 ± 0.30	3.51 ± 0.11	3.39 ± 0.08
8	4.97 ± 0.18	3.41 ± 0.34	4.92 ± 0.08
9	–[a]	5.94 ± 2.56	8.98 ± 0.70
cisplatin	5.29 ± 1.85	2.03 ± 1.61	3.13 ± 1.49

[a]Activity above the studied concentrations

small human lung carcinoma), T47D (human breast cancer), and SW707 (human rectal adenocarcinoma) cells (Table II). Antiproliferative activities of compounds **2**, **7**, **8** against A549, compound **2** against T47D, and compounds **2** and **7** against SW707 cells were similar to those of cisplatin, and compound **2** was the most active against all tested cell lines. It was especially active against HCV29T and T47D cells, showing inhibition at a concentration of 1 μg/mL (2-3 μM).

By analyzing the structures of compounds and their activity, it was found that the presence of a hydrophobic substituent (Cl, Me) or Me(Et)O(CO)- at positions 5, 6 and/or 7 of the 1*H*-benzimidazole ring enhanced antiproliferative activity compared to the unsubstituted parent compound bib-1,3-diol (Table I). Especially beneficial properties were shown for the compounds with two Me substituents in positions 5 and 6 or 6 and 7. The SAR analysis shows that the additional substituent in the resorcinol moiety of 5,6-dimethylbenzimidazoles decreases antiproliferative activity. This was especially evident in the case of the derivatives with the third hydroxyl group (**10**, **11**). This was a general trend observed across different groups of compounds with the 2,4-dihydroxyphenyl substituent. At the same time, this group of derivatives does not confirm the earlier finding that the presence of a Cl-atom or Me (Et, *i*-Pr) substituents in position 5 of the resorcinol moiety of resorcinol azoles improves their anticancer properties (Brough et al., 2005; Kreusch et al., 2005).

In conclusion, a synthetic method for 1*H*-benzimidazoles which offers short reaction times, relatively large-scale synthesis, easy and quick isolation of the products, and good yields has been developed. It provides the opportunity to obtain a wide range of the compounds with (modified) resorcinol substituents, and in this way it makes it possible to add new analogues to the library of 1*H*-benzimidazoles. The presented results of biological studies indicate a high level of antiproliferative activity against human cancer cell lines for several of these compounds (IC_{50} = 2-3 μM). Therefore further studies including design, synthesis, and analysis of subsequent derivatives as well as extension of anticancer activity will be conducted.

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