Res Chem Intermed (2018) 44:6169–6182
https://doi.org/10.1007/s11164-018-3483-0

Synthesis and biological activity of novel benzoazoles, benzoazines and other analogs functionalized by 2,4-dihydroxyphenyl moiety

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Received: 8 December 2017 / Accepted: 22 May 2018 / Published online: 31 May 2018

Abstract Novel benzoazoles, benzoazines, benzothiazoles, benzothiazines and other related compounds possessing a 2,4-dihydroxyphenyl moiety were prepared. The compounds were obtained by the reaction of sulfinylbis[(2,4-dihydroxyphenyl)methanethione]s with the appropriate heterocyclic amines or hydrazines. The structures of the compounds were proved by IR, ^{1}H NMR, and mass spectral data. Human cancer lines, *Candida* species and phytopathogenic fungi were used for the evaluation of biological potency of compounds. Additionally, drug-like properties were estimated in silico.

Keywords Benzothiazine · Benzothiazole · Benzoazine · Antiproliferative activity · Antifungal activity

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Introduction

Nitrogen and sulfur heterocycles have received a great deal of attention in the literature due to their role as active pharmacophores. Benzothiazines are a group of bioactive compounds known to have anticancer, diuretic, and anti-hypertensive activities [1, 2]. The representatives of this heterocyclic class are assumed to possess biological activities since they might provide the heteroatoms as potential hydrogen bond acceptors and the fused phenyl ring for possible π–π interactions. In particular, 1,2-benzothiazine and 1,3-benzothiazine derivatives have exhibited promising pharmacological properties: anti-inflammatory, antitumor and antiproliferative activity [3, 4], as well as antomicrobial activity [5–8]. 4*H*-1,4-benzothiazines exhibited antifungal potency against *Aspergillus fumigates* and *Candida albicans* [9, 10]. The derivatives of 12(*H*)-quino[3,4-*b*][1,4]benzothiazine showed antiproliferative activity against two cancer cell lines, SNB-19 and C-32 [11], while 6-substituted 9-fluoroquino[3,2-*b*]benzo[1,4]thiazines exhibited cytotoxicity as well as antiproliferative actions against human peripheral blood mononuclear cells stimulated with phytohemagglutinin A (PHA) [12].

Coupling of 2-alkylthiobenzimidazole with the β-lactam ring produced compounds with antibacterial and antifungal activities [13]. Attachment of other heterocycles like β-lactam, thiadiazole and oxadiazole to the benzimidazole skeleton resulted in hybrid compounds of potent antibacterial and/or antifungal properties [14–16]. A pyridobenzimidazole derivative with unique activities, called D75-4590, is a specific inhibitor of β-1,6-glucan synthesis and exhibits potent activities against various *Candida* species [17]. The series of methyl-4*H*-1-benzopyran-4-ones carrying mono- or diamidinobenzimidazoles at different positions have been described for antibacterial and antifungal activities [18]. Methylbenzimidazoles are an important class of compounds for cytotoxic activity against human tumour cell lines [19].

Recently, there have been various approaches to study the role of the benzothiazole moiety as antimicrobial [20–23], anticancer [24–27] and antifungal agents [22, 28–30]. It has been shown that some benzothiazole dimers were free from irritation, teratogenicity and sensitivity properties compared to monomers and that the increase in the hydrogen donor count is conductive for cytotoxic activity of derivatives against the HL-60 cell lines [31]. The novel N-alkylbromobenzothiazoles have been evaluated for their anticancer potency [32]. The derivatives of imidazo[2,1-*b*][1,3]benzothiazole have been reported for their strong inhibitory activity against bacterial and fungal strains [33].

Modifications on the heterocycle nucleus resulted in a large number of compounds characterized by diverse pharmacological activities. The biological profiles of new generations of benzothiazoles, benzimidazoles and benzthiazines represent much progress as regards the older compounds. Looking into the medicinal importance of these compounds' moiety, it was thought worth synthesizing some newer derivatives and screening them for their biological activities.

To extend our studies in this area, a set of new heterocyclic analogs with 2,4-dihydroxyphenyl functionality were designed and obtained. Following from previous studies, this group of compounds exhibits strong antifungal and antiproliferative activity [34–39]. This paper presents the in vitro antifungal potency of compounds against the panel phytopathogenic fungi and *Candida* strains as well as cytotoxic potency against human cancer cell lines. Some properties of compounds in silico were estimated to explain the influence of the structure of the compounds on their biological properties.

Experimental

Chemistry

General

Melting points (m.p.) were determined using a BÜCHI B-540 (Flawil, Switzerland) melting point apparatus. The elemental analysis (C, H, N) was performed on a Perkin-Elmer 2400. The IR spectra were measured with a Perkin-Elmer FT-IR 1725X spectrophotometer (in KBr) or a Varian 670-IR FT-IR spectrometer (ATR) in the range of 600–4000 cm^{-1}. The NMR spectra (1D NMR) were recorded in DMSO-d_6 using a Varian Mercury 400, Bruker DRX 500 (Bruker Daltonics, Billerica, MA, USA) or Tesla BS 567A (100 MHz). Chemical shifts (δ, ppm) have been described in relation to tetramethylsilane and coupling constants (J) are expressed in Hz. The MS spectra (EI, 70 eV) were recorded using the apparatus AMD-604.

The purity of the compounds was examined by HPLC Knauer (Berlin, Germany) with a dual pump, a 20-μL simple injection valve and a UV–visible detector (275 and 330 nm). The 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 the aqueous phase (acetate buffer, pH 4, 20 nM). The flow rate was 0.4 ml/min at room temperature. The retention time of an unretained solute (t_o) was determined using KCl. The log k values for 80 or 65% of MeOH (v/v) in the mobile phase are presented. They were calculated as $\log k = \log(t_R - t_o)/t_o$, where t_R is the retention time of a solute, and t_o the retention time of an unretained solute.

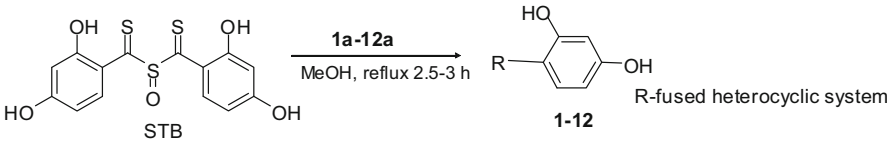


Fig. 1 Synthesis scheme of compounds **1–12**

General procedure for the synthesis of compounds 1–12

A mixture of the corresponding amine or hydrazine (5 mmol) (**1a**–**12a**) and STB (10 mmol) in MeOH (80 mL) (Fig. 1) was heated to reflux for 2.5–3 h. The hot mixture was filtered via a Büchner funnel. Next, the formed solid was filtered off (**2**, **9**–**11**), and either the filtrate was concentrated (compounds **3**–**8**) or water was added to afford the solid (**1**, **12**) which was filtered. Finally, the product was recrystallized from MeOH (compounds **1**, **2**, **5**, **9**, **10**), from MeOH/H_2O (3:1) (**4**, **11**) or from MeOH/H_2O (2:1) (**3**, **6–8**, **12**), respectively.

2-(2,4-Dihydroxyphenyl)-9b-methoxy-[1,3,4]thiadiazolo[2,3-a]isoindol-5(9bH)-one ***1*** IR (KBr) ν_{max}: 3460 (OH), 3024 (C(Ar)-H), 2950 (CH), 1702 (C=O), 1661 (C=N), 1623 (C=N), 1604 (C=C), 1576 (C=C), 1521 (C=C), 1494, 1472, 1452, 1435, 1349, 1322, 1302, 1280, 1217 (C–OH), 1181, 1141, 1123, 1096, 1024, 997, 985, 969, 871, 829, 804, 795, 759, 729, 714 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 11.14 (s, 1 H, HO–C–(2)), 10.08 (s, 1 H, HO–C(4)), 8.08 (d, J = 8.7 Hz, 1 H, H–C(6)), 7.89 (m, 1 H, H–C(Ar)), 7.80 (m, 1 H, H–C(Ar)), 7.72 (td, J = 7.5 and 1.5 Hz, 1 H, H–C(Ar)), 7.68 (td, J = 7.5 and 1.4 Hz, 1 H, H–C(Ar)), 6,51 (d, J = 2.3 Hz, 1 H, H–C(3)), 6.46 (dd, J = 8.7 and 2.3 Hz, 1 H, H–C(5)), 3.71 (s, 3 H, CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ: 167.7 (C=O), 164.2, 163.5, 161.3, 156.4, 131.6, 131.5, 130.8, 130.3, 129.3, 129.0, 128.9, 108.4, 108.2, 102.3, 52.3 (CH_3) ppm; EI-MS (m/z, %): 328 (M^+, 48), 296 (72), 268 (3), 162 (100), 149 (5), 132 (23), 130 (14), 119 (2), 104 (70), 102 (3), 90 (2), 76 (18), 63 (2), 51 (10), 40 (7).

4-(4-Hydroxy-6-methyl-4-phenyl-4H-benzo[d][1,3]thiazin-2-yl)benzene-1,3-diol ***2*** IR (ATR) ν_{max}: 3251 (OH), 2942, 1618 (C=N), 1593 (C=C), 1531, 1490, 1445 (C-H), 1340, 1257, 1209 (C–OH), 1182, 1128, 1053, 1019, 982, 920, 882, 845, 810, 746, 697, 654 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 14.55 (s, 1 H, HO–C(thiazine), 11.82 (s, 1 H, HO–C(3)), 10.90 (s, 1 H, HO–C(1)), 7.85 (d, J = 9.0 Hz, 1 H, H–C(Ar)), 7.74 (m, 1 H, H–C(Ar)), 7.57 (m, 2 H, H–C(Ar)), 7.49 (d, J = 8.77 Hz, 1 H, H–C(Ar)), 7.40 (m, 2 H, H–C(Ar)), 7.25 (m, 1 H, H–C(Ar)), 6.45 (d, J = 8.2 Hz, 1 H, H–C(Ar)), 6.37 (m, 2 H, H–C(Ar)), 1.70 (s, 3 H, CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ: 207.8, 164.7, 163.0 162.9, 162.6, 140.5, 139.5, 136.3, 129.9, 129.7, 128.6, 128.1, 126.3, 119.6, 114.3, 110.9, 108.7, 107.9, 103.2, 57.9, 19.5 (CH_3) ppm; ESI–MS (m/z): 362.1 [[M-H]$^-$].

4-(Perfluorobenzo[d]thiazol-2-yl)benzene-1,3-diol ***3*** IR (KBr) ν_{max}: 3445 (OH), 3350 (OH), 1626 (C=N), 1595 (C=N), 1497 (C=C), 1493, 1333, 1291, 1256, 1228 (C–OH), 1173, 1139 (C–F), 1090, 1041, 1013, 980, 946, 876, 794, 749 cm^{-1}; ^1H NMR (100 MHz, DMSO-d_6) δ: 11.39 (s, 1 H, HO–C(3) (exchangeable in D_2O)), 10.31 (s, 1 H, HO–C(1) (exchangeable in D_2O)), 8.05 (d, J = 8.7 Hz, 1 H, H–C(5)), 6.51 (d, J = 2.3 Hz, 1 H, H–C(2)), 6.49 (m, 1 H, H–C(6)) ppm; EI-MS (m/z, %): 315 (M^+, 100), 259 (7), 258 (32), 226 (4), 144 (6), 69 (3), 40 (5).

4-(6-Amino-5-chlorobenzo[d]thiazol-2-yl)benzene-1,3-diol ***4*** IR (KBr) ν_{max}: 3443 (OH), 3383 (OH), 2928 (CH), 2850 (CH), 1628 (C=N), 1591 (C=N), 1543 (C=C), 1511 (C=C), 1470, 1384, 1345, 1277, 1230, 1121 (C–OH), 1178 (C–Cl), 991, 871,

853, 794, 728 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 11.86 (s, 1 H, HO–C(3)), 10.37 (s, 1 H, HO–C(1)), 8.30 (s, 1 H, H–C(benzothiazole)), 8.21 (m, 2 H, H–C(benzothiazole)), H–C(5)), 6.42 (m, 2 H, H–C(2, 6)) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ: 162.2, 147.2, 135.9, 134.4, 130.1, 128.7, 127.3, 115.8, 112.3 108.1, 106.5, 102.6, 99.5 ppm; EI-MS (m/z, %): 292 (M^+, 100), 258 (5), 223 (6), 176 (18), 153 (30), 147 (5), 124 (4), 97 (6), 69 (5), 53 (3).

4-(5-(5-(2,4-Dihydroxyphenyl)-1,3,4-thiadiazol-2-yl)-1H-benzo[d]imidazol-2-yl)benzene-1,3-diol ***5*** IR (ATR) ν_{max}: 3402 (OH), 3134 (OH), 2940 (OH), 1631 (C=N), 1598 (C=C), 1525 (C=C), 1496, 1469, 1424, 1315, 1301, 1257 (C–OH), 1163, 1139, 1107, 1050, 1018, 981, 964, 846, 800, 752, 691, 659, 633 cm^{-1}. 1H NMR (400 MHz, DMSO-d_6) δ: 11.56 (s, 1 H, OH), 11.28 (s, 1 H, OH), 10.82 (s, 1 H, OH), 10.17 (s, 1 H, OH), 8.39 (d, J = 1.7 Hz, 1 H, H–C(benzoimidazole)(4)), 8.10 (m, 3 H, H–C(Ar)), 7.91 (d, J = 8.4 Hz, 1 H, H–C(6)), 6.75 (d, J = 2.2 Hz, 1 H, H–C(3)), 6.59 (m, 2 H, H–C(2,6)), 6.48 (dd, J = 8.7 and 2.2 Hz, 1 H, H–C(5)) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ: 164.7, 161.1, 160.3, 157.3, 156.6, 155.6, 132.0, 130.8, 130.6, 129.3, 127.7, 121.2, 120.7, 120.6, 118.9, 112.4, 112.2, 108.6, 108.5, 102.6, 56.1 ppm; ESI–MS (m/z): 419 $[M + H]^+$.

4-(5,7,8-Trifluoro-1H-benzo[e][1,3,4]thiadiazin-3-yl)benzene-1,3-diol ***6*** IR (KBr) ν_{max}: 3223 (OH), 3088 (H–C(Ar)), 1653 (C=N), 1615 (C=C), 1516 (C=C), 1488, 1464, 1416, 1395, 1373, 1315, 1262, 1225 (C–OH), 1180, 1168, 1116, 1076, 985, 948, 920, 844, 825, 816, 777, 712 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 11.93 (s, 1 H, HO–C(3)), 10.18 (s, 1 H, HO–C(1)), 8.92 (s, 1 H, NH), 7.89 (d, J = 9.4 Hz, 1 H, H–C(5)), 7.24 (m, 1 H, H–C(benzothiadiazine)), 6.37 (m, 2 H, H–C(2, 6)) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ: 192.0, 162.1, 157.8, 146.8, 144.9, 138.0, 135.9, 133.3, 127.3 113.9, 107.9, 102.9, 96.5 ppm; EI-MS (m/z, %): 312 (M^+, 100), 260 (8), 187 (61), 165 (86), 153 (27), 150 (49), 135 (89), 108 (22), 99 (13), 88 (7), 80 (12), 69 (16), 52 (16), 39 (11).

4-(5,6,7,8-Tetrafluoro-1H-benzo[e][1,3,4]thiadiazin-3-yl)benzene-1,3-diol ***7*** IR (KBr) ν_{max}: 3234 (OH), 1622 (C=N), 1521 (C=C), 1464, 1404, 1347, 1225 (C–OH), 1173, 1125, 1023, 985, 842, 811, 737, 712 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 11.96 (s, 1 H, HO-C(3)), 10.11 (s, 1 H, HO-C(1)), 8.83 (s, 1 H, NH), 7.82 (d, J = 9.4 Hz, 1 H, H–C(5)), 6.35 (d, J = 2.3 Hz, 1 H, H–C(2)), 6.33 (m, 1 H, H–C(6)) ppm; EI-MS (m/z, %): 330 (M^+, 100), 318 (6), 312 (7), 243 (3), 196 (52), 183 (54), 168 (54), 155 (24), 153 (24), 135 (100), 124 (13), 108 (29), 99 (10), 80 (14), 77 (6), 69 (23), 63 (9), 52 (19), 44 (11), 39 (14).

4-(6,8-Dichloro-1H-benzo[e][1,3,4]thiadiazin-3-yl)benzene-1,3-diol ***8*** IR (KBr) ν_{max}: 3397 (OH), 3214 (OH), 3063 (C(Ar)-H), 1626 (C=N), 1601 (C=C), 1509 (C=C), 1447, 1382, 1352, 1332, 1265, 1231 (C–OH), 1179, 1127, 1106, 1077, 986, 944, 887, 861, 851, 824, 799, 774, 725 cm^{-1}; ^1H NMR (100 MHz, DMSO-d_6) δ: 12.10 (s, 1 H, HO-C(3) (exchangeable in D_2O)), 10.21 (s, 1 H, HO-C(1) (exchangeable in D_2O)), 8.05 (d, 1 H, H–C(5)), 7.50 (m, 2 H, H–C(benzothiadiazine) (6, 8)), 6.38 (m, 2 H, H–C(2, 6)) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.7, 157.1, 152.3, 146.3, 138.7, 134.0, 128.7, 126.4, 124.5, 113.2, 109.3, 107.9,

102.6 ppm; EI-MS (m/z, %): 327 (M^+, 84), 294 (3), 210 (4), 195 (37), 167 (9), 158 (9), 153 (100), 136 (21), 124 (7), 108 (5), 97 (8), 69 (5), 63 (4), 52 (5), 39 (4).

2-(2,4-Dihydroxyphenyl)-4H-benzo[d][1, 3]thiazin-4-one 9 (previously the compound was obtained from 2-aminobenzanilide [5])

4-(1H-Perimidin-2-yl)benzene-1,3-diol ***10*** IR (KBr) ν_{max}: 3412 (OH), 3057 (C(Ar)-H), 2846 (CH), 1622 (C=N), 1533 (C=C), 1482, 1446, 1300, 1229 (C–OH), 1150, 1032, 891, 843, 815, 791, 717 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.60 (s (broad), 2 H, H–C(1, 3), 10.39 (s, 1 H, NH), 7.73 (d, J = 8.7 Hz, 1 H, H–C(5)), 7.25 (m, 4 H, H–C(Ar)), 6.80 (m, 2 H, H–C(Ar)), 6.47 (d, J = 2.1 Hz, 1 H, H–C(2)), 6.44 (dd, J = 8.7 and 2.3 Hz, 1 H, H–C(6)) ppm; EI-MS (m/z, %): 276 (M^+, 100), 247 (6), 229 (4), 219 (15), 206 (27), 166 (5), 152 (3), 140 (7), 138 (5), 115 (7), 88 (3), 69 (2), 63 (3), 51 (2), 39 (2), 36 (6).

4-(Benzimidazo[1,2-c]chinazolin-6-yl)benzene-1,3-diol ***11*** IR (KBr): ν_{max} = 3273 (OH), 1640 (C=N), 1620 (C=N), 1600 (C=C), 1521 (C=C), 1449, 1341, 1301, 1230, 1193, 1120, 979, 818, 771 cm^{-1}; ^{1}H NMR (100 MHz, DMSO-d_6) δ: 10.03 (s, 1 H, HO–C(3)), 9.93 (s, 1 H, HO–C(1)), 8.65 (dd, J = 7.9 and 1.3 Hz, 1 H, H–C(Ar)), 7.97 (dd, J = 7.9 and 1.1 Hz, 1 H, H–C(Ar)), 7.93 (d, J = 8.1 Hz, 1 H, H–C(5)), 7.89 (td, J = 8.3 and 1.5 Hz, 1 H, H–C(Ar)), 7.77 (td, J = 8,1 and 1.1 Hz, 1 H, H–C(Ar)), 7.49 (td, J = 7.9 and 1.2 Hz, 1 H, H–C(Ar)), 7.36 (d, J = 8.3 Hz, 1 H, H–C(Ar)), 7.26 (td, J = 7.9 and 1.1 Hz, 1 H, H–C(Ar)), 6.83 (d, J = 8.4 Hz, 1 H, H–C(Ar)), 6.59 (d, J = 2.2 Hz, 1 H, H–C(2)), 6.53 (dd, J = 8.2 and 2.2 Hz, 1 H, H–C(6) ppm; EI-MS (m/z, %): 327 (M^+, 92), 326 (100), 310 (13), 298 (6), 281 (5), 280 (5), 270 (5), 256 (5), 163 (8), 141 (3), 135 (4).

4-(3-Chlorodibenzo[b,g][1,4,6]oxathiazocin-6-yl)benzene-1,3-diol ***12*** IR (KBr): ν_{max} = 3255 (OH), 3065 (OH), 1678 (C=N), 1619 (C=N), 1594 (C=N), 1500 (C=C), 1472 (C=C), 1452, 1385, 1343, 1308, 1255, 1127, 1099, 1057, 1007, 988, 836, 807, 749 cm^{-1}; ^{1}H NMR (100 MHz, DMSO-d_6) δ: 11.88 (s, 1 H, HO–C(3)), 10.25 (s, 1 H, HO–C(1)), 8.32–8.09 (m, 1 H, H–C(Ar)), 7.75–7.68 (m, 1 H, H–C(Ar)), 7.48–7.18 (m, 4 H, H–C(Ar)), 6.95–6.70 (m, 2 H, H–C(Ar)), 6.43–6.34 (m, 2 H, H–C(2, 6)) ppm; EI-MS (m/z, %): 369 (M^+, 10), 333 (5), 257 (11), 253 (100), 244 (89), 220 (26), 218 (82), 202 (6), 192 (13), 183 (6), 162 (28), 137 (83), 109 (12), 91 (9), 80 (60), 77 (5), 69 (7), 65 (15) 63 (16) 53 (9), 52 (7), 44 (6), 39 (11), 36 (4).

Computational methods

The Clog P values and molar refractivity MR were calculated using the ChemDraw Ultra 10.0 according to the fragmentation method introduced by Crippen [40]. The tPSA was calculated by Virtual Computational Chemistry Laboratory [41]. The polar surface area (tPSA) was estimated by the atom-based method [42].

Biological assays

Antiproliferative assay

The following human cell lines were applied in the antiproliferative assay in vitro: T47D (breast cancer), SW707 (rectal adenocarcinoma), and A549 (nonsmall cell lung carcinoma) from the American Type Culture Collection (Rockville, MD, USA), and HCV29T (bladder cancer) from the Fibiger Institute, Copenhagen, Denmark. The SRB test measuring the cell proliferation inhibition in the in vitro culture was applied [43]. The experiments were repeated at least 3 times. The IC_{50} values were calculated by Cheburator 0.9.0 software [44]. The details were described previously [45].

Activity of the compounds against phytopathogenic fungi

In vitro tests estimating the inhibition of mycelium growth in the agar culture medium caused by the compound under investigation were performed. The five strains of phytopathogenic fungi: *Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium culmorum*, and *Phytophthora cactorum* were used. The solutions (suspensions) were prepared with concentrations making it possible to obtain 200 and 20 μg/mL of the studied substance after dilution with the agar culture medium (PDA). Petri scale pans were used into which the agar culture medium and the studied substance were poured. When the culture medium set, the infectious material of the tested fungus in the form of agar discs overgrown with mycelium were placed at three sites on its surface. After 3–5 days (temperature 22 ± 1 °C) depending on the mycelium culture, the linear growth of the mycelium was measured. The compound action was determined from the percentage of mycelium growth inhibition compared with the control using the equation: $J = (C - T)/C\ 100\%$, where J is the percentage of mycelium growth inhibition, C the zone of mycelium growth in the control combination (mm), and T the zone of the mycelium growth in the combination with the compound (mm). Carbendazim (Sarfun 500 SC; Organica, Chemical Comp., Nowa Sarzyna, Poland) and procymidone (Sumilex 500 SC; Sumitomo Chemical Comp, Japan) were used as standards. The results are given in the five-degree scale, determining the percentage of mycelium growth inhibition compared with the control. Biological studies were carried out in the Institute of Industrial Organic Chemistry in Warsaw with the SPR/BFF/01/b procedures (certificate GLP-OECD—1997) [36].

Antifungal activity of the compounds against the fungi Candida

Fifty strains of *Candida albicans* taken from the mouth cavity of patients suffering from tumors were used as the selective material. Itraconazole and fluconazole were administered for prophylaxis or due to the symptoms of candidosis. The isolates resistant to the antifungal drugs were chosen for the dilution method testing the compounds. In addition, the strain was used for comparison with the American Type

Culture Collection (University Boulevard, Manassas, VA, USA) *C. albicans* (ATCC 10231).

Six isolates of non-*albicans Candida* (two isolates of *C. tropicalis*, one each of *C. glabrata*, *C. krusei*, *C. paratropicalis*, and *C. tropicalis*) ($n = 6$) were tested. The drug resistance by Fungitest® was also determined for them. The MIC values were determined by the agar dilution procedure according to the National Committee for Clinical Laboratory Standards reference document M27 [46]. The details were presented by Matysiak et al. [47]. Itraconazole (Pliva, Krakow, Poland) and fluconazole (Janssen-Cilag) tested under the same experimental conditions were used as the references. MIC values were expressed as the average value from 10 measurements for *C. albicans* and from 6 for non-*albicans Candida*.

Results and discussion

Chemistry

Using the reaction with sulfinylbis[(2,4-dihydroxyphenyl)methanethione] (**STB**) suitable nitrogen bases—amines, hydraznes or hydrazides—new compounds with an additional heterocyclic ring have been obtained (Fig. 1; Table 1). Initially, the process was carried out according to the electrophilic substitution to lead to the corresponding thioamides. The final compounds were afforded by the elimination of HF molecule (compounds **3**, **4**, **6**, **7**) or HCl (**8**, **12**) leading to thiazole or thiazine rings, respectively. Formation of an azine ring was an effect of H_2S elimination (compounds **5**, **10**, **11**). In the case of the substrates possessing the carbonyl group, cycloaddition took place and a thiazine or thiazole ring was obtained with the additional hydroxyl group (**1**, **2**). Formation of the fused 4*H*-3,1-benzothizin-4-on system was an effect of the elimination of the corresponding amine (**9**) [5].

The synthesis was performed in methanol under reflux (2–3 h) with moderate to good yields (64–88%), as outlined in Fig. 1. The composition and properties of products **1**–**12** are summarized in Table 2. Purity of the compounds was monitored by the reversed-phase HPLC (CP-18, methanol–water mobile phase). The log k values of the compounds for the selected system are collected in Table 2. The other analytical data of the compounds confirmed the proposed constructions.

The OH substituents of the compounds were usually registered as broad singlets at ca. 10–12.5 ppm by ^{1}H NMR spectroscopy. OH protons were exchangeable in D_2O. Similar data for other derivatives with the benzenediol moiety were obtained [40]. A strong band in the region of about 1630–1600 cm^{-1} was recorded which corresponds to the ν (C=N) bond in the IR spectra. The bands in the range of ca. 3500–3150 cm^{-1} confirmed the presence of OH groups. The C=O moiety was observed for compounds **1** and **10**. In the case of compounds **2** and **5**, the carbonyl group of the substrates was not observed. In the EI-MS, the molecular ion peak M^+ of varying intensity was registered.

Table 1 Structures of the compounds and substrates applied in the synthesis

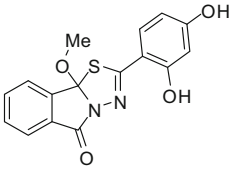
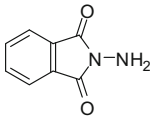
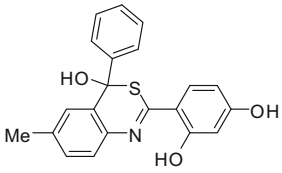
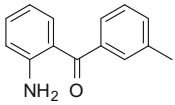
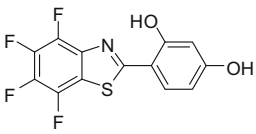
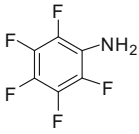
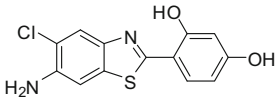
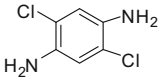
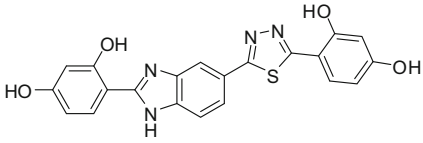
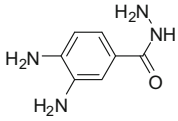
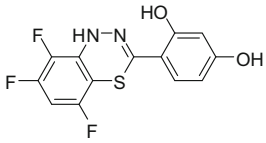
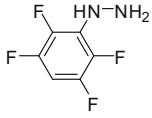
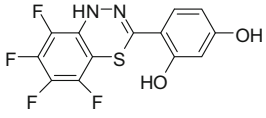
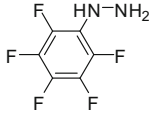
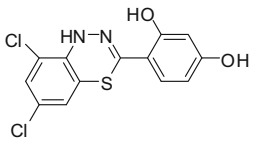
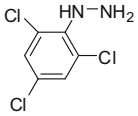
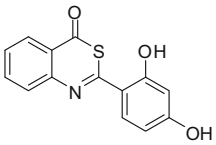
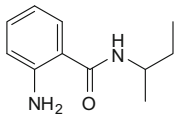
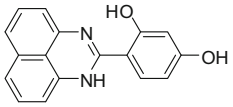
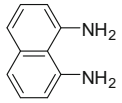
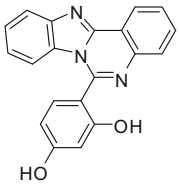
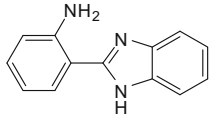
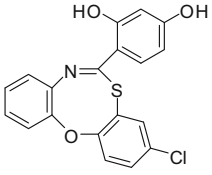
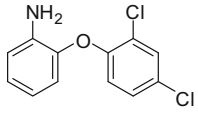
No.	Product	No.	Substrate	Yield (%)
1.		1a		77
2.		2a		62
3.		3a		65
4.		4a		65
5.		5a		70
6.		6a		73
7.		7a		77

Table 1 continued

No.	Product	No.	Substrate	Yield (%)
8.		8a		69
9.		9a		79
10.		10a		80
11.		11a		70
12.		12a		80

Biological activity

Cytotoxic activity in vitro of two compounds, **3** and **11**, was assessed against four human cancer cells. Their potency was expressed as IC_{50} [μM] which is the concentration of the substance that inhibited the proliferation rate of tumour cells by 50% as compared to the untreated control cells. The results of the tests are presented in Table 3. They show that both compounds display substantial activity against all studied cells, similar to that of cisplatin.

Antifungal potency of the selected compounds was assessed in vitro against five strains of phytopathogenic fungi (Table 4). The research was carried out at two different concentrations: 20 and 200 μg/mL. They show that the antifungal potency of compounds is varied, and that the highest activity was found for compound **7** with the 5,6,7,8-tetrafluoro-1*H*-benzo[*e*][1,3,4]thiadiazine ring. At the concentrations of 200 and 20 μg/mL the derivative showed fungistatic action against *A.*

Table 2 Characterization data of compounds **1–12**

No.	Formula	*M*	Elemental analysis (calcd.)/(found)/ %			M.p. °C	log *k*
			C	H	N		
1.	$C_{16}H_{12}N_2O_4S$	328.34	58.53	3.68	8.53	193–195	0.027[b]
			58.60	3.66	8.56		
2.	$C_{21}H_{17}NO_3S$	363.43	69.40	4.71	3.85	123–124	− 0.277[b]
			69.51	4.70	3.87		
3.	$C_{13}H_5F_4NO_2S$	315.24	49.53	1.60	4.44	197–199	0.313[b]
			49.60	1.59	4.46		
4.	$C_{13}H_9N_2O_2SCl$	292.74	53.34	3.10	9.57	246–248	0.440[b]
			53.46	3.09	9.60		
5.	$C_{21}H_{14}N_4O_4S$	418.43	60.28	3.37	13.39	220–221	− 0.357[a]
			60.21	3.35	13.43		
6.	$C_{13}H_7F_3N_2O_2S$	312.27	50.00	2.26	8.97	146–147	− 1.107[a]
			49.88	2.27	9.00		
7.	$C_{13}H_6F_4N2O_2S$	330.25	47.28	1.83	8.48	123–125	− 0.570[a]
			47.35	1.84	8.44		
8.	$C_{13}H_8N_2O_2SCl$	327.19	47.72	2.46	8.56	185–186	− 0.402[a]
			47.80	2.45	8.59		
10.	$C_{17}H_{12}N_2O_2$	276.29	73.90	4.38	10.14	210–212	− 0.175[a]
			74.01	4.40	10.10		
11.	$C_{20}H_{13}N_3S$	327.10	73.38	4.00	12.84	294–296	− 0.091[a]
			73.30	4.02	12.80		
12.	$C_{19}H_{12}ClNO_3S$	369.82	61.71	3.27	3.45	210–212	− 0.175[a]
			59.70	3.25	3.47		

[a]The log *k* values for 80% of MeOH (v/v) in the mobile phase

[b]The log *k* values for 65% of MeOH (v/v) in the mobile phase

Table 3 Antiproliferative activity of compounds **3** and **11** against human cancer cell lines

Compound	Cell line/IC_{50}^{a}/(μM)			
	HCV29T	A 549	T 47D	SW 707
3.	28.30 ± 8.63	19.38 ± 3.87	16.94 ± 5.14	19.60 ± 4.54
11.	7.28 ± 2.04	5.56 ± 3.73	10.73 ± 0.80	13.85 ± 0.61
Cisplatin	10.33 ± 3.20	22.57 ± 17.89	21.56 ± 7.57	34.77 ± 16.53

[a]IC_{50} indicates the compound concentration that inhibits the proliferation rate of tumor cells by 50% as compared to the control untreated cells. The values are the mean ± SD of nine independent experiments

Table 4 Antifungal potency of compounds against phytopathogenic fungi

Compound	Estimation of mycelium growth inhibition[a]/(μg/mL)									
	A. alternata		*B. cinerea*		*R. solani*		*F. culmorum*		*P. cactorum*	
	200	20	200	20	200	20	200	20	200	20
2.	1	0	0	0	0	0	0	0	0	0
4.	2	1	2	1	0	0	2	0	2	1
5.	0	0	0	0	1	0	0	0	0	0
7.	2	1	2	1	2	0	2	1	2	0
9.	2	1	2	0	2	0	0	0	1	0
10.	0	0	0	0	0	0	0	0	0	0
Carbendazim	–[b]	–[b]	–[b]	–[b]	3	3	3	3	3	3
Procymidone	2	2	3	3	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]

[a]The results are given in the five-degree scale determining the percentage of mycelium growth inhibition compared with the control: 0 = 0–20%; 1 = 21–40%; 2 = 41–60%; 3 = 61–80%; 4 = 81–100%

[b]Study was not performed

alternate, *P. cactorum* and *B. cinerea* at the levels of 41–60% and 21–40%, respectively. The compounds under consideration show lower potency compared to the previously described analogs with the 2,4-dihydroxyphenyl moiety [36].

Antifungal activity of the compounds against pathogenic fungi was also estimated. It was expressed as the average MIC values against several clinical strains of *C. albicans* and non-*albicans Candida* as well as against the reference strain of *C. albicans* (Table 5). The results show that the non-*albicans Candida*

Table 5 Antifungal activity against *C. albicans* and non-*albicans Candida* strains expressed in the average MIC values (μg/mL)[a]

Compound	*C. albicans* ATCC 10231[b]	*C. albicans*[c] (n = 10)	Non-*albicans Candida*[d] (n = 6)
1.	100.0 ± 0.0	95.0 ± 15.8	75.0 ± 38.7
4.	200.0 ± 0.0	200.0 ± 0.0	83.3 ± 60.6
6.	100.0 ± 0.0	90.0 ± 45.9	23.96 ± 15.0
7.	50.0 ± 0.0	50.0 ± 0.0	47.9 ± 30.01
8.	200.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
12.	100.0 ± 0.0	70.0 ± 25.2	83.3 ± 60.6
Itraconazole	200.0 ± 0.0	47.5 ± 7.9	27.5 ± 7.9
Fluconazole	200.0 ± 0.0	27.5 ± 7.9	12.5 ± 0.0

[a]*MIC* the minimal inhibitory concentration caused full inhibition of growth in relation to the control (μg/mL)

[b]MIC ± SD (mean ± standard deviation of three independent experiments)

[c]MIC ± SD (MIC values expressed as the average value from 10 isolates ± standard deviation)

[d]MIC ± SD (MIC values expressed as the average value from 6 isolates ± standard deviation)

Table 6 Molecular descriptors in silico of compounds

Compound	HBD	HBA	C logP	MlogP	NR	RB	tPSA (Å^2)	MR (cm^3/mol)
1.	2	6	2.00	1.96	4	2	82.36	84.67
2.	3	4	3.39	2.91	4	2	73.05	104.09
3.	2	3	3.36	2.93	3	1	53.35	68.76
4.	4	4	3.21	1.86	3	1	79.37	76.57
5.	5	8	2.81	1.33	5	3	135.38	115.72
6.	3	4	1.92	2.80	3	1	64.85	72.74
7.	3	4	1.92	2.93	3	1	64.85	73.14
8.	3	4	2.99	2.93	3	1	64.85	80.73
9.	2	4	1.29	1.78	3	1	69.89	71.81
10.	3	4	4.49	3.00	4	1	64.85	80.43
11.	2	5	4.12	3.58	5	1	70.65	93.19
12.	2	4	5.89	3.08	4	1	66.49	97.34

HBD the number of H-bond donors, *HBA* the number of H-bond acceptors, *Clog P* the octanol–water partition coefficients, *MlogP* the Moriguchi octanol–water partition coefficient, *RB* the number of rotatable bonds, *NR* the number of rings; *tPSA* the polar surface area, *MR* the molar refractivity

clinical isolates are more sensitive to the considered compound than those of *C. albicans*. The strongest antifungal activity was observed for compound **7**.

To estimate drug-likeness of the compounds, their molecular descriptors were calculated in silico (Table 6) and Lipinki's rule of five was applied [48]. The partition coefficient of compounds was determined in two different ways: log P and clog P (Table 6). The results show that the compounds with the exception of compound **12** meet the Lipinki's criterion and possess drug-like properties.

References

1. R. Fringuelli, L. Milanese, F. Schiaffella, Mini Rev. Med. Chem. **5**, 1061 (2005)
2. J. Wang, Q.L. Xu, J.M. Li, E.L. Zhang, M.H. Hu, W.F. Ye, W.J. Huang, Chin. J. Org. Chem. **34**, 2040 (2014)
3. S.I. Mohammed, B.A. Craig, A.J. Mutsaers, N.W. Glickman, P.W. Snyder, A.E. deGortari, D.L. Schlittler, D.L. Schlittler, K.T. Coffman, P.L. Bonney, D.W. Knapp, Mol. Cancer Ther. **2**, 183 (2003)
4. A.N. Matralis, E.I. Bavavea, S. Incerpi, J.Z. Pedersen, A.P. Kourounakis, Curr. Med. Chem. **24**, 1214 (2017)
5. J. Matysiak, Bioorg. Med. Chem. **14**, 2613 (2006)
6. D.A. Lega, V.P. Chernykh, L. Zaprutko, A.K. Gzella, L.A. Shemchuk, Chem. Heterocycl. Compd. **53**, 219 (2017)
7. M. Shafiq, I.U. Khan, M.N. Arshad, W.A. Siddiqui, Asian J. Chem. **23**, 2101 (2011)
8. B.S. Rathore, M. Kumar, Bioorg. Med. Chem. **14**, 5678 (2006)
9. B.J. Khairnar, R.S. Salunke, P.B. Patil, S.A. Patil, R.J. Kapade, P.S. Girase, B.R. Chaudhari, E-J. Chem. **9**, 318 (2012)

10. S. Mor, P. Pahal, B. Narasimhan, Eur. J. Med. Chem. **53**, 176 (2012)
11. A. Zieba, M. Latocha, A. Sochanik, Med. Chem. Res. **22**, 4158 (2013)
12. M. Jelen, K. Pluta, M. Zimecki, B. Morak-Mlodawska, J. Artym, M. Kocieba, Eur. J. Med. Chem. **89**, 411 (2015)
13. K.G. Desai, K.R. Desai, Bioorg. Med. Chem. **14**, 8271 (2006)
14. C. Gill, G. Jadhav, M. Shaikh, R. Kale, A. Ghawalkar, D. Nagargoje, M. Shiradkar, Bioorg. Med. Chem. Lett. **18**, 6244 (2008)
15. K.F. Ansari, C. Lal, J. Chem. Soc. **121**, 1017 (2009)
16. K.F. Ansari, C. Lal, Eur. J. Med. Chem. **44**, 4028 (2009)
17. A. Kitamura, K. Someya, M. Hata, R. Nakajima, M. Takemura, Antimicrob. Agents Chemother. **53**, 670 (2009)
18. H. Goker, D.W. Boykin, S. Yildiz, Bioorg. Med. Chem. **13**, 1707 (2005)
19. C.B. Zhang, Y. Liu, Z.F. Liu, S.Z. Duan, M.Y. Li, W. Chen, Y. Li, H.B. Zhang, X.D. Yang, Bioorg. Med. Chem. Lett. **27**, 1808 (2017)
20. B. Soni, M.S. Ranawat, R. Sharma, A. Bhandari, S. Sharma, Eur. J. Med. Chem. **45**, 2938 (2010)
21. S. Rubino, R. Busa, A. Attanzio, R. Alduina, V. Di Stefano, M.A. Girasolo, S. Orecchio, L. Tesoriere, Bioorg. Med. Chem. **25**, 2378 (2017)
22. S. Maddila, S. Gorle, N. Seshadri, P. Lavanya, S.B. Jonnalagadda, Arab. J. Chem. **9**, 681 (2016)
23. M. Singh, S.K. Singh, M. Gangwar, G. Nath, S.K. Singh, RSC Adv. **4**, 19013 (2014)
24. A. Belal, M.A. Abdelgawad, Res. Chem. Intermediat. **43**, 3859 (2017)
25. A.K. El-Damasy, J.H. Lee, S.H. Seo, N.C. Cho, A.N. Pae, G. Keum, Eur. J. Med. Chem. **115**, 201 (2016)
26. B. Mavroidi, M. Sagnou, K. Stamatakis, M. Paravatou-Petsotas, M. Pelecanou, C. Methenitis, Inorganica Chim. Acta **444**, 63 (2016)
27. G.J. Kumar, S.N. Kumar, D. Thummuri, L.B.S. Adari, V.G.M. Naidu, K. Srinivas, V.J. Rao, Med. Chem. Res. **24**, 3991 (2015)
28. S.Z. Zhao, L.Y. Zhao, X.Q. Zhang, C.C. Liu, C.Z. Hao, H.L. Xie, B. Sun, D.M. Zhao, M.S. Cheng, Eur. J. Med. Chem. **123**, 514 (2016)
29. N.H. Cano, M.S. Ballari, A.G. Lopez, A.N. Santiago, J. Agric. Food Chem. **63**, 3681 (2015)
30. Y. Liu, Y. Wang, G.Q. Dong, Y.Q. Zhang, S.C. Wu, Z.Y. Miao, J.Z. Yao, W.N. Zhang, C.Q. Sheng, Medchemcomm **4**, 1551 (2013)
31. S. Gupta, N. Ajmera, N. Gautam, R. Sharma, D.C. Gautam, Indian J. Chem. Sect B **48**, 853 (2009)
32. R.K. Gill, G. Singh, A. Sharma, P.M.S. Bedi, A.K. Saxena, Med. Chem. Res. **22**, 4211 (2013)
33. T.H. Al-Tel, R.A. Al-Qawasmeh, R. Zaarour, Eur. J. Med. Chem. **46**, 1874 (2011)
34. J. Matysiak, A. Niewiadomy, B. Senczyna, A. Zabinska, J.K. Rozylo, J. AOAC Int. **87**, 579 (2004)
35. J. Matysiak, A. Skrzypek, M. Karpinska, U. Glaszcz, A. Niewiadomy, B. Paw, B. Senczyna, J. Wietrzyk, D. Klopotowska, J. Enzyme Inhib. Med. Chem. **31**, 166 (2016)
36. J. Legocki, J. Matysiak, A. Niewiadomy, M. Kostecka, J. Agric. Food Chem. **51**, 362 (2003)
37. J. Matysiak, A. Niewiadomy, B. Paw, I. Dybala, Arch. Pharm. (Weinheim) **344**, 340 (2011)
38. J. Matysiak, R. Los, A. Malm, M.M. Karpinska, U. Glaszcz, B. Rajtar, M. Polz-Dacewicz, M. Trojanowska-Wesolowska, A. Niewiadomy, Arch. Pharm. (Weinheim) **345**, 302 (2012)
39. J. Matysiak, A. Niewiadomy, Bioorg. Med. Chem. **11**, 2285 (2003)
40. A.K. Ghose, G.M. Crippen, J. Chem. Inf. Comput. Sci. **27**, 21 (1987)
41. http://www.vcclab.org
42. P. Ertl, B. Rohde, P. Selzer, J. Med. Chem. **43**, 3714 (2000)
43. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl Cancer Inst. **82**, 1107 (1990)
44. D. Nevozhay, PLoS ONE **9**, e106186 (2014)
45. J. Matysiak, A. Opolski, Bioorg. Med. Chem. **14**, 4483 (2006)
46. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard. CLSI document M27-A. (Clinical and Laboratory Standards Institute, Wayne, 1997)
47. A. Niewiadomy, A. Skrzypek, J. Matysiak, U. Glaszcz, J. Wietrzyk, E. Krajewska-Kulak, Acta Pol. Pharm. **72**, 943 (2015)
48. C.A. Lipinski, Drug Discov. Today Technol. **1**, 337 (2004)