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Original article

Synthesis and antiproliferative activity of some 5-substituted 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles

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Abstract

A series of new 5-substituted 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles has been synthesised and evaluated for their antiproliferative activity. The compounds were prepared by the reaction of the sulphinylbis(2,4-dihydroxythiobenzoyl) (STB) wit hydrazides or carbazates. The panel substitution included alkyl, alkoxy, aryl and heteroaryl derivatives. The structures of compounds were identified from the elemental, IR, ^{1}H NMR and MS spectra analysis. The highest antiproliferative activity against the cells of human cancer lines for 2-(2,4-dihydroxyphenyl)-5-(4-methoxybenzyloxy)-1,3,4-thiadiazole was found with ID_{50} values comparable (HCV29T and SW707) or significantly lower (T47D) than for cisplatin applied as the reference compound. The influence of 5-substiution type of 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles on antiproliferative activity is discussed.

Keywords: 2-(2,4-Dihydroxyphenyl)-1,3,4-thiadiazoles; Synthesis; Antiproliferative activity; In vitro studies

1. Introduction

Cancer remains a major public health issue at the beginning of the 21st century.

Chemotherapy is one of the ways to fight against cancer. Significant side effects such as nausea, vomiting, diarrhoea, hair loss, serious infections (mostly due to leucopoenia) and others often are accompanying chemotherapy. Therefore, the need for accelerated development of new, more effective as well as less toxic chemotherapeutic agents has appeared.

1,3,4-Thiadiazoles have been of great interest as antitumour compounds for several scores of years. At first the attention was focused on 2-aminothidiazole (ATDA) and its simple derivatives [1–6]. ATDA was introduced into phase II clinical trials in patients with different tumours: renal [7], colon [8], ovarian [9] and others [10–12]. However, due to stomatitis and hyperuricemia, the clinical applicability of this agent was limited [13–16].

Than, much more complex structures based on 1,3,4-thiadiazole ring were synthesised and tested. They included among others, the derivatives of the fused system like imidazo[2,1b]-1,3,4-thiadiazole [17,18]. For this type of compounds, the activity against 60 lines of human cancer cells was found in in vitro conditions. High potency of (E,E)-2,5-bis[4-(3-dimethylaminopropoxy)styryl]-1,3,4-thiadiazole against A549 lung cancer was demonstrated [19]. Some Fe(II)/Fe(III) complexes containing 1,3,4-thiadiazole derivatives as co-ligands have also been prepared and tested, however without much success [20].

Recently, strong antitumour effects against different melanoma cell lines of 4-phenyl-5-(cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides were found. The compounds decreased the viability and proliferation rate of MEL-85, SK-MEL, A3058 and MEWO cell lines [21–23]. In vivo this effect was confirmed in mice [23].

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Thiadiazole-2-sulphonamide derivatives possessing potent carbonic anhydraze isozyme properties also act as effective in vitro tumour cell growth inhibitors (leukaemia, non-small lung cancer, melanoma, ovarian, renal, prostate and breast cancer cell lines) [24–27].

Acetazolamide (5-acethylamide-1,3,4-thiadiazole-2-sulphonamide) used as diuretic agent might also function as an inhibitor of cancer growth in combination with different cytotoxic agents, such as alkylating agents, nucleosides analogues and platinum derivatives [28].

Variously N-substituted 2-amino-5-(2,4-dihydroxyphenyl-1,3,4-thiadiazoles prepared in our laboratory also exhibit a wide spectrum of antiproliferative activity. Some of them show the ability to inhibit proliferation rate of the tumour cells at a level comparable to that of cisplatin [29]. Extending the research in this area, we decided to obtain the derivatives which contain the substituent at 5-position of 1,3,4-thiadiazole ring combined in different way from that by mean of amine atom of nitrogen [30].

The aim of this work is to investigate the effect of substitution at 5-position of 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles on antiproliferative activity against different human tumour cell lines. A wide range of compounds includes alkyl, aryl, heteroaryl derivatives in which the substituent is directly connected with C-5 of thiadiazole ring or by oxygen atom, $–OCH_2–$ or $–CH_2O–$ moiety.

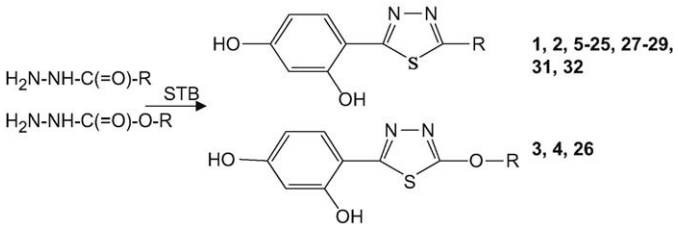
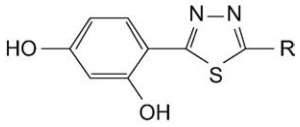


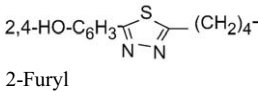
Fig. 1. Synthesis scheme of 5-substituted 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles.

2. Chemistry

5-Substituted 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles were obtained according to Fig. 1. Their chemical structures are shown in Table 1. Compounds were prepared by the reaction of the sulphinylbis(2,4-dihydroxythiobenzoyl) (**STB**) with the commercially available hydrazides (**1**, **2**, **5**–**25**, **27**–**29**, **31**, **32**) or carbazates (compounds **3**, **4**, **26**) in the endocyclising process [30]. **STB** was obtained from 2,4-dihydroxybenzenecarbodithioic acid and $SOCl_2$ in diethyl ether [31]. The synthesis of derivatives **4**, **6**, **17**, **18**, **21**, **26**, **30** and **31** has been previously reported in [30]. Compound **30** was obtained from corresponding amidrazone and **STB** [32]. Purity of compounds was monitored by reversed-phase (RP-18) HPLC chromatography (methanol–water).

Table 1
Structure and antiproliferative activity of 5-substituted 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles against the cells of HCV29T bladder cancer cell line expressed as ID_{50} (μg ml^{-1})[a]



Numbers	Substituent	ID_{50} (μg ml^{-1})[a]	Numbers	Substituent	ID_{50} (μg ml^{-1})
1.	H	34.5 ± 1.5	17.	1-Naphthyl	23.8 ± 1.2
2.	$CH_3(CH_2)_{14}$	NEG[b]	18.	$C_6H_5–CH_2$	35.0 ± 0.5
3.	CH_3O	49.4 ± 2.5	19.	1-Naphthylmethyl	28.0 ± 0.0
4.	C_2H_5O	48.0 ± 14.2	20.	3-F–C_6H_4–OCH_2	39.4 ± 2.5
5.	$HO(CH_2)_3$	43.0 ± 5.2	21.	4-F–C_6H_4–OCH_2	31.8 ± 4.3
6.	C_6H_5	51.0 ± 3.0	22.	4-Cl–(2-CH_3)–C_6H_3–OCH_2	22.8 ± 2.7
7.	4-$(CH_3)_3$ C–C_6H_4	3.7 ± 0.6	23.	2-Cl–C_6H_4–OCH_2	38.5 ± 5.0
8.	2-Br–C_6H_4	29 ± 6.5	24.	2,4-Cl–$C_6H_3OCH_2$	94.9 ± 5.0
9.	3,5-CH_3O–(4-HO)–C_6H_2	36.9 ± 3.0	25.	4-NO_2–C_6H_4–OCH_2	92.7 ± 7.0
10.	3-C_2H_5O–C_6H_4	34.5 ± 0.5	26.	4-CH_3O–C_6H_4–CH_2O	1.1 ± 2.2
11.	4-C_2H_5O–C_6H_4	32.6 ± 1.4	27.	C_6H_5–CH(OH)	21.6 ± 2.3
12.	3-CH_3–(2-HO)–C_6H_3	16.7 ± 12.6	28.		11.7 ± 1.0
13.	2-HO–C_6H_4	34.0 ± 1.4	29.	2-Furyl	39.2 ± 1.
14.	2,4-HO–C_6H_3	33.6 ± 10.0	30.	2-Pyridyl	38.5 ± 1.0
15.	3,5-HO–C_6H_3	46.0 ± 0.0	31.	4-Pyridyl	NEG[b]
16.	4-C_6H_5–C_6H_4	17.1 ± 5.8	32.	Benzo[b]thiophen-2-yl	23.0 ± 1.7
	Cisplatin	0.7 ± 1.5			

[a] ID_{50} (μg ml^{-1}) indicates the compound concentration that inhibits the proliferation rate of tumour cells by 50% as compared to the control untreated cells. The values are the mean ± S.D. of nine independent experiments.

[b] NEG – negative in the studied concentrations (up to 100 μg ml^{-1}).

All these derivatives were characterised by spectral and elemental analysis data which confirmed their structure. Interpretation of ^{1}H NMR spectra were limited to protons connected with heteroatoms and other than aromatic ones. The data show bands in the range 11.95–10.81 and 10.19–9.09 ppm characteristic of 2-COH and 4-COH protons in the resorcinol moiety, respectively. The IR spectra of compounds show absorption bands in the region of 1520, 1490, 1385, 1235, 1040 and 865, which is typical of 1,3,4-thiadiazole ring vibration [33].

Mass spectra of compounds gave molecular ion peaks with different intensities confirming their molecular weights. Compounds **3**, **9** and **22** lack the molecular ion. The fragmentation pathway in all derivatives involved the cleavage of the S–C_5 and N–N bonds of 1,3,4-thiadiazole ring with formation of ion 167 *m*/*z* (Fig. 2). Elimination of sulphur gave ion 135 *m*/*z*. The cleavage of C_2–N_3 and C_5–S bonds was also observed (153 *m*/*z*). Similar fragmentation pattern of 1,3,4-thiadiazole ring were reported by Frański et al. [34]. Other fragmentations depend on the type of C-5 substitution of thiadiazole ring. For example elimination of phenoxy radical with formation of ion 207 *m*/*z* is characteristic of the compounds with phenoxym ethyl substituents (**20**–**25**). The mass fragmentation pathway of compound **23** is shown in Fig. 2.

3. Results and discussion

Compounds of structure presented in Table 1 have been evaluated for their antiproliferative activity against HCV29T cells (human bladder cancer). The cytotoxic activity in vitro was expressed as ID_{50} (μg ml^{-1}), the concentration of compound that inhibit proliferation rate of the tumour cells by 50% as compared to the control untreated cells.

Cisplatin was used as a reference drug. The results of screening are summarised in Table 1. The selected compounds were also tested against A549 cells (human non-small lung carcinoma), T47D cells (human breast cancer) and SW707 cells (human rectal adenocarcinoma) (Table 2).

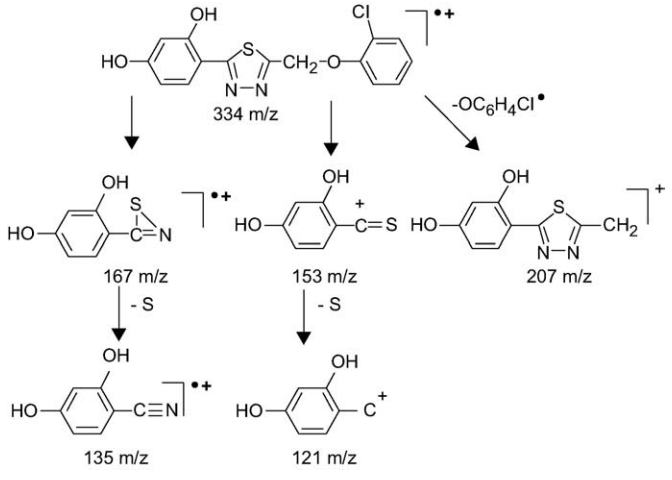


Fig. 2. The mass fragmentation pathway of 5-substituted 2-(2,4-dihydroxyphenyl)-1,34-thiadiazoles (compound **23**).

Table 2
Antiproliferative activity of some 5-substiuted 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles against the cells of human SW707, A549 and T47D lines

Compounds	Cell line/ID_{50} (μg ml^{-1})[a]		
	SW707	A549	T47D
7	4.5 ± 1.0	12.8 ± 5.1	4.0 ± 0.9
8	27.1 ± 3.7	28.5 ± 5.1	9.7 ± 5.3
12	16.7 ± 3.0	5.9 ± 1.1	5.6 ± 2.3
16	5.0 ± 1.6	6.6 ± 0.1	8.8 ± 3.9
17	18.7± 1.5	21.5 ± 1.5	4.8 ± 1.4
19	28.2 ± 0.9	24.5 ± 2.5	14.2 ± 1.9
22	45.7 ± 7.5	32.1 ± 7.3	8.8 ± 0.3
23	36.5 ± 4.0	42.9 ± 4.8	10.4 ± 5.6
26	5.0 ± 5.7	7.9 ± 1.8	3.0 ± 1.5
27	32 ± 1.7	41.4 ± 10.4	42.2 ± 8.1
28	4.7 ± 11	11.6 ± 5.0	NEG[b]
29	NEG[b]	NEG[b]	40.9 ± 1.1
Cisplatin	4.9 ± 1.5	3.3 ± 1.4	6.2 ± 1.5

[a] ID_{50} (μg ml^{-1}) indicates the compound concentration that inhibits the proliferation rate of tumour cells by 50% as compared to the control untreated cells. The values are the mean ± S.D. of nine independent experiments.
[b] NEG – negative in the studied concentrations (up to 100 μg ml^{-1}).

Antiproliferative effect of presented compounds is diverse, however, most of them exhibit weak activity. Derivatives **7** and **26** of different structures prove to be the most active. They exhibited higher inhibitory activity against T47D cells than cisplatin. Compounds **2** and **31** did not reveal any cytotoxic activity (ID_{50} > 100 μg ml^{-1}) against HCV29T, as well as **28** against T47D and **29** against SW707 and A549.

In the terms of structure–activity relationships, alkyl derivatives (**2**–**5**) show weak antiproliferative effect with ID_{50} at the level of 40–50 μg ml^{-1}. Their activity is lower than unsubstituted parent compound (**1**). Derivative **2** with the long hydrophobic pentadecyl chain did not reveal any cytotoxic activity (ID_{50} > 100 μg ml^{-1}) against the cells of HCV29T line (Table 1).

Generally, activity of aryl derivatives (**6**–**17**) is slightly higher as compared with that of alkyl derivatives, but it is still not satisfactory. Phenyl derivative (**6**) exhibits lower activity than compounds with substitution of aryl ring (**7**–**15**). However, the effect of type of aryl ring substituent on the activity is insignificant. Only compound **7** with a large volumetric *tert*-butyl substituent in *para*-position satisfies the cytotoxic activity criterion against breast cancer cells (ID_{50} ~4 μg ml^{-1}), for new synthetic compounds [35].

Joining the aryl ring by means of the –CH_2– (**18**, **19**) or –OCH_2– (**20**–**25**) link, giving possibility of accepting a definitely larger number of conformers by a molecule, does not effect favourably their activity. The reverse effect is observed for compounds **24** and **25**. However, benzyloxy derivative (**26**) is the most active compound of the presented derivatives. It exhibits about two times lower ID_{50} value towards T47D cells than cisplatin, and the activity against HCV29T and SW707 cells is only slightly lower. At the same time, 1-naphthyl (**17**) and 1-naphthylmethyl (**19**) derivatives revealed higher inhibitory effects than corresponding phenyl (**6**) and benzyl (**18**) ones.

Interesting level of activity reveal compound **28** with the double 2-(2,4-dihydroxyphenyl) moiety, especially against

SW707 cells. Substitution of C-5 ring of 1,3,4-thiadiazole directly by mean of the heterocyclic ring does not give the expected effect on tumour growth inhibition (**29**–**32**). The highest activity from this group exhibits benzo[b]thiophen-2-yl derivative (**32**) with ID_{50} 23 μg ml^{-1} against HCV29T cells (Table 2). Some compounds did not reveal any cytotoxic activity (ID_{50} > 100 μg ml^{-1}) (Tables 1 and 2).

The obtained results indicated that for antiproliferative activity of 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles it is not necessary to link the substituent at C-5 by means of amine atom of nitrogen. However, this element is an essential factor of activity as N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles generally exhibited stronger inhibition of proliferation rate of tumour cells. Probably –CH_2–O– group (compound **26**) is also favourable link of the aryl substituent like amine atom of nitrogen. The explicit statement is difficult as it was the only derivative of this kind. However, it may point to the essential function of heteroatom directly linked to C-5 ring of thiadiazole. The presence of another atom of high electronegativity in the vicinity of C-5 ring probably causes formation of a strong electron gap at this atom of carbon which may be essential in the ligand–receptor interactions. Another factor to be considered is possibility of hydrogen bond formation in the compounds of the structures where heteroatom (N or O) is a acceptor of hydrogen atom. However, it should be stated that joining aryl substituents by means of the link of reverse order of atoms (–O–CH_2–) does not promote antiproliferative activity.

4. Experimental protocols

4.1. Chemistry

The melting point (m.p.) was determined using a Sanyo melting point apparatus. The elemental analysis was performed in order to determine C, H and N contents (Perkin–Elmer 2400). Analyses of C, H and N were within ±0.4% of the values. The vibrational spectra were recorded with a Perkin–Elmer FT-IR 1725X spectrophotometer (in potassium bromide). Thy were made in the range of 600–4000 cm^{-1}. ^{1}H NMR spectra were made using the Bruker DRX 500 instrument (500 MHz), internal standard, tetramethylsilane (TMS), solutions in DMSO, shift (ppm). The spectra MS (EI, 70 eV) were recorded using the apparatus AMD-604.

4.1.1. 2-(2,4-Dihydroxyphenyl)-1,3,4-thidiazole (*1*)

Formic acid hydrazide (Lancaster) (0.01 mol) and **STB** (0.0075 mol) were added to methanol (60 ml) and heated to boiling (3 h). During the synthesis there was removed the compound which was filtered, washed with water and crystallised from aqueous (1:1) methanol (60 ml). M.p. 246–247 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.03 (s, 1H, 2-COH) 10.02 (s, 1H, 4-COH), 9.45 (s, 1H, C_{thia}H); IR (KBr) $\tilde{\nu}$: 3113 (OH), 1632 (C=N), 1602 (C=C), 1524, 1472, 1424, 1328, 1265, 1232, 1167 (C–OH), 1133, 1057 (N=C–S–C=N), 980, 966, 937, 915, 833 cm^{-1}; MS (*m*/*z*, %): 194 (M^+, 100), 167 (14), 153 (6), 136 (13), 135 (24) 119 (3), 108 (6), 107 (4), 84 (3), 83 (5), 80 (4), 60 (5). Anal. $C_8H_6N_2O_2S$ (C, H, N).

4.1.2. 2-(2,4-Dihydroxyphenyl)-5-(pentadecyl)-1,3,4-thiadiazole (*2*)

Palmitic acid hydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered. The removed compound from the filtrate was crystallised from methanol (75 ml). M.p. 120–122 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 10.95 (s, 1H, 2-COH), 9.95 (s, 1H, 4-COH), 3.04 (t, 2H, CH_2), 1.7–1.23 (m, 29H, CH_2, CH_3); IR(KBr): 3144 (OH), 2920, 2848 (CH_2), 1685 (C=N), 1602 (C=C), 1469, 1440, 1339, 1317, 1284, 1218, 1189 (C–OH), 1121, 1073, 1015 (N=C–S–C=N) 987, 932, 875, 848, 812 cm^{-1}; MS (*m*/*z*, %) 404 (M^+, 2), 403 (5), 390 (9), 375 (6), 361 (8), 374 (10), 319 (8), 305 (7), 291 (7), 277 (17), 263 (15), 252 (4), 235 (11), 221 (86), 208 (100), 195 (2), 169 (6), 153 (14), 136 (10), 115 (4), 106 (2), 80 (3), 69 (6), 55 (20), 41 (22). Anal $C_{23}H_{36}N_2O_2S$ (C, H, N).

4.1.3. 2-(2,4-Dihydroxyphenyl)-5-methoxy-1,3,4-thiadiazole (*3*)

Methyl carbazate (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from aqueous (5:1) methanol (120 ml). M.p. 195–197 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.58 (s, 1H, 2-COH), 10.17 (s, 1H, 4-COH), 3.64 (s 3H, CH_3); IR (KBr) $\tilde{\nu}$: 3234 (OH), 1697 (C=N), 1624 (C=C), 1591, 1508, 1462, 1356, 1323, 1267, 1234, 1182 (C–OH), 1120, 1042 (N=C–S–C=N), 985, 969, 937, 865, 809 cm^{-1}; MS (*m*/*z*, %): 210 (81), 192 (12), 184 (4), 167 (3), 153 (36), 150 (25), 137 (57), 121 (25), 108 (13), 94 (59), 81 (27), 66 (51), 64 (100), 52 (27), 39 (40). Anal. $C_9H_8N_2O_3S$ (C, H, N).

4.1.4. 2-(2,4-Dihydroxyphenyl)-5-ethoxy-1,3,4-thiadiazole (*4*), 2-(2,4-dihydroxyphenyl)-5-phenyl-1,3,4-thiadiazole (*6*), 2-(2,4-dihydroxyphenyl)-5-(1-naphthyl)-1,3,4-thiadiazole (*17*), 5-benzyl-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (*18*), 2-(2,4-dihydroxyphenyl)-5-(4-fluorophenoxymethyl)-1,3,4-thiadiazole (*21*), 2-(2,4-dihydroxyphenyl)-5-(4-methoxybenzyloxy)-1,3,4-thiadiazole (*26*), 2-(2,4-dihydroxyphenyl)-5-(2-pyridyl)-1,3-4-thiadiazole (*30*) and 2-(2,4-dihydroxyphenyl)-5-(4-pyridyl)-1,3,4-thiadiazole (*31*)

Compounds **4**, **6**, **17**, **18**, **21**, **26**, **30**, **31** were prepared according to the procedure already described in [30,32].

4.1.5. 2-(2,4-Dihydroxyphenyl)-5-(3-hydroxypropyl)-1,3,4-thidiazole (*5*)

4-Hydroxybutyric acid hydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered. The removed compound was filtered, washed by water and crystal-

lised from aqueous (1:1) methanol (60 ml). M.p. 210–212 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 9.68 (s, 1H, 2-COH), 9.08 (s, 1H, 4-COH), 5.01 (broad band, 5H, OH, CH_2), 3.36 (m, 2H, CH_2); IR (KBr) $\tilde{\nu}$: 3296, 3225, 3194 (OH), 2960 (CH_2), 1618 (C=N), 1565 (C=C), 1514, 1363, 1301, 1274, 1206 (C–OH), 1126, 1064, 1013 (N=C–S–C=N), 989, 914, 822 cm^{-1}; EI-MS (*m*/*z*, %): 252 (M^+, 26), 221 (13), 208 (100), 179 (2), 167 (3), 153 (10), 136 (13), 119 (2), 108 (4), 80 (4), 69 (4), 52 (5), 39 (5). Anal. $C_{11}H_{12}N_2O_3S$ (C, H, N).

4.1.6. 2-(2,4-Dihydroxyphenyl)-5-(4-tert-butylphenyl)-1,3,4-thiadiazole (7)

4-Tert-butylbenzhydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from methanol (70 ml). M.p. 243–245 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.13 (s, 1H, 2-COH), 10; (s, 1H, 4-COH), 1.33 (s, 9H); IR (KBr) $\tilde{\nu}$: 3306 (OH), 2959, 2867 (CH_3), 1626 (C=N), 1597 (C=C), 1472, 1445, 1410 1364, 1315, 1251, 1228 (C–OH), 1168, 1441, 1019, 992, 962, 934 cm^{-1} ; MS (*m*/*z*, %): 326 (M^+, 76), 311 (100), 297 (22), 283 (12), 176 (2), 167 (5), 155 (70), 141 (16), 135 (4), 116 (11), 111 (2), 97 (3), 81 (4), 69 (7), 57 (6), 43 (5). Anal. $C_{18}H_{18}N_2O_2S$ (C, H, N).

4.1.7. 5-(2-Bromophenyl)-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (8)

2-Bromobenzhydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered and the filtrate was concentrated to dry. The removed compound was washed with water and crystallised from methanol (80 ml). M.p. 207–209 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.18 (s, 1H, 2-COH) 10.12 (s, 1H, 4-COH); IR (KBr) $\tilde{\nu}$: 3094 (OH), 1631 (C=N), 1595 (C=C), 1562, 1526, 1474, 1430, 1331, 1257, 1177 (C–OH), 1163, 1138, 1126, 1107, 1065, 1025 (N=C–S–C=N), 995, 982, 964, 915 cm^{-1}; MS (*m*/*z*, %): 350, (M^+, 100), 215 (6), 201 (6), 184 (2), 167 (92), 137 (2), 134 (22), 119 (9), 107 (9), 102 (5), 90 (4), 80 (6), 69 (5), 52 (6), 39 (5). Anal. $C_{14}H_9BrN_2O_2S$ (C, H, N).

4.1.8. 2-(2,4-Dihydroxyphenyl)-5-(4-hydroxy-3,5-dimethoxyphenyl)-1,3,4-thiadiazole (9)

4-Hydroxy-3,5-dimethoxybenzhydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (60 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from aqueous (1:1) methanol (60 ml). M.p. 325–327 ° C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.03 (s, 1H, 2-COH), 9.97 (s, 1H, 4-COH), 9.35 (s, H, 4′-COH), 3.81 (s, 6H, CH_3); IR (KBr) $\tilde{\nu}$: 3378 (OH), 2949, 2889 (CH_3), 1613 (C=N), 1532 C=C, 1465, 1419, 1376, 1337, 1300, 1264, 1200 (C–OH), 1157, 1135, 1104, 1000, 979, 961, 918, 898, 851, 836 cm^{-1}; MS (*m*/*z*, %): 304 (7), 303 (17), 302 (100), 273 (3), 168 (6), 167 (37), 153 (11), 151 (4), 138 (8), 135 (7), 119 (4), 107 (5), 106 (2), 80 (3), 69 (2), 39. Anal. $C_{16}H_{14}N_2O_5S$ (C, H, N).

4.1.9. 2-(2,4-Dihydroxyphenyl)-5-(3-ethoxyphenyl)-1,3,4-thiadiazole (10)

3-Ethoxybenzhydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (60 ml) and heated to boiling (4 h). During the synthesis the removed compound was filtered, washed with water and crystallised from methanol (60 ml). M.p. 190–191 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.09 (s, 1H, 2-COH), 10.06 (s, 1H, 4-COH), 4.16–4.12 (q, 2H, CH_2), 1.39–1.36 (t, 3H, CH_3); IR (KBr) $\tilde{\nu}$: 3528, 3140 (OH), 2983, 2924 (CH_3), 1597 (C=N), 1526 (C=C), 1479, 1442, 1414, 1351, 1321, 1267, 1241, 1203 (C–OH), 1105, 1042 (N=C–SC=N), 983, 928, 907, 840 cm^{-1}: MS (*m*/*z*, %): 314 (M^+, 100), 286 (10), 179 (5), 167 (39), 153 (7), 151 (12), 143 (5), 137 (5), 134 (5), 119 (10), 107 (4), 93 (3), 80 (3), 65 (4), 52 (3), 39 (4). Anal. $C_{16}H_{14}N_2O_3S$ (C, H, N).

4.1.10. 2-(2,4-Dihydroxyphenyl)-5-(4-ethoxyphenyl)-1,3,4-thiadiazole (11)

4-Ethoxybenzhydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered. Water (100 ml) was added to the filtrate and the filtrate was left at room temperature (48 h). The removed compound was crystallised from aqueous (2:1) methanol (75 ml). M.p. 250–252 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.09 (s, 1H, 2-COH), 10.05 (s, 1H, 4-COH), 4.22–4.01 (q, 2H, CH_2), 1.43–1.29 (t, 3H, CH_3); IR (KBr) $\tilde{\nu}$: 3114 (OH), 2984 (CH_3), 2927 (CH_2), 1609 (C=N), 1522 (C=C), 1452, 1421, 1395, 1310, 1261, 1179 (C–OH), 1140, 1013, 1046 (N=C–S–C=N), 982, 963, 923 cm^{-1}; MS (*m*/*z*, %): 314 (M^+, 100), 298 (30), 286 (18), 241 (4), 213 (3), 184 (5), 167 (27), 149 (29), 137 (19), 134 (5), 121 (16), 107 (3), 93 (5), 65 (5). Anal. $C_{16}H_{14}N_2O_3S$ (C H, N).

4.1.11. 2-(2,4-Dihydroxyphenyl)-5-(2-hydroxy-3-methylphenyl)-1,3,4-thiadiazole (12)

2-Hydroxy-3-methylbenzhydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from methanol (100 ml). M.p. 271–273 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.34 (s, 1H, 2-COH), 11.24 (s, 1H, 2′-COH), 10.13 (s, 1H, 4-COH), 2.28 (s, 3H, CH_3); IR (KBr) $\tilde{\nu}$: 3344 (OH), 1629 (C=N), 1596 (C=C), 1527, 1463, 1409, 1347, 1243 (C–OH), 1134, 1141, 1098, 1083, 972, 843, 812 cm^{-1}; MS (*m*/*z*, %): 300 (M^+, 100), 271 (8), 167 (18), 165 (13), 153 (8), 151 (5), 150 (4), 132 (22), 121 (3), 97(2), 77 (9), 65 (4), 52 (7), 39 (7). Anal. $C_{15}H_{12}N_2O_3S$ (C, H, N).

4.1.12. 2-(2,4-Dihydroxyphenyl)-5-(2-hydroxyphenyl)-1,3,4-thiadiazole (13)

2-Hydroxybenzhydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to

boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from methanol (100 ml). M.p. 289–291 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.11 (s, 1H, 2-COH), 11.06 (s, 1H, 2′-COH), 10.02 (s, 1H, 4-COH); IR (KBr): 3387, 3024 (OH), 1631 (C=N), 1612 (C=C), 1529, 1456, 1420, 1375, 1311, 1265, 1197 (C–OH), 1161, 1137, 1118, 1102, 1039 (N=C–S–C=N), 1002, 984, 963 cm^{-1}; MS (*m*/*z*, %): 286 (M^+, 100), 258 (3), 167 (33), 151 (26), 139 (2), 136 (10), 134 (22), 119 (9), 107 (9), 102 (5), 90 (4), 80 (6), 69 (5), 52 (6), 39 (5). Anal. $C_{14}H_{10}N_2O_3S$ (C, H, N).

*4.1.13. 2,5-Bis(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (**14**)*

2,4-Dihydroxybenzhydrazide (Lancaster) (0.01 mol) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from methanol (100 ml). M.p. > 350 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.05 (s, 2H, 2-COH), 9.99 (s, 2H, 4-COH); IR (KBr): 3377, (OH), 1631 (C=N), 1612 (C=C), 1523, 1467, 1418, 1374, 1298, 1262, 1201 (C-OH), 1158, 1134, 1105, 1002 (N=C–S–C=N), 979, 961, 850 cm^{-1}; MS (*m*/*z*, %): 302 (M^+, 100), 273 (5), 167 (57), 153 (22), 139 (2), 135 (16), 119 (7), 97 (5), 80 (9), 69 (8), 63 (4), 52 (12), 39 (10). Anal. $C_{14}H_{10}N_2O_4S$ (C, H, N).

*4.1.14. 2-(2,4-Dihydroxyphenyl)-5-(3,5-dihydroxyphenyl)-1,3,4-thiadiazole (**15**)*

3,5-Dihydroxybenzhydrazide (Lancaster) (0.01 mol) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from methanol (50 ml). M.p. 293–295 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.11 (s, 1H, 2-COH), 10.07 (s, 1H, 4-COH), 9.66 (s, 2H, 3,5-COH); IR (KBr) $\tilde{\nu}$: 3455, 3322 (OH), 1625 (C=N), 1601 (C=C), 1520, 1484, 1450, 1416-1342, 1315, 1275, 1249, 1233 (C–OH), 1177, 1137, 1112, 1013 (N=C–S–C=N), 988, 969, 861, 851, 823 cm^{-1}; MS (*m*/*z*, %): 302 (M^+, 100), 273 (4), 168 (8), 167 (55), 153 (14), 151 (5), 136 (5), 135 (13), 119 (5), 109 (3), 107 (6), 106 (3), 81 (4), 80 (4), 69 (6), 52 (5), 39 (4). Anal. $C_{14}H_{10}N_2O_4S$ (C, H, N).

*4.1.15. 5-(4-Biphenyl)-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (**16**)*

4-Biphenylcarboxylic acid hydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from aqueous (5:1) methanol (120 ml). M.p. 254–256 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.20 (s, 1H, 2-COH), 10.15 (s, 1H, 4-COH); IR (KBr) $\tilde{\nu}$: 3336, (OH), 2928, 1603 (C=N, C=C), 1474, 1424, 1317, 1273, 1210, 1185 (C–OH), 1105, 998, 982, 965 cm^{-1}; MS (*m*/*z*, %): 346 (M^+, 100), 330 (4), 317 (2), 302 (2), 211 (8), 197 (95), 179 (6), 165 (1), 152 (4), 135, 102 (5), 97, 80, 69, 39. Anal. $C_{20}H_{14}N_2O_2S$ (C, H, N).

*4.1.16. 2-(2,4-Dihydroxyphenyl)-5-(1-naphthylmethyl)-1,3,4-thiadiazole (**19**)*

1-Naphthaleneacethydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered and the filtrate was concentrated to dry. The removed compound was washed with water, filtered, crystallised from aqueous (3:1) methanol (60 ml). M.p. 218–220 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 10.81 (s, 1H, 2-COH), 9.95 (s, 1H, 4-COH), 4.90 (s, 2H, CH_2); IR (KBr) $\tilde{\nu}$: 3157 (OH), 1599 (C=N, C=C), 1512, 1463, 1423, 1399, 1314, 1284, 1241 (C-OH), 1196, 1168, 1133, 1112, 1090, 1041 (N=C–S–C=N), 1018, 985, 967, 905, 840 cm^{-1}; MS (*m*/*z*, %): 334 (M^+, 100), 333 (80), 201 (5), 200 (13), 199 (70), 198 (15), 172 (8), 171 (10), 167 (18), 166 (42), 153 (7), 141 (23), 139 (10), 135 (5), 115 (16), 107 (10), 91 (7), 51 (4). Anal. $C_{19}H_{14}N_2O_2S$ (C, H, N).

*4.1.17. 2-(2,4-Dihydroxyphenyl)-5-(3-fluorophenoxymethyl)-1,3,4-thiadiazole (**20**)*

2-(3-Fluorophenoxy)acetic acid hydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered. The removed compound was filtered, washed with water and crystallised from aqueous (1:1) methanol (75 ml). M.p. 151–153 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.17 (s, 1H, 2-COH), 10.19 (s, 1H, 4-COH), 5.56 (s, 2H, CH_2); IR (KBr) $\tilde{\nu}$: 3214 (OH), 1620 (C=N), 1508, 1505 (C=C), 1460, 1420, 1367, 1323, 1292, 1247, 1195 (C–OH), 1121 (C–F), 1097, 1052 (N=C–S–C=N), 1005, 981, 883, 824 cm^{-1}; MS (*m*/*z*, %): 318 (M^+, 16), 207 (100), 167 (6), 154 (5), 153 (60), 135 (3), 93 (7), 95 (3). Anal. $C_{15}H_{11}FN_2O_3S$ (C, H, N).

*4.1.18. 5-(4-Chloro-2-methylphenoxymethyl)-2-(2,4-dihydroxyphenyl)-1,3,4-thidiazole (**22**)*

2-(4-Chloro-2-methylphenoxy)acetic acid hydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered and the filtrate was concentrated to dry. The removed compound was washed with water and crystallised from methanol (70 ml). M.p. 204–206 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.11 (s, 1H, 2-COH), 10.09 (s, 1H, 4-COH), 5.69 (s, 2H, CH_2), 2.19 (s, 3H, CH_3); IR (KBr): 3368 (OH), 2924 (CH_3), 1632 (C=N), 1601 (C=C), 1526, 1493, 1461, 1412, 1364, 1296, 1251, 1222, 1188 (C–OH), 1132, 1048 (N=C–S–C=N), 987, 968 cm^{-1}; MS (*m*/*z*, %): 334 (10), 299 (12), 207 (100), 167 (6), 153 (73), 141, 135 (4), 124 (2), 99 (4), 73 (2), 63 (4), 51 (2), 39 (4). Anal. $C_{16}H_{13}ClN_2O_3S$ (C, H, N).

*4.1.19. 5-(2-Chlorophenoxymethyl)-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (**23**)*

2-(2-Chlorophenoxy)acetic acid hydrazide (0.01 mol) (Alfa Aesar) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered and the filtrate was concentrated to dry. The removed compound was washed with water and crystallised from methanol

(50 ml). M.p. 202–204 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.19 (s, 1H, 2-COH), 10.0 (s, 1H, 4-COH), 5.69 (s, 2H, CH_2); IR (KBr) $\tilde{\nu}$: 3363 (OH), 2925 (CH_2), 1631 (C=N, C=C), 1525, 1492 (C=C), 1410, 1359, 1298, 1251, 1223, 1187 (C-OH), 1133, 1047 (N=C–S–C=N), 991, 880 cm^{-1}; MS (*m*/*z*, %): 334 (M^+, 10), 299 (14), 207 (100), 167 (6), 153 (58), 135 (3), 124, 99, 97 (2), 77 (1), 69 (2), 45 (1), 39 (2). Anal. $C_{15}H_{11}ClN_2O_3S$ (C, H, N).

4.1.20. 5-(2,4-Dichlorophenoxymethyl)-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole ***(24)***

2-(2,4-Dichlorophenoxy)acetic acid hydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (70 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from methanol (70 ml). M.p. 216–218 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.08 (s, 1H, 2-COH), 10.05 (s, 1H, 4-COH), 5.70 (s, 2H, CH_2); IR (KBr) $\tilde{\nu}$: 3418, (OH), 2925 (CH_2), 1634 (C=N), 1600 (C=C), 1527, 1481, 1395, 1370, 1345, 1291, 1267, 1247, 1219 (C–OH), 1134, 1106 (C–Cl) 1065 (N=C–S–C=N), 1032, 987, 969, 867, 840, 822 cm^{-1}; MS (*m*/*z*, %): 369 (M^+, 2), 368 (11) 333 (4), 209 (5), 208 (12), 207 (100), 178 (2), 167 (4), 155 (3), 154 (4), 153 (47), 135 (2). Anal. $C_{15}H_{10}Cl_2N_2O_3S$ (C, H, N).

4.1.21. 2-(2,4-Dihydroxyphenyl)-5-(4-nitrophenoxymethyl)-1,3,4-thiadiazole ***(25)***

2-(4-Nitrophenoxy)acetic acid hydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (70 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from aqueous (1:1) methanol (60 ml). M.p. 243–245 °C; ^{1}H NMR (DMSO-d6, TMS): 11.09 (s, 1H, 2-COH), 10.06 (s, 1H, 4-COH), 5.76 (s, 2H, CH2); IR (KBr) $\tilde{\nu}$: 3541, 3381, 3191), (OH 2920 (CH_2), 1609 (C=N), 1592 (C=C), 1513, 1497 (C=C), 1473, 1461, 1422, 1342, 1299, 1260 (NO_2), 1200 (C–OH) 1176, 1139, 1110, 1043 (N=C–S–C=N), 999, 983, 868, 851, 840, 809 cm^{-1}; MS (*m*/*z*, %): 345 (M^+, 25), 208 (13), 207 (100), 167 (6), 154 (6), 153 (68), 135 (3), 124, 97 (3), 77 (1) 69 (3), 39 (2). Anal. $C_{15}H_{11}N_3O_5S$ (C, H, N).

4.1.22. 2-(2,4-Dihydroxyphenyl)-5-(dyhydroxybenzyl)-1,3,4-thiadiazole ***(27)***

Mandelic acid hydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered. The removed compound was filtered, washed with water and crystallised from aqueous (1:1) methanol (50 ml). M.p. 124–126 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 10.95 (s, 1H, 2-COH), 10.00 (s, 1H, 4-COH), 6.12 (s, 1H, OH), 4.19–4.059 (m, 1H, C_{alif}H); IR (KBr) $\tilde{\nu}$: 3431 (OH), 2960 (C_{alif}–H), 1627 (C=N, C=C), 1450, 1408, 1315, 1260 (C–OH), 1106, 1019 (N=C–S–C=N), 803 cm^{-1}; MS (*m*/*z*, %): 300 (M^+, 100), 284 (7), 271 (4), 223 (6), 214 (1), 195 (22), 167 (7), 153 (9), 135 (26), 119 (2), 105 (31), 91 (2), 77 (22), 64 (5), 51 (7), 34 (4). Anal. $C_{15}H_{12}N_2O_3S$ (C, H, N).

4.1.23. 1,4-Bis[5-(2,4-dihydroxyphenyl)-(1,3,4-thiadiazol-2-yl)]butan ***(28)***

Adipic acid dihydrazide (0.01 mol) (Aldrich) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). During the synthesis the removed compound was filtered, washed with water and crystallised from methanol (60 ml). M.p. 270–272 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 10.91 (s, 2H, 2-COH), 9.97 (s, 2H, 4-COH), 3.15–3.07 (m, 4H, CH_2), 1.87-1.85(m, 4H, CH_2); IR (KBr)$\tilde{\nu}$: 3260, 3057 (OH), 2963, 2835 (CH_2), 1616 (C=N), 1597 C=C, 1505, 1460, 1419, 1346, 1319, 1250, 1178 (C–OH), 1125, 1028 (N=C–S–C=N), 964, 927, 862 cm^{-1}; MS (*m*/*z*, %): 442 (M^+, 100), 426 (5), 413 (3), 302 (9), 294 (5), 274 (18), 267 (1), 234 (34), 221 (100), 208 (37), 195 (2), 167 (6), 153 (12), 136 (14), 119 (1), 100 (5), 97 (2), 80 (2), 52 (3), 39 (3). Anal. $C_{20}H_{18}N_4O_4S_2$ (C, H, N).

4.1.24. 2-(2,4-Dihydroxyphenyl)-5-(2-furyl)-1,3,4-thiadiazole ***(29)***

2-Furoic acid hydrazide (0.01 mol) (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered and water (25 ml) was added to the filtrate. The filtrate was left at room temperature (24 h). The removed compound was crystallised from methanol (70 ml). M.p. 252–254 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.19 (s, 1H, 2-COH), 10.10 (s, 1H, 4-COH), 7.28–7.25 (d, 2H, OCH), 6.52–6.41 (m, 2H, C_{fur}H); IR (KBr) $\tilde{\nu}$: 3122 (OH), 1635 (C=N), 1604 (C=C), 1530, 1491, 1474, 1435, 1331, 1263, 1236, 1177 (C–OH), 1142, 1114, 1075, 1044 (N=C–S–C=N), 1013, 985, 967, 893, 847, 821 cm^{-1}; MS (*m*/*z*, %): 260 (M^+, 100), 231 (2), 203 (2), 167 (20), 153 (11), 147, 135 (4), 125 (18), 111 (7), 108 (3), 97 (5), 83 (1), 80 (4), 70 (3), 52 (3), 40 (3), 39 (30). Anal. $C_{12}H_8N_2O_3S$ (C, H, N).

4.1.25. 5-(Benzo[b]thiophen-2-yl)-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole ***(32)***

Benzo[b]thiophene-2-carboxylic acid hydrazide (Lancaster) and **STB** (0.0075 mol) were added to methanol (50 ml) and heated to boiling (3 h). The mixture was hot filtered and water (25 ml) was added to the filtrate. The filtrate was left at room temperature (24 h). The removed compound was filtered, washed with water and crystallised from aqueous (1:1) methanol (60 ml). M.p. 274–276 °C; ^{1}H NMR (DMSO-d_6, TMS): δ 11.34 (s, 1H, 2-COH), 10.19 (s, 1H, 4-COH); IR (KBr) $\tilde{\nu}$: 3137 (OH), 1635 (C=N), 1597 (C=C), 1530, 1472, 1440, 1413, 1336, 1257, 1181 (C–OH), 1140, 1001, 1016 (N=C–S–C=N), 985, 970, 908, 844, 822 cm^{-1}; MS (*m*/*z*, %): 326 (M^+, 100), 297 (2), 191 (11), 177 (7), 167 (17), 153 (3), 135 (1), 115 (2), 101, 89 (1), 69 (1). Anal. $C_{16}H_{10}N_2O_2S_2$ (C, H, N).

4.2. Antiproliferative assay in vitro

The following lines of human cancer cells established in vitro were applied: T47D (breast cancer), SW707 (rectal adenocarcinoma), A549 (non-small lung carcinoma) from the

American Type Culture Collection (Rockville, MD, USA) and HCV29T (bladder cancer) from the Fibiger Institute, Copenhagen, Denmark. They were maintained in the Cell Culture Collection of the Institute of Immunology and Experimental Therapy wroclaw.

Twenty-four hours before addition of the tested agents, the cells were plated in 96-well plates (Sarstedt, USA) at a density of 10^4 cells per well. All cell lines were maintained in the optiMEM medium supplement with 2 mM glutamine (Gibco, Warsaw, Poland), streptomycin (50 μg ml^{-1}), penicillin (50 U ml^{-1}) (Polfa, Tarchomin, Poland) and 5% foetal calf serum (Gibco, Grand Island, USA). The cells were incubated at 37 °C in the humid atmosphere saturated with 5% CO_2. The solutions of compounds (1 mg ml^{-1}) were prepared *ex tempore* by dissolving the substance in 100 μl of DMSO completed with 900 μl of tissue culture medium. Afterwards the compounds were diluted in the culture medium to reach the final concentrations ranging from 0.1 to 100 μg ml^{-1}. The solvent (DMSO) in the highest concentration used in the test did not reveal any cytotoxic activity. Cisplatin was applied as a test referential agent. The cytotoxicity assay was performed after 72 h exposure of the cultured cells at the concentration ranging from 0.1 to 100 μg ml^{-1} of the tested agents. The SRB test measuring the cell proliferation inhibition in in vitro culture was applied [36]. The cells attached to the plastic were fixed with cold 50% trichloroacetic acid (TCA) (Aldrich-Chemie, Germany) added on the top of the culture medium in each well. The plates were incubated at 4 °C for 1 h and then washed five times with tap water. The background optical density was measured in the wells filled with culture medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulphorhodamine B (SRB, Sigma, Germany) dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 min. The unbound dye was removed by rinsing (four times) with 1% acetic acid, and the protein-bound dye was extracted with 10 mM unbuffered Tris base (tris (hydroxymethyl) aminomethane, POCh, Gliwice, Poland) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Uniskan II (Labsystems, Helsinki, Finland). The compounds were tested in triplicates per experiment. The experiments were repeated at least three times.

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