One-Pot Synthesis of New (1,3-Thiazolo[5,4-*b*]pyridin-2-yl)benzenediols and Their Antiproliferative Activities against Human Cancer Cell Lines

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A one-pot synthesis of new 4-(1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diols has been described. The compounds were prepared by the reaction of sulfinylbis[(2,4-dihydroxyphenyl)methanethione] derivatives, with various substituents in the aryl rings, with 2-chloropyridin-3-amines. Their structures were deduced from IR and, ^{1}H- and ^{13}C-NMR spectroscopic, mass spectrometric, and elemental analyses. The antiproliferative properties of some of the products against human cancer cell lines were comparable to those of cisplatin. Structure–activity analysis showed that the presence of hydrophobic substituents in both heterocyclic fused and phenyl rings of the compounds improves their biological effects. Further, an additional OH group in the resorcinol moiety reduced the antiproliferative activity.

Introduction. – The thiazole and fused-thiazole heterocycles are important structural components of biologically active molecules, and, as a result, they serve as attractive targets for developing new, effective synthesis methods [1][2]. Because of their pharmaceutical importance in the area of drug discovery, we have been interested in developing an efficient protocol to prepare fused-thiazole scaffolds of 1,3-thiazolo[5,4-*b*]pyridine that is relatively unexplored with regard to its biological activity.

Several synthetic methods for the preparation of 1,3-thiazolo[5,4-*b*]pyridine derivatives have been well-documented, but all approaches have some restraints. The most common route involves the reaction of 2-chloropyridin-3-amine with alkyl, aralkyl, or aryl isothiocyanate in absolute EtOH [3][4]. *Krayushkin* and co-workers described the oxidation of compounds, in which a monothioxamide fragment is linked to a heterocyclic ring leading to the formation of fused thiazole derivatives with a carboxamide group. This approach was applied to the synthesis of previously unknown derivatives, and its sensitivity to electronic factors was noticed [5–8]. 1,3-Thiazolo[5,4-*b*]pyridine-2-carboxamides were also prepared by the reaction of 2-nitropyridin-3-amine with dithioesters containing a carbamoyl group [9]. A one-step synthesis from appropriately substituted chloro-nitro-pyridine, and thioamide or thiourea has been recently described by *Sahasrabudhe et al.* In particular, the reaction was used to prepare a large number of 6-nitrothiazolo[5,4-*b*]pyridine derivatives with various substituents in 2-position [10]. Treatment of 4-chloro-*N*-(2-chloropyridin-3-yl)- or 4-chloro-*N*-(4-chloropyridin-3-yl)-1,2,3-dithiazol-5*H*-imines with catalytic amounts of tetraalkylammonium iodide gave 1,3-thiazolo[5,4-*b*]pyridine-2-carbonitriles [11].

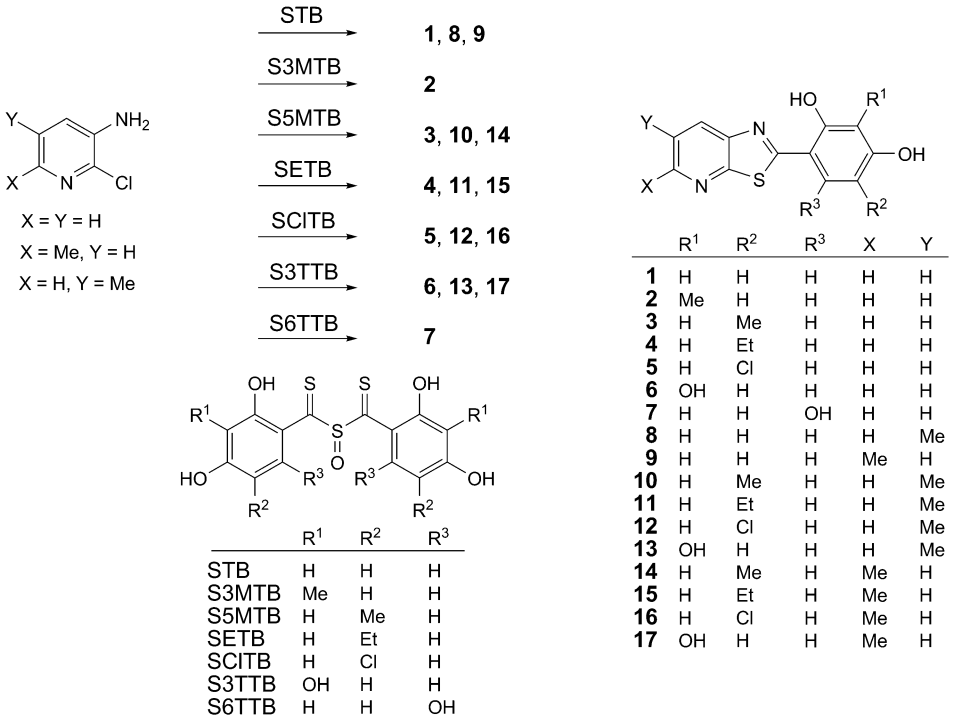
The formation of 1,3-thiazolo[4,5-*b*]pyridine, *via* transformation of 1,2,4-triazine rings [12] or 2-(tetrazolylsulfanyl)pyridin-3-amines containing an aryl substituent in the tetrazole moiety under acidic conditions, has been also reported [13]. *Couture* and *Grandclaudon* described the conversion of the C=O group of *N*-(2-chloropyridin-3-yl)benzamides to the C=S group with *Lawesson*'s reagent, followed by ring closure providing desired 1,3-thiazolo[5,4-*b*]pyridines [14]. This method was applied by *Lee et al.* [15]. Other derivatives were prepared from ethyl 5-amino-2-(methylsulfanyl)-thiazole-4-carboxylate and benzylidinemalononitrile [16].

There is little information available about biological activity of 1,3-thiazolo[5,4-*b*]pyridines. For example, 3-cyclopentyl-*N*-(5-methoxythiazolo[5,4-*b*]pyridin-2-yl)-2-[4-(4-methylpiperazine-1-sulfonyl)phenyl]propionamide activates the GK enzyme *in vitro* at low nanomolar concentrations and significantly reduces glucose levels [17]. Thiazolo[5,4-*b*]pyridines with a MeO group at C(5) show potent inhibitory activities for Aβ42 fibrillization at the micromolar level for *Alzheimer*'s disease treatment [15], whereas 5-chloro[1,3]thiazolo[5,4-*b*]pyridin-2-amines are H_3 receptor antagonists [4]. 2,6-Difluoro-3-methoxybenzamide derivatives are potent antistaphylococcal compounds with suboptimal drug-like properties [18]. They act as inhibitors of the bacterial cell-division protein, FtsZ. Other derivatives were described as useful antimicrobial agents effective against a variety of human and veterinary pathogens including, among others, *Gram*-positive and *Gram*-negative aerobic and anaerobic bacteria, as well as mycobacteria [19][20]. 1,3-Thiazolo[5,4-*b*]pyridines act also as amplifiers of phleomycin [21].

Additionally, our early studies showed that 2-(2,4-dihydroxyphenyl)benzo-1,3-thiazoles possess promising biological properties [22]. Therefore, we decided to prepare the isosteric fused ring with the heteroatom N as the potential atom interacting with the molecular target containing a 2,4-dihydroxyphenyl moiety. The 2,4-dihydroxyphenyl substituent is mainly modified at C(5) by the hydrophobic substituents Cl, Me, and Et according to the literature. It was pointed out that they interact with a hydrophobic pocket of HSP90 protein as a molecular target of resorcyl derivatives [23]. These groups of compounds are commonly described as promising anticancer drugs of non-standard mechanism of action. Some of them are in the stage of clinical trials [24]. Additionally, for comparison, we decided to prepare compounds with a third OH group in a resorcinol moiety in two different positions. The Me substituents at C(5) or/and C(6) of the fused heterocyclic ring might protect its metabolically sensitive positions and change the lipophilicity of compounds [25]. The compounds were designed and synthesized as potential anticancer substances.

Results and Discussion. – *Chemistry.* Based on similarities with the reaction of 2-nitropyridin-3-amines with thioamides (thioureas) [9], and the transformation of *N*-(2-chloropyridin-3-yl)benzamides by treatment with *Lawesson*'s reagent [15], which leads to corresponding 1,3-thiazolo[5,4-*b*]pyridines, a synthesis of analogs making use of 2-chloropyridin-3-amine, or the 5- or 6-Me derivatives is proposed (*Scheme 1*). This type of reagents enables the reaction with some electrophilic reagents elaborated by us, *i.e.*, sulfinylbis[(2,4-dihydroxyphenyl)methanethione] derivatives (STB, S3MTB, S5MTB, SETB, SClTB, S3TTB, and S6TTB; *Scheme 1*) [26], towards thioamidation and then cyclization by elimination of HCl (*Scheme 2*). The presence of the halogen atom at

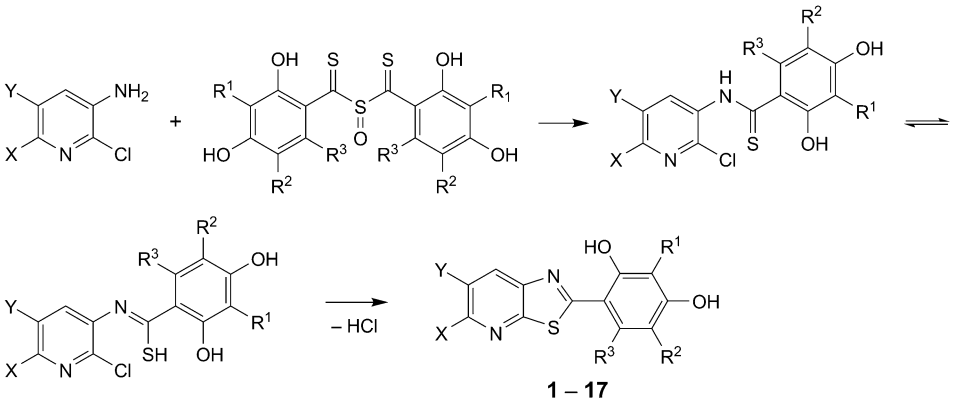
Scheme 1. *Synthesis of 4-(1,3-Thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diols*



	R^1	R^2	R^3
STB	H	H	H
S3MTB	Me	H	H
S5MTB	H	Me	H
SETB	H	Et	H
SClTB	H	Cl	H
S3TTB	OH	H	H
S6TTB	H	H	OH

	R^1	R^2	R^3	X	Y
1	H	H	H	H	H
2	Me	H	H	H	H
3	H	Me	H	H	H
4	H	Et	H	H	H
5	H	Cl	H	H	H
6	OH	H	H	H	H
7	H	H	OH	H	H
8	H	H	H	H	Me
9	H	H	H	Me	H
10	H	Me	H	H	Me
11	H	Et	H	H	Me
12	H	Cl	H	H	Me
13	OH	H	H	H	Me
14	H	Me	H	Me	H
15	H	Et	H	Me	H
16	H	Cl	H	Me	H
17	OH	H	H	Me	H

Scheme 2. *Reaction Mechanism of the Formation of 4-(1,3-Thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diols*



C(2) of pyridin-3-amine is a necessary condition for the reaction. The yield and purity of products depend on the kind of substituents (X, Y) in this ring, and also on the kind

of substituent R in the dihydroxyphenyl moiety of the electrophilic reagent. The cyclization process depends on amide–thiolimine isomerization and ionization ability of the SH group regulated by ring substituents. The reaction promotes a tendency of the compounds with thioamide moiety toward transition into the thiolimine forms compared with the analogous ones with an O-atom [27]. Electrophilic reagents were obtained according to the method described previously, *i.e.*, by treatment of the corresponding dithioic acids with $SOCl_2$ [26].

In the ^{1}H-NMR spectrum, the unsubstituted 1,3-thiazolo[5,4-*b*]pyridine ring in **1**–**7** shows three *doublet*s of *doublet*s at *ca.* 8.56 ($J = 4.6$, 1.4 Hz), 8.32 ($J = 8.2$, 1.4 Hz), and 7.55 ppm ($J = 8.2$, 4.6 Hz), corresponding to H–C(5), H–C(7), and H–C(6), respectively. The 5-Me derivatives exhibit the characteristic two *doublet*s at 8.27 and 7.45 ppm with $J = 8.3$ Hz, and the 6-Me derivatives most often show two *doublet*s at 8.27 and 7.45 ppm with $J = 1.3$ Hz. The signals of the β-resorcinol moiety in the case of additional substitution at C(5) appear as two characteristic *singlet*s at *ca.* 7.9 and 6.6 ppm, corresponding to H–C(3) and H–C(6), respectively. The OH groups are sometimes invisible in the background on the base line.

In the IR spectrum, there are strong bands in the region of *ca.* 3400–3100 and 1635–1610 cm^{-1}, corresponding to ν(O–H) and ν(C=N). The mass spectra of the compounds showed molecular-ion peaks, however, with various intensities.

Antiproliferative Activity. The 1,3-thiazolo[5,4-*b*]pyridines compiled in *Scheme 1* have been evaluated for their antiproliferative activities against human bladder cancer HCV29T cells. The cytotoxic activity *in vitro* was expressed as IC_{50}, the concentration of the compound to inhibit proliferation rate of the tumor cells by 50% as compared to the untreated cells. Cisplatin was used as a reference drug. The results of the screening are collected in *Table 1*.

Table 1. *Antiproliferative Activity of 4-(1,3-Thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diols against the Human Cancer Cell Line HCV29T Expressed as* IC_{50}[a])

Compound	IC_{50} [μg/ml]	Compound	IC_{50} [μg/ml]
1	8.34 ± 1.27	**10**	4.24 ± 1.80
2	3.39 ± 0.24	**11**	4.45 ± 1.12
3	6.70 ± 0.54	**12**	3.30 ± 1.11
4	2.37 ± 0.37	**13**	10.64 ± 0.58
5	7.61 ± 0.44	**14**	6.29 ± 1.03
6	22.14 ± 2.83	**15**	6.75 ± 2.14
7	39.82 ± 2.36	**16**	3.29 ± 1.27
8	5.61 ± 1.20	**17**	18.40 ± 3.16
9	4.09 ± 0.26	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 cells. The values are the means ± S.D. of nine independent experiments.

Activity of compounds clearly depends on the substitution pattern of both rings. Significantly, the highest effect against HCV29T cells was exhibited by compounds **2**, **4**, **12**, and **16** (*Table 1*). The lowest antiproliferative effect was detected for the compounds with a third OH group in the resorcinol moiety of **6**, **7**, and **17**. Compounds

2, **4**, **9**, **10**, **12**, and **16**, as the most active derivatives of this set of tested 1,3-thiazolo[5,4-*b*]pyridines, meet the cytotoxic-activity criterion against HCV29T cancer cell line for potentially new antiproliferative drugs ($IC_{50} \leq 4$ μg/ml) [28].

The selected compounds of the highest activity against HCV29T were also tested against A549 (human non-small lung carcinoma), T47D (human breast cancer), and SW707 (human rectal adenocarcinoma) cells (*Table 2*). Antiproliferative activities of compounds **4**, **12**, and **16** against A549 cells were at the level of cisplatin. At the same time, compound **4** met the cytotoxicity criterion against this cell line.

Table 2. *Antiproliferative Activity of Some Compounds against the Human Cancer Cell Lines T47D, A549, and SW707, Expressed as* IC_{50}

Compound	IC_{50} [μg/ml]		
	T47D	A 549	SW707
4	4.29 ± 0.84	3.32 ± 1.19	10.88 ± 3.39
12	4.81 ± 2.05	6.14 ± 0.24	10.78 ± 1.54
16	6.28 ± 3.19	4.45 ± 0.52	11.64 ± 2.31
Cisplatin	2.03 ± 1.61	5.29 ± 1.85	3.13 ± 1.49

Analyzing the structure of compounds and their activity, it was found that the presence of a Me substituent at C(5) or C(6) of the 1,3-thiazolo[5,4-*b*]pyridine ring increases the antiproliferative activity of compounds. The same effect is observed where a Me (Et) substituent or a Cl-atom is present in the resorcinol moiety, compared to the unsubstituted parent compound **1**. The most beneficial is the presence of an Et substituent at C(5) of the resorcinol moiety in **4**. These effects are not additive when two substituents are introduced into both rings; it is especially evident in the case of Et derivatives (*i.e.*, compounds **4** and **11** or **15**). Introducing a Me group to the fused ring of the compounds with three OH substituents in the aryl ring significantly increases the activity (*i.e.*, compounds **6** and **13** or **17**). This analysis reveals that the hydrophobic substituent ($\pi > 0$) in the resorcinol ring increases antiproliferative activity. Similar effects were observed for 2-(2,4-dihydroxyphenyl)-4*H*-3,1-benzothiazines [29]. It confirms the earlier finding that the presence of a Cl-atom or Et substituent at C(5) of the resorcinol moiety of resorcyl-azoles enhances their anticancer properties [23][30][31].

Conclusions. – In conclusion, an efficient one-pot synthesis of new 4-(1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diols has been developed. The studies indicated high level of antiproliferative activities of some products. Structure–activity relationship analyses indicated that the presence of hydrophobic substituents at C(5) or C(6) of the fused ring, or at C(5) of the phenyl residue (especially the Et group) improves the antiproliferative activities against human cancer cell lines. Taking into account these findings, further studies including design, synthesis, and analysis of subsequent derivatives, as well as new groups of compounds with the 2,4-dihydroxyphenyl substituent will be carried out.

Experimental Part

General. M.p.: *BÜCHI B-540* (Switzerland) melting-point apparatus; uncorrected. IR Spectra: *Perkin–Elmer FT-IR 1725X* spectrophotometer (in KBr); $\tilde{\nu}$ in cm^{-1}; recorded in the range of 600–4000 cm^{-1}. ^{1}H-NMR Spectra: *Bruker DRX 500* instrument in (D_6)DMSO, $CDCl_3$ or CD_3OD solns.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS (70 eV): *AMD-604*; in *m*/*z* (rel. %). Elemental analysis: *Perkin–Elmer 2400*; within ±0.4% of the theoretical values.

General Procedure for the Synthesis of Compounds **1**–**17**. A mixture of 2-chloropyridin-3-amine (*Sigma–Aldrich*; compounds **1**–**7**) or 6-chloro-3-methylpyridin-5-amine (*Sigma–Aldrich*; compounds **8**, **10**–**13**), or 6-chloro-2-methylpyridine-5-amine (*Acros Organics*; compounds **9**, **14**–**17**; 0.002 mol), and the corresponding electrophile (0.0015 mol; *Scheme 1*) in MeOH (10 ml) was heated to reflux for 3 h. The hot mixture was filtered. The filtrate was concentrated, and the formed solid was filtered and crystallized from MeOH (5 ml).

*4-(1,3-Thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**1**). Yield 89%. M.p. 252–253°. IR: 3068 (OH), 3024 (arom. C–H), 1632 (C=N), 1599 (C=C), 1561 (C=C), 1224 (C–OH). ^{1}H-NMR ((D_6)DMSO): 11.48 (*s*, HO–C(3)); 10.23 (*s*, HO–C(1)); 8.54 (*dd*, *J* = 4.7, 1.4, H–C(5′)); 8.35 (*dd*, *J* = 8.2, 1.4, H–C(7′)); 7.89 (*s*, H–C(5)); 7.55 (*dd*, *J* = 8.2, 4.7, H–C(6′)); 6.49 (*d*, *J* = 1.7, H–C(2)); 6.45 (*dd*, *J* = 8.7, 1.8, H–C(6)). EI-MS: 244 (100, M^+), 216 (10), 187 (37), 174 (3), 155 (4), 137 (2), 122 (2), 108 (11), 93 (3), 82 (5), 69 (4), 51(4), 39 (7). Anal. calc. for $C_{12}H_8N_2O_2S$ (244.27): C 59.00, H 3.30, N 11.47; found: C 59.22, H 3.29, N 11.50.

*2-Methyl-4-(1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**2**). Yield 87%. M.p. 287–289°. IR: 3240 (OH), 3092 (OH), 3035 (arom. C–H), 2925 (CH), 2856 (CH), 1624 (C=N), 1598 (C=C), 1557 (C=C), 1507 (C=C), 1214 (C–OH). ^{1}H-NMR (CD_3OD): 12.25 (*s*, HO–C(3)); 10.43 (*s*, HO–C(1)); 8.55 (*dd*, *J* = 4.7, 1.4, H–C(5′)); 8.35 (*dd*, *J* = 8.2, 1.5, H–C(7′)); 7.58 (*m*, H–C(6′), H–C(5)); 6.53 (*d*, *J* = 8.7, H–C(6)); 2.07 (*s*, Me). EI-MS: 258 (100, M^+), 229 (25), 201 (6), 175 (4), 129 (5), 106 (3), 83 (3), 65 (3), 39 (6). Anal. calc. for $C_{13}H_{10}N_2O_2S$ (258.30): C 60.45, H 3.90, N 10.85; found: C 60.28, H 3.89, N 10.89.

*4-Methyl-6-(1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**3**). Yield 79%. M.p. 241–243°. IR: 3236 (OH), 3128 (OH), 2922 (CH), 2855 (CH), 1615 (C=N), 1526 (C=C), 1505 (C=C), 1215 (C–OH). ^{1}H-NMR ((D_6)DMSO): 10.30 (*s*, HO–C(3)); 8.54 (*dd*, *J* = 4.7, 1.4, H–C(5′)); 8.31 (*dd*, *J* = 8.2, 1.4, H–C(7′)); 7.89 (*s*, H–C(5)); 7.55 (*dd*, *J* = 8.2, 4.7, H–C(6′)); 6.60 (*s*, H–C(2)); 2.13 (*s*, Me). EI-MS: 258 (100, M^+), 229 (12), 201 (10), 187 (13), 120 (5), 106 (4), 83 (4), 69 (4), 39 (6). Anal. calc. for $C_{13}H_{10}N_2O_2S$ (258.30): C 60.45, H 3.90, N 10.85; found: C 60.57, H 3.91, N 10.82.

*4-Ethyl-6-(1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**4**). Yield 89%. M.p. 264–265°. IR: 3427 (OH), 3106 (arom. C–H), 2964 (C–H), 2923 (C–H), 2856 (C–H), 1609 (C=N), 1500 (C=C), 1205 (C–OH). ^{1}H-NMR ((D_6)DMSO): 10.34 (*s*, HO–C(3)); 8.56 (*dd*, *J* = 4.7, 1.4, H–C(5′)); 8.36 (*dd*, *J* = 8.2, 1.4, H–C(7′)); 7.91 (*s*, H–C(5)); 7.58 (*q*, *J* = 8.2, 4.7, H–C(6′)); 6.64 (*s*, H–C(2)); 2.56 (*m*, MeCH_2); 1.17 (*t*, *Me*CH_2). ^{13}C-NMR ((D_6)DMSO): 165.8; 160.5; 156.0; 155.0; 145.3; 143.8; 129.9; 128.4; 123.3; 122.0; 109.7; 102.4; 22.2; 14.3. EI-MS: 272 (55, M^+), 257 (100, $[M - Me]^+$), 229 (3), 187 (7), 136 (3), 83 (3), 69 (3), 39 (3). Anal. calc. for $C_{14}H_{12}N_2O_2S$ (272.31): C 61.74, H 4.44, N 10.28; found: C 61.80, H 4.43, N 10.31.

*4-Chloro-6-(1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**5**). Yield 86%. M.p. 234–237°. IR: 3022 (OH, arom. C–H), 1619 (C=N), 1598 (C=C), 1192 (C–OH). ^{1}H-NMR ((D_6)DMSO): 11.68 (*s*, HO–C(1)); 10.73 (*s*, HO–C(3)); 8.56 (*dd*, *J* = 4.7, 1.4, H–C(5′)); 8.36 (*dd*, *J* = 8.2, 1.4, H–C(7′)); 7.91 (*s*, H–C(5)); 7.58 (*q*, *J* = 8.2, 4.7, H–C(6′)); 6.72 (*s*, H–C(2)). EI-MS: 278 (100, M^+), 243 (11), 215 (8), 187 (23), 136 (7), 121 (5), 95 (4), 69 (6), 51 (5), 39 (4). Anal. calc. for $C_{14}H_7ClN_2O_2S$ (278.71): C 51.71, H 2.53, N 10.05; found: C 51.90, H 2.52, N 10.02.

*4-(1,3-Thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,2,3-triol* (**6**). Yield 76%. M.p. 337–340°. IR: 3042 (OH, arom. C–H), 1629 (C=N), 1606 (C=C), 1192 (C–OH). ^{1}H-NMR ((D_6)DMSO): OH signals not visible in the background on the base line; 8.56 (*dd*, *J* = 4.7, 1.4, H–C(5′)); 8.36 (*dd*, *J* = 8.2, 1.4, H–C(7′)); 7.57 (*q*, *J* = 8.2, 4.7, H–C(6′)); 7.51 (*d*, *J* = 8.7, H–C(5)); 6.56 (*d*, *J* = 8.7, H–C(6)). ^{13}C-NMR ((D_6)DMSO): 166.7; 155.2; 150.3; 147.4; 145.3; 144.5; 133.1; 129.8; 122.0; 119.4; 110.9; 108.9. EI-MS: 260 (100, M^+), 231 (4), 203 (3), 187 (3), 176 (4), 161 (3), 67 (7), 39 (3). Anal. calc. for $C_{12}H_8N_2O_3S$ (260.27): C 55.38, H 3.10, N 10.76; found: C 55.52, H 3.09, N 10.71.

*2-(1,3-Thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3,5-triol* (**7**). Yield 69%. M.p. 285–287°. IR: 3338 (OH), 2923 (CH), 2854 (CH), 1621 (C=N), 1222 (C–OH). EI-MS: 260 (100, M^+), 231 (3), 203 (4), 190 (4), 176 (10), 163 (5), 116 (6), 69 (4), 39 (3). ^{1}H-NMR ((D$_6$)DMSO): 12.53 (*s*, HO–C(1), HO–C(3)); 10.23 (*s*, HO–C(5)); 8.56 (*dd*, *J* = 4.6, 1.4, H–C(5′)); 8.32 (*dd*, *J* = 8.2, 1.4, H–C(7′)); 7.55 (*dd*, *J* = 8.2, 4.6, H–C(6′)); 6.01 (*s*, H–C(4,6)). Anal. calc. for $C_{12}H_8N_2O_3S$ (260.27): C 55.38, H 3.10, N 10.76; found: C 55.20, H 3.08, N 10.72.

*4-(6-Methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**8**). Yield 86%. M.p. 268–270°. IR: 3428 (OH), 3069 (OH), 2921 (CH), 2859 (CH), 1635 (C=N), 1594 (C=C), 1510 (C=C), 1212 (C–OH). ^{1}H-NMR ((D$_6$)DMSO): OH signals not visible in the background on the base line; 8.44 (*d*, *J* = 1.3, H–C(5′)); 8.19 (*m*, H–C(7′)); 8.00 (*d*, *J* = 8.7, H–C(5)); 6.54 (*d*, *J* = 2.3, H–C(2)); 6.47 (*dd*, *J* = 2.3, 8.7, H–C(6)); 2.46 (*s*, Me). EI-MS: 258 (100, M^+), 230 (8), 201 (31), 169 (3), 137 (2), 115 (5), 69 (4), 38 (9). Anal. calc. for $C_{13}H_{10}N_2O_2S$ (258.30): C 60.45, H 3.90, N 10.85; found: C 60.31, H 3.93, N 10.81.

*4-(5-Methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**9**). Yield 82%. M.p. 261–263°. IR: 3430 (OH), 3334 (OH), 3153 (OH), 2859 (CH), 1636 (C=N), 1600 (C=C), 1580 (C=C), 1540 (C=C), 1183 (C–OH). ^{1}H-NMR ((D$_6$)DMSO): OH signals not visible in the background on the base line; 8.29 (*d*, *J* = 8.3, H–C(7′)); 7.99 (*d*, *J* = 8.7, H–C(5)); 7.47 (*d*, *J* = 8.3, H–C(6′)); 6.54 (*d*, *J* = 1.7, H–C(2)); 6.48 (*dd*, *J* = 8.7, 1.8, H–C(6)); 2.65 (*s*, Me). ^{13}C-NMR ((D$_6$)DMSO): 164.9; 162.3; 158.4; 153.9; 153.6; 143.7; 130.5; 129.8; 122.2; 110.2; 108.7; 102.6; 22.4. EI-MS: 258 (100, M^+), 246 (13), 230 (13), 213 (46), 201 (22), 169 (2), 53 (4), 36 (17). Anal. calc. for $C_{13}H_{10}N_2O_2S$ (258.30): C 60.45, H 3.90, N 10.85; found: C 60.57, H 3.93, N 10.80.

*4-Methyl-6-(6-methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**10**). Yield 88%. M.p. 253–256°. IR: 3142 (OH), 3009 (arom. C–H), 2922 (C–H), 1625 (C=N), 1209 (C–OH). ^{1}H-NMR ((D$_6$)DMSO): 12.30 (*s*, HO–C(1)); 10.50 (*s*, HO–C(3); 8.40 (*d*, *J* = 1.3, H–C(5′)); 8.15 (*m*, H–C(7′)); 7.52 (*d*, *J* = 8.7, H–C(5)); 6.61 (*d*, *J* = 8.7, H–C(6)); 2.64 (*s*, Me); 2.06 (*s*, Me). ^{13}C-NMR ((D$_6$)DMSO): 172.9; 163.7; 160.5; 155.1; 150.0; 148.0; 135.3; 132.0; 130.3; 114.3; 112.0; 111.6; 21.1; 11.5. EI-MS: 272 (100, M^+), 243 (30), 215 (12), 201 (7), 189 (5), 160 (3), 151 (3), 136 (4), 97 (4), 78 (2), 36 (11). Anal. calc. for $C_{14}H_{12}N_2O_2S$ (272.32): C 61.75, H 4.44, N 10.29; found: C 61.93, H 4.42, N 10.17.

*4-Ethyl-6-(6-methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**11**). Yield 78%. M.p. 215–217°. IR: 3220 (OH), 3150 (OH), 2857 (CH), 1612 (C=N), 1527 (C=C), 1511 (C=C), 1183 (C–OH). ^{1}H-NMR ((D$_6$)DMSO): 10.27 (*s*, HO–C(3)); 8.40 (*d*, *J* = 1.3, H–C(5′)); 8.17 (*m*, H–C(7′)); 7.84 (*s*, H–C(5)); 6.59 (*s*, H–C(2)); 2.55 (*q*, *J* = 7.5, MeC*H*$_2$); 2.45 (*s*, Me); 1.16 (*t*, *J* = 7.5, *Me*CH$_2$). EI-MS: 286 (M^+, 55), 271, (100, $[M-\text{Me}]^+$), 243 (6), 215 (4), 201 (8), 143 (3), 97 (3), 69 (4), 39 (3). Anal. calc. for $C_{15}H_{14}N_2O_2S$ (286.35): C 62.92, H 4.93, N 9.78; found: C 63.04, H 4.91, N 9.81.

*4-Chloro-6-(6-methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**12**). Yield 86%. M.p. 227–229°. IR: 3013 (OH, arom. C–H), 1627 (C=N), 1609 (C=C), 1188 (C–OH). ^{1}H-NMR ((D$_6$)DMSO): 11.60 (*s*, HO–C(1)); 10.90 (*s*, HO–C(3); 8.43 (*d*, H–C(5′)); 8.18 (*m*, H–C(7′)); 8.15 (*s*, H–C(5)); 6.86 (*s*, H–C(2)); 2.44 (*s*, Me). EI-MS: 292 (100, M^+), 264 (6), 258 (4), 201 (21), 187 (2), 132 (3), 122 (3), 95 (3), 69 (5), 51 (4), 39 (5). Anal. calc. for $C_{13}H_9ClN_2O_2S$ (292.74): C 53.34, H 3.10, N 9.57; found: C 53.57, H 3.09, N 9.62.

*4-(6-Methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,2,3-triol* (**13**). Yield 72%. M.p. 310–313°. IR: 3435 (OH), 3045 (arom. C–H), 2922 (C–H), 1631 (C=N), 1598 (C=C), 1548 (C=C), 1183 (C–OH). ^{1}H-NMR ((D$_6$)DMSO): 11.95 (*s*, HO–C(3)); 9.13 (*s*, HO–C(1)); 8.73 (*s*, HO–C(2)); 8.40 (*d*, *J* = 1.5, H–C(5′)); 8.15 (*m*, H–C(7′)); 7.41 (*d*, *J* = 8.7, H–C(5)); 6.53 (*d*, *J* = 8.7, H–C(6)); 2.45 (*s*, Me). ^{13}C-NMR ((D$_6$)DMSO): 167.2; 153.6; 149.8; 147.3; 147.1; 144.4; 133.0; 131.4; 128.3; 119.2; 110.7; 108.5; 17.8. EI-MS: 274 (100, M^+), 245 (5), 209 (7), 201 (6), 190 (8), 175 (7), 164 (4), 137 (3), 123 (4), 95 (2), 81 (2), 65 (1), 53 (2), 39 (2). Anal. calc. for $C_{13}H_{10}N_2O_3S$ (274.30): C 56.92, H 3.67, N 10.21; found: C 57.13, H 3.66, N 10.18.

*4-Methyl-6-(5-methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**14**). Yield 81%. M.p. 251–254°. IR: 3116 (OH), 2922 (C–H), 1624 (C=N), 1504 (C=C), 1236 (C–OH). ^{1}H-NMR ($CDCl_3$): 12.30 (*s*, HO–C(1)); 10.31 (*s*, HO–C(3)); 8.23 (*d*, *J* = 8.3, H–C(7′)); 7.53 (*d*, *J* = 8.7, H–C(5)); 7.44 (*d*, *J* = 8.4, H–C(6′)); 7.60 (*d*, *J* = 8.7, H–C(2)); 2.61 (*s*, Me); 2.07 (*s*, Me). EI-MS: 272 (100, M^+), 243 (22), 227 (7), 215 (10), 201 (6), 188 (3), 65 (4), 51 (3), 39 (7). Anal. calc. for $C_{14}H_{12}N_2O_2S$ (272.32): C 61.75, H 4.44, N 10.29; found: C 61.92, H 4.42, N 10.32.

*4-Ethyl-6-(5-methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**15**). Yield 77%. M.p. 270–273°. IR: 3385 (OH), 3078 (arom. C–H), 2969 (CH), 1619 (C=N), 1503 (C=C), 1250 (C–OH). ^{1}H-NMR ((D$_6$)DMSO): 10.32 (*s*, HO–C(3)); 8.33 (*d*, *J* = 8.3, H–C(7′)); 7.87 (*s*, H–C(5)); 7.49 (*d*, *J* = 8.4, H–C(6′)); 6.63 (*s*, H–C(2)); 2.67 (*s*, Me); 2.52 (*m*, Me*CH*$_2$); 1.16 (*t*, *Me*CH$_2$). ^{13}C-NMR ((D$_6$)DMSO): 165.1; 160.5; 156.7; 152.5; 152.4; 144.4; 131.4; 128.2; 123.3; 122.6; 109.5; 102.3; 22.1; 21.5; 14.3. EI-MS: 286 (54, M^+), 271 (100, $[M-\text{Me}]^+$), 243 (7), 215 (4), 201 (7), 143 (7), 69 (3), 36 (29). Anal. calc. for $C_{15}H_{14}N_2O_2S$ (286.35): C 62.92, H 4.93, N 9.78; found: C 63.18, H 5.00, N 9.69.

*4-Chloro-6-(5-methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,3-diol* (**16**). Yield 85%. M.p. 278–280°. IR: 2995 (C–H), 1633 (C=N), 1584 (C=C), 1525 (C=C), 1185 (C–OH), 1100 (C–Cl). ^{1}H-NMR ((D$_6$)DMSO): 11.80 (*s*, HO–C(1)); 11.10 (*s*, HO–C(3)); 8.27 (*d*, *J* = 8.3, H–C(7′)); 8.14 (*s*, H–C(5)); 7.45 (*d*, *J* = 8.3, H–C(6′)); 6.87 (*d*, H–C(2)); 2.64 (*s*, Me). ^{13}C-NMR ((D$_6$)DMSO): 162.5; 157.1; 156.6; 153.5; 153.7; 143.7; 130.9; 128.4; 122.3; 112.2; 111.5; 103.8; 22.4. EI-MS: 292 (100, M^+), 265 (8), 259 (5), 201 (24), 132 (2), 121 (4), 94 (6), 69 (7), 51 (3), 39 (6). Anal. calc. for $C_{13}H_9ClN_2O_2S$ (292.74): C 53.34, H 3.10, N 9.57; found: C 53.48, H 3.09, N 9.54.

*4-(5-Methyl-1,3-thiazolo[5,4-*b*]pyridin-2-yl)benzene-1,2,3-triol* (**17**). Yield 82%. M.p. 319–321°. IR: 3450 (OH), 3127 (OH), 1630 (C=N), 1227 (C–OH). ^{1}H-NMR ((D$_6$)DMSO): 9.42 (*s*, HO–C(2)); 8.31 (*d*, *J* = 8.3, H–C(7′)); 7.46 (*m*, H–C(6′), H–C(5)); 6.54 (*d*, *J* = 8.7, H–C(6)); 2.65 (*s*, Me). EI-MS: 274 (100, M^+), 245 (4), 217 (4), 201 (6), 190 (7), 175 (6), 137 (3), 123 (4), 81 (2), 39 (2). Anal. calc. for $C_{13}H_{10}N_2O_3S$ (274.30): C 56.92, H 3.67, N 10.21; found: C 57.13, H 3.65, N 10.24.

Bioassays. The following human cell lines were used *in vitro:* T47D (breast cancer), SW707 (rectal adenocarcinoma), and A549 (nonsmall cell lung carcinoma) from the *American Type Culture Collection* (*Rockville*, Maryland, USA), and HCV29T (bladder cancer) from the Fibiger Institute, DK-Copenhagen. Twenty-four hours before the addition of the agents to be tested, the cells were plated in 96-well plates (Sarstedt, USA) at a density of 10^4 cells/well. All cell lines were maintained in the opti-MEM medium supplement with 2 mM glutamine (*Gibco*; PL-Warsaw), streptomycin (50 μg/ml), penicillin (50 U/ml; *Polfa*; PL-Tarchomin), and 5% fetal calf serum (*Gibco*, Grand Island, USA). The cells were incubated at 37° in a humid atmosphere saturated with 5% CO_2. The solns. of compounds (1 mg/ml) were prepared *ex tempore* by dissolving the substance in 100 μl of DMSO completed with 900 μl of tissue culture medium. Then, the compounds were diluted in the culture medium to reach the final concentrations ranging from 0.1 to 100 μg/ml. The solvent (DMSO) at the highest concentration used in the test did not reveal any cytotoxic activity. Cisplatin was applied as a test reference 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 of the tested agents. The sulforhodamine B (SRB) test to determine the cell proliferation inhibition in *in vitro* culture was applied [32]. The cells attached to the plastic were fixed with cold 50% CCl_3CO_2H (*Aldrich-Chemie*, Germany) added on the top of the culture medium in each well. The plates were incubated at 4° 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 CCl_3CO_2H was stained with 0.4% SRB (*Sigma*; Germany) dissolved in 1% AcOH (POCh, PL-Gliwice) for 30 min. The unbound dye was removed by rinsing (four times) with 1% AcOH, and the protein-bound dye was extracted with 10 mM unbuffered *Tris* base (tris(hydroxymethyl)amino-methane; POCh, PL-Gliwice) for determination of the optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Uniskan II (*Labsystems*, FI-Helsinki). The compounds were tested in triplicates per experiment. The experiments were repeated at least three times.

Cisplatin was used for comparison. It is not characterized by great stability, but it has been used as the reference drug in biological studies of new potential anticancer agents [33]. It was reported that it is stable for 24 h in the admixtures containing NaCl concentrations of 0.3% or larger [34]. Our studies showed that its biological effect starting with the 24-h incubation was stable and did not depend on incubation time.

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