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Original article

Synthesis and biological activity of new 2-amino-8-chloro-5,5-dioxo[1,2,4]triazolo[2,3-*b*][1,4,2]benzodithiazines

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Abstract

Two series of 1-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-4-arylsemicarbazides **6**–**17** and 2-arylamino-8-chloro-5,5-dioxo[1,2,4]triazolo [2,3-*b*][1,4,2]benzodithiazines **18**–**26** were prepared in order to evaluate their biological activity. Compounds **6** and **18**–**26** were tested for their in vitro cytotoxic potency against 12 human cancer cell lines. The compounds **6** and **19** were inactive, whereas triazolobenzodithiazines **18**, **20**–**26** possess tumor growth inhibitory properties. The prominent methyl 8-chloro-2-(4-chlorophenylamino)-5,5-dioxo[1,2,4]triazolo[2,3-*b*][1,4,2]benzodithiazine-7-carboxylate (**21**) exhibited potency higher or comparable to cisplatin. Moreover, compounds **6**, **9**, **19** and **23**–**25** with structure similar to other chemotherapeutic agents were tested for their antibacterial activity and exhibited MIC and MBC against *Staphylococcus aureus* (3.9–31.5 μg ml).
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Keywords: Triazolo[2,3-*b*] [1,4,2]benzodithiazines; Cytotoxic activity; Antibacterial activity

1. Introduction

Cancer is one of the most feared diseases in the modern society. A variety of approaches have been taken to cancer chemotherapy, and many antitumor drugs have been developed for clinical use, but cancer still remains one of the leading causes of human mortality. Recently, aryl and heteroarylsulfonamides have been attracted attention as anticancer agents [1–7]. Our extensive research program aimed at the synthesis of 1,1-dioxo-3-methylthio-1,4,2-benzodithiazines and their subsequent transformation into 4-chloro-2-mercaptobenzenesulfonamides (MBSAs) with the nitrogen atom of sulfonamide moiety attached to variety of heterocyclic ring system. These compounds, depending on structure, showed either antiarrhythmic [8–10], anti-HIV-1 (Fig. 1 MBSAs structure **I** – integrase inhibitors) [11–15] or anticancer [16–20] activities. Furthermore, a number of different cyclic analogues of 2-MBSAs (Fig. 1 **II**–**V**) have been displayed to act as anticancer agents [21–25]. In our previous study we have reported that variously substituted 1,2,4-triazolo[4,3-*b*][1,4,2]benzodithiazines caused considerable growth inhibition on distinct tumor cell lines [24]. To explore in details the structure–activity relationships of this class of compounds, analogues triazolobenzodithiazines have been obtained with variations at both positions 7 and 2. To investigate the importance of the substituent at position 7 on the cytotoxic activity, compounds lacking this substituent have been prepared. Derivatives with various substituted benzene or naphthalene rings at position 2 have also been synthesized. Hence, in search for even more potent derivatives and for structure activity relationship studies we synthesized a new series of 2-amino-8-chloro-5,5-dioxo [1,2,4]triazolo[2,3-*b*][1,4,2] benzodithiazines.

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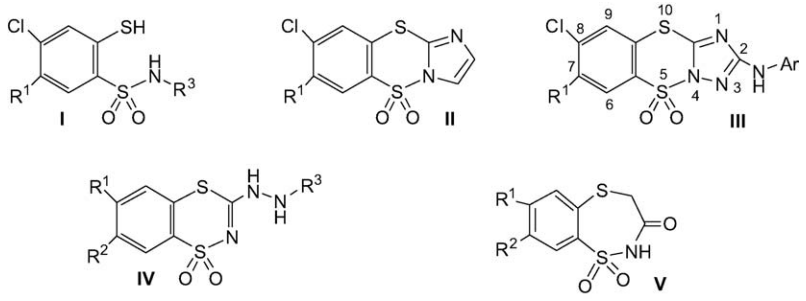


Fig. 1.

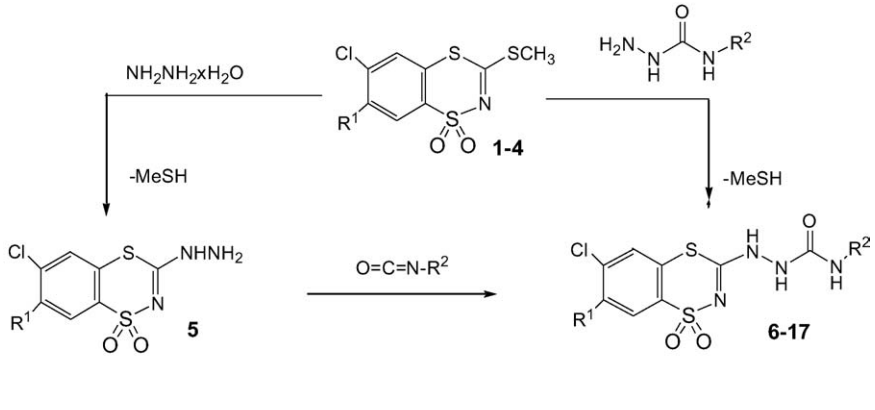
2. Results and discussion

2.1. Chemistry

The key intermediates for the syntheses of 6-chloro-1,1-dioxo-3-methylthio-1,4,2-benzodithiazines (**1**–**4**) and (6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-yl)hydrazine (**5**) were obtained according to the methods described previously (see Section 4). The reaction of hydrazine **5** with the corresponding isocyanates have led to the formation of the expected 1-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-4-arylsemicarbazides **6**, **7**. In turn, nucleophilic displacement of the 3-methylthio group of the appropriate 6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine **1**–**4** by 4-arylsemicarbazide proceeded with methylthiol evolution to give semicarbazides **8**–**17** (Scheme 1). Treatment of semicarbazides **7**, **10**–**17** with an excess of phosphorus oxychloride under reflux gave rise to the target 2-amino-8-chloro-5,5-dioxo[1,2,4]triazolo[4,3-*b*][1,4,2]benzoditiazines **18**–**26**. A probable mechanism of the intramolecular ring closure was demonstrated in the our previous study [24].

2.2. Biology

Primary screening of compounds **6** and **18**–**26** for in vitro cytotoxic activity took place on six different human cancer cell



Compounds	R^1	R^2
1, 5, 6	Me	3,4-diClPh
1, 5, 7	Me	1-naphthyl
4, 8	4-ClPhNHCO	4-ClPh
4, 9	4-ClPhNHCO	4-MePh
1, 10	Me	3,5-diClPh
1, 11	Me	4-MeOPh
3, 12	COOMe	4-ClPh
3, 13	COOMe	4-MeOPh
2, 14	H	4-MePh
2, 15	H	Ph
2, 16	H	4-MeOPh
2, 17	H	4-ClPh

Compounds	R^1	R^2
7, 18	Me	1-naphthyl
10, 19	Me	3,5-diClPh
11, 20	Me	4-MeOPh
12, 21	COOMe	4-ClPh
13, 22	COOMe	4-MeOPh
14, 23	H	4-MePh
15, 24	H	Ph
16, 25	H	4-MeOPh
17, 26	H	4-ClPh

Scheme 1. Syntheses of the 1-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-4-arylsemicarbazides (**6**–**17**) and 8-chloro-2-arylamino-5,5-dioxo [1,2,4]triazolo[2,3-*b*][1,4,2]benzodithiazines (**18**–**26**).

Table 1
IC_{50} values (μM) for the inhibition of in vitro cell growth of human cancer cell lines by compounds **18**, **20**–**26**[a,b]

Tumor cell line	IC_{50} (μM) for compounds								
	18	**20**	**21**	**22**	**23**	**24**	**25**	**26**	Cisplatin
RT-4	32.6 ± 11.8	11.6 ± 1.5	3.5 ± 0.3	5.4 ± 1.2	12.3 ± 0.4	11.1 ± 2.0	13.4 ± 8.1		3.7
RT-112	20.1 ± 0.8	5.6 ± 0.2	1.7 ± 0.5	2.2 ± 0.7	Nd[c]	Nd	6.4 ± 2.3; 6.0 ± 1.1	Nd	2.14
5637	14.9 ± 1.8	5.8 ± 1.4	0.6 ± 0.1	1.9 ± 0.1	6.9 ± 0.4	7.4 ± 1.8			0.31
KYSE-70	28.6 ± 3.2	7.0 ± 0.5	9.2 ± 1.1	9.3 ± 0.2	Nd	Nd	Nd	1a7 ± 2.5	1.49
KYSE-510	> 20	10.3 ± 0.4	16.1 ± 1.6	13.4 ± 0.1	Nd	Nd	Nd	3.0 ± 0.1	0.88
KYSE-520	15.5 ± 1.9	7.4 ± 1.5	2.2 ±0.3	2.3 ± 0.4	8.5 ± 0.5	8.9 ± 1.6		5.4 ± 0.7	5.07
YAPC	29.7 ± 4.6	8.4 ± 0.1	4.3 ± 0.5	5.1 ± 0.5	Nd	Nd	Nd	Nd	6.03
DAN-G	15.9 ± 1.4	6.6 ± 1.6	3.5 ± 0.5	4.5 ± 0.4	Nd	Nd	Nd	Nd	1.39
LCLC-103H	9.6 ± 1.0	5.5 ± 0.3	2.5 ± 0.2	4.0 ± 1.6	6.7 ± 0.9	8.9 ± 1.6	4.1 ± 0.3	3.4 ± 0.5	1.63
A-427	17.3 ± 1.1	5.0 ± 0.8	6.2 ± 1.6	6.3 ± 1.0	6.1 ± 0.8	8.9 ± 0.2	4.0 ±0.2	2.5 ±0.3	Nd
MCF-7	20.4 ± 1.7	6.1 ± 0.5	2.1 ± 0.2	3.1 ± 0.5	Nd	Nd	Nd	Nd	0.74
SISO	20.0 ± 0.8	6.2 ± 1.0	6.3 ± 0.8	8.6 ± 1.8	9.5 ± 0.4	14.8 ± 2.5	6.8 ± 0.1	4.5 ± 0.7	4.52
Average[d]	20.42	7.12	4.84	5.50	8.32	10.0	6.80	4.92	2.14
R.S.D.[e] (%)[c]	16	14	17	16	7	16	29	16	91

[a] Compounds **6** and **19** were inactive.
[b] Values are averages of three independent determinations ± 1 SD.
[c] Not determined.
[d] Averaged IC50 values over all tested cancer cell lines.
[e] relative standard deviation.

lines. Compounds that showed enough activity at 20 μM to inhibit cell growth by more than 50% in one or more of the cell lines were further investigated. Secondary screening to determine potency was performed on a panel of 12 human cancer cell lines: three bladder cell lines: RT-4, RT-112, and 5637; three esophagus cell lines: KYSE-70, -510 and -520; two pancreas lines: YAPC and DAN-G; two lung cancer cell lines: LCLC-103H and A-427; a cervical cancer line: SISO and the breast cancer cell line: MCF-7 [26]. Table 1 lists the average IC_{50} values calculated from the dose–response data obtained from three independent experiments. The IC_{50} is the concentration required to inhibit cell growth by 50% compared to the untreated control over a 96 h treatment period. The following conclusion may be drawn from the structure–activity relationship study. The semicarbazide **6** and compound **19** were inactive. The most active compounds **21** and **22** possess at position 7 of the triazolobenzodithiazine ring system electron-withdrawing group such as the ester group or only the hydrogen atom (**26**). The presence of electron-donating group at this position decreases cytotoxicity (**18**–**20**). Replacement of the ester group in **22** (R^1 = COOMe) for methyl (**20**, $R^1 = CH_3$) caused a reasonable decrease of anticancer activity or total loss of it as in the case of **19**. Interestingly, the lack of substituent at position 7 leads to compounds with a good potency (e.g. **23**–**26**), suggesting that position 7 is not critical and thus is free for variations (Table 1).

Substitution of the exocyclic nitrogen atom at position 2 by naphthalene ring resulted in compound **18** with diminished selectivity toward leukemia cells lines compared to a benzene ring as reported by us previously in [24]. The presence of 4-chlorophenyl moiety at this position (**21**, R^2 = 4-ClPh) enhanced activity against the cell lines of urinary bladder cancer (5637, IC_{50} = 0.57 μM, RT-112, IC_{50} = 1.72 μM)). In some cell lines compound **21** shows similar (RT-4) or even higher (YAPC, KYSE-520, RT-112) activity than cisplatin (Table 1). Replacement of the chloro atom for the methoxy group results in a slight reduction in activity of **22** but in case of some cell lines (esophagus cancer, KYSE-520, pancreatic cancer, YAPC) growth inhibitory activity comparable to cisplatin was also found. Generally, it can be stated, that electronic character of substituent on the exocyclic nitrogen atom at position 2 of the triazolobenzodithiazine ring system influence on cytotoxicity of tested compounds **19**–**26**.

Several compounds derived from hydrazidehydrazones, semicarbazides and cyclic thiosemicarbazides derivatives are known to possess antibacterial, antifungal and antimycobacterial activities [27,28]. Therefore, in order to extend biological study on semicarbazides and triazolobenzodithiazines antibacterial activity was investigated in vitro on bacterial strains: *Escherichia coli* (NCTC 8196), *Staphylococcus aureus* (NCTC 4163) and *Pseudomonas aeruginosa* (NCTC 6749) [29]. Compounds **8**, **18**, **20**, **21**, **22** and **26** were inactive (MIC > 62. 5 μg ml^{-1}) while **6**, **9**, **19**, **23**, **24** and **25** showed activity against gram-positive *S. aureus* with MIC and MBC values of the range from 3.9 to 31.5 μg ml^{-1} (Table 2).

Table 2
Antibacterial activity of compounds **6**, **9**, **19** and **23**–**25** against *S. aureus* NCTC 4163

Compounds	MIC (μg ml^{-1})	MBC (μg ml^{-1})
6	7.81	62.5
9	15.25	–
19	31.5	31.5
23	3.9	3.9
24	15.62	15.62
25	15.62	15.62

3. Conclusion

A number of 2-amino-8-chloro-5,5-dioxo[1,2,4]triazolo[2,3-*b*][1,4,2]benzoditiazines were synthesized and evaluated for their antiproliferative activities on human cancer cells. Compounds **21** and **22** were the most potent of all derivatives tested and displayed inhibitory activity against cancer cell lines similar or higher than cisplatin. The results we have obtained hitherto indicated that the active compounds possess electron-withdrawing substituents at both positions 2 and 7 of the triazolobenzodithiazine scaffold. Moreover, the combined data obtained for both series [24] suggest that further modifications of the triazolobenzodithiazine substituents may lead to discovery of active compounds with selectivity against various cancer types.

4. Experimental protocols

4.1. Chemistry

Melting points are uncorrected and were determined on a Büchi SMP-20 apparatus. The IR spectra were recorded on 1600 FTIR Perkin Elmer spectrometer as potassium bromide pellets and frequencies are expressed in cm^{-1}. The ^{13}C NMR and ^{1}H NMR spectra were obtained on a Varian Gemini (200 MHz) or Varian Unity Plus (500 MHz) spectrometers in dimethyl sulfoxide-d6. The chemical shift values δ are expressed in ppm relative to tetramethylsilane as internal standard and coupling constants (*J*) are in Hertz. The analytical results for C, H, and N were within ± 0.4% of the theoretical values. The starting 6-chloro-3-methylthio-1,4,2-benzodithiazines **1**, **3** [30,31], **2** [23], **4** [32] and hydrazine **5** [33] were obtained by the previously described methods.

4.1.1. General procedure for preparation of 1-(6-chloro-1,1-dioxo-7-R^1-1,4,2-benzodithiazin-3-yl)-4-R^3-semicarbazides (**6**, **7**)

The suspension of the corresponding 6-chloro-1,1-dioxo-7-methyl-1,4,2-benzodithiazin-3-ylhydrazine **6** (5 mmol) and the appropriate isocyanate (5 mmol) in dry solvent (60 ml benzene or THF) was stirred at room temperature for 70 h, followed by reflux for 2 h. The solid that obtained was collected by filtration, washed (2 × 10 ml), dried and purified by crystallization from acetone (1:40). In this manner, the following semicarbazides were obtained.

*4.1.1.1. 1-(6-Chloro-1,1-dioxo-7-methyl-1,4,2-benzodithiazin-3-yl)-4-(3,4-dichlorophenyl)semicarbazide (**6**)*. Yield: 1.82 g, 78%, m.p. 232–234 °C; IR (KBr) 3280, 3108, 3210 (NH), 1687 (CONH), 11591, 1566, 1528 (C=N, aromat.), 1302, 1155 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 7.41–7.59 (m, 2H, aromat.), 7.81–7.89 (m, 1H, aromat.), 7.95 (s, 1H, H-5 benzodithiazine), 8.01 (s, 1H, H-8 benzodithiazine), 9.21 (s, 1H, HN-2), 9.72 (s, 1H, HN-4), 11.28 (s, 1H, HN-1) ppm. Anal. ($C_{15}H_{11}C_{13}N_4O_3S_2$) C, H, N.

*4.1.1.2. 1-(6-Chloro-1,1-dioxo-7-methyl-1,4,2-benzodithiazin-3-yl)-4-(1-naphthyl)semicarbazide (**7**)*. Yield: 1.74 g, 78%, m.p. 214–215 °C; IR (KBr) 3277, 3170 (NH), 1709, 1674 (CONH), 1560 (C=N, aromat.), 1345, 1150 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 7.42–8.14 (m, 9H, aromat), 9.13 (s, 1H, HN-2), 9.35 (s, 1H, HN-4), 11.31 (s, 1H, HN-1) ppm. Anal. ($C_{19}H_{15}ClN_4O_3S_2$) C, H, N.

4.1.2. General procedure for the preparation of 1-(6-chloro-1,1-dioxo-7-R^1-1,4,2-benzodithiazin-3-yl)-4-phenylsemicarbazides (**8**–**17**)

A stirred mixture of the appropriate 3-methylthiobenzodithiazine **1**–**3** or **4** (5 mmol), the proper arylsemicarbazide (5 mmol), and methanol (100 ml) was refluxed until the evolution of MeSH had ceased (40–50 h) [CAUTION: because of high toxicity, MeSH should be trapped in an aqueous NaOH solution]. The precipitate was filtered off, washed with methanol and dried. In this manner the following semicarbazide were obtained.

*4.1.2.1. 1-[6-Chloro-1,1-dioxo-7-(4-chlorophenylcarbamoyl)-1,4,2-benzodithiazin-3-yl]-4-(4-chlorophenyl)semicarbazide (**8**)*. Yield: 2.67 g, 93%, m.p. 284–286 °C; IR (KBr) 3350, 3250, 3207 (NH), 1684, 1660 (CONH), 1310, 1164 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.31–7.60 (m, 4H, aromat.), 7.70–7.80 (m, 4H, aromat.), 8.19 (s, 2H, H-5, H-8 benzodithiazine), 9.14 (s, 1H, HN-2), 9.64 (s, 1H, HN-4), 10.87 (s, 1H, NHCO), 11.49 (br.s, 1H, HN-1) ppm. ^{13}C NMR (DMSO-d_6) δ 120.53, 120.76, 121.66, 124.82, 126.58, 128.09, 128.92, 129.06, 129.70, 130.29, 132.18, 134.02, 137.07, 137.73, 138.26 (aromat.), 154.64 (C=N), 163.06, 169.60 (CONH) ppm. Anal. ($C_{21}H_{14}Cl_3N_5O_4S_2$) C, H, N.

*4.1.2.2. 1-[6-Chloro-1,1-dioxo-7-(4-chlorophenylcarbamoyl)-1,4,2-benzodithiazin-3-yl]-4-(4-methylphenyl)semicarbazide (**9**)*. Yield: 2.50 g, 93%, m.p. 265–267 °C; IR (KBr) 3348, 3215 (NH), 1684, 1660 (CONH), 1310, 1164 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H, CH_3), 7.08–7.77 (m, 8H, aromat.), 8.16 (s, 2H, H-5), 8.19 (s, 1H, H-8 benzodithiazine), 8.97 (s, 1H, HN-2), 9.37 (s, 1H, HN-4), 10.73 (s, 1H, NHCO), 11.41 (s, 1H, HN-1) ppm. ^{13}C NMR (DMSO-d_6) δ 20.63 (CH_3), 119.12, 119.33, 121.66, 124.79, 128.08, 129.07, 129.42, 129.69, 130.33, 131.85, 132.29, 133.98, 136.62, 137.03, 137.73 (aromat.), 154.68 (C=N), 163.07, 169.64 (CONH) ppm. Anal. ($C_{22}H_{17}Cl_2N_5O_4S_2$) C, H, N.

*4.1.2.3. 1-(6-Chloro-1,1-dioxo-7-methyl-1,4,2-benzodithiazin-3-yl)-4-(3,5-dichlorophenyl)semicarbazide (**10**)*. Yield: 1.90 g, 82%, m.p. 242–244 °C; IR (KBr) 3300, 3210 (NH), 1705 (CONH), 1600 (C=N), 1300, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 7.16–7.25 (m, 1H, aromat.), 7.55–7.65 (m, 2H, aromat.), 7.96 (s, 1H, H-5), 8.02 (s, 1H, H-8), 9.31 (s, 1H, HN-2), 9.79 (s, 1H, HN-4), 11.30 (br.s, 1H, HN-1) ppm. ^{13}C NMR (DMSO-d_6) δ 19.58 (CH_3), 117.0, 117.22, 122.03, 126.63, 127.68, 128.54, 130.16, 134.29,

134.33, 137.45, 137.85, 137.95, 141.86 (aromat.), 154.58 (C=N), 169.80 (CONH) ppm. Anal. ($C_{15}H_{11}Cl_3N_4O_3S_2$) C, H, N.

*4.1.2.4. 1-(6-Chloro-1,1-dioxo-7-methyl-1,4,2-benzodithiazin-3-yl)-4-(4-methoxyphenyl)semicarbazide (**11**)*. Yield: 1.85 g, 87%, m.p. 238–240 °C; IR (KBr) 3300, 3250, 3190 (NH), 1680 (CONH), 1602 (C=N), 1320, 1150 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 6.81–6.42 (m, 4H, aromat.), 7.95 (s, 1H, H-5), 8.0 (s, 1H, H-8), 8.86 (s, 1H, HN-2), 9.2 (s, 1H, HN-4), 11.19 (br.s, 1H, HN-1) ppm. Anal. ($C_{16}H_{15}ClN_4O_4S_2$) C, H, N.

*4.1.2.5. 1-(6-Chloro-1,1-dioxo-7-methoxycarbonyl-1,4,2-benzodithiazin-3-yl)-4-(4-chlorophenyl)semicarbazide (**12**)*. Yield: 1.95 g, 82%, m.p. 229–230 °C; IR (KBr) 3348, 3215 (NH), 1740, 1720, 1701 (CO), 1670 (CONH), 1604 (C=N), 1325, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.90 (s, 3H, OCH_3), 7.29–7.58 (m, 4H, phenyl), 8.18 (s, 1H, H-5), 8.37 (s, 1H, H-8), 9.10 (s, 1H, HN-2), 9.60 (s, 1H, HN-4), 11.48 (s, 1H, HN-1) ppm. Anal. ($C_{16}H_{12}Cl_2N_4O_5S_2$) C, H, N.

*4.1.2.6. 1-(6-Chloro-1,1-dioxo-7-methoxycarbonyl-1,4,2-benzodithiazin-3-yl)-4-(4-methoxyphenyl)semicarbazide (**13**)*. Yield: 1.80 g, 78%, m.p. 206–207 °C; IR (KBr) 3322, 3205 (NH), 1732 (CO), 1695 (CONH), 1603 (C=N), 1300, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.70 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.82–6.91 (m, 2H, phenyl), 7.31–7.41 (m, 2H, phenyl), 8.18 (s, 1H, H-5), 8.36 (s, 1H, H-8), 8.92 (s, 1H, HN-2), 9.25 (s, 1H, HN-4), 11.42 (s, 1H, HN-1) ppm. Anal. ($C_{17}H_{15}ClN_4O_6S_2$) C, H, N.

*4.1.2.7. 1-(6-Chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-4-(4-methylphenyl)semicarbazide (**14**)*. Yield: 0.78 g, 40%, m.p. 193–195 °C; IR (KBr) 3296, 3190 (NH), 1682 (CONH), 1601 (C=N), 1320, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.23 (s, 3H, CH_3), 7.01–7.41 (m, 4H, aromat.), 7.69 (dd, $J_{5,7}$ = 1.95 Hz, $J_{8,7}$ = 8.42 Hz, 1H, H-7), 7.99 (d, J = 8.3 Hz, 1H, H-8), 8.0 (d, J = 1.95 Hz, 1H, H-5), 8.90 (s, 1H, HN-2), 9.29 (s, 1H, HN-4), 11.27 (s, 1H, HN-1) ppm. ^{13}C NMR (DMSO-d_6) δ 20.33 (CH_3), 118.82, 119.03, 126.01, 127.98, 129.09, 129.41, 131.10, 131.50, 136.34, 136.65 (C aromat.), 154.42 (C=N), 169.48 (CONH) ppm. Anal. ($C_{15}H_{13}ClN_4O_3S_2$) C, H, N.

*4.1.2.8. 1-(6-Chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-4-phenylsemicarbazide (**15**)*. Yield: 0.59 g, 31%, m.p. 178–180 °C; IR (KBr) 3340, 3195 (NH), 1690 (CONH), 1600 (C=N), 1316, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.94–7.53 (m, 5H, phenyl), 7.69 (dd, $J_{5,7}$ = 1.95 Hz, $J_{8,7}$ = 8.42 Hz, 1H, H-7), 7.95–8.04 (m, 2H, H-5, H-8), 8.96 (s, 1H, HN-2), 9.4 (s, 1H, HN-4), 11.29 (s, 1H, HN-1) ppm. Anal. ($C_{14}H_{11}ClN_4O_3S_2$) C, H, N.

*4.1.2.9. 1-(6-Chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-4-(4-methoxyphenyl)semicarbazide (**16**)*. Yield: 0.91 g, 48%, m.p. 197–199 °C; IR (KBr) 3300, 3230, 3189 (NH), 1676 (CONH), 1600 (C=N), 1320, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.70 (s, 3H, OCH_3), 6.80–7.45 (m, 4H, aromat.), 7.69 (dd, $J_{5,7}$ = 1.83 Hz, $J_{8,7}$ = 8.50 Hz, 1H, H-7), 7.97 (s, 1H, H-8), 8.01(s, 1H, H-5), 8.88 (s, 1H, HN-2), 9.21 (s, 1H, HN-4), 11.26 (s, 1H, HN-1) ppm. Anal. ($C_{15}H_{13}ClN_4O_4S_2$) C, H, N.

*4.1.2.10. 1-(6-Chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)-4-(4-chlorophenyl)semicarbazide (**17**)*. Yield: 1.4 g, 87%, m.p. 227–228 °C; IR (KBr) 3346, 3220 (NH), 1690 (CONH), 1596 (C=N, aromat.), 1300, 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.28–7.58 (m, 4H, aromat.), 7.66–7.75 (m, 1H, H-7), 7.94–8.05 (m, 2H, H-8, H-5), 9.07 (s, 1H, HN-2), 9.57 (s, 1H, HN-4), 11.30 (br.s, 1H, HN-1) ppm. Anal. ($C_{14}H_{10}Cl_2N_4O_3S_2$) C, H, N.

*4.1.3. General procedure for the preparation of 8-chloro-2-arylamino-7-R^1-5,5-dioxo[1,2,4]triazolo[2,3-b][1,4,2] benzodithiazines (**18**–**26**)*

A stirred mixture of the appropriate semicarbazide **7**, **10**–**17** (5 mmol) and phosphorus oxychloride (30 ml) was refluxed for 18 h. After cooling, the resulting solution was poured onto crashed ice (200 g) and stirred at room temperature for 10 h. The precipitate that deposited was collected by filtration, washed thoroughly with several portions of water (pH 7), dried and crystallized from dimethylformamide (1:9). In this manner the following triazolobenzodithiazines were obtained.

*4.1.3.1. 8-Chloro-7-methyl-2-(1-naphthylamino)-5,5-dioxo[1,2,4]triazolo[2,3b][1,4,2]benzodithiazine (**18**)*. Yield: 0.65 g, 30%, m.p. 215–216 °C; IR (KBr) 3405 (NH), 1625 (C=N), 1567, 1555 (aromat.), 1353, 1182 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.52–8.0 (m, 6H, aromat.), 8.27–8.36 (m, 2H, H-9, aromat.), 8.41 (s, 1H, H-6), 9.95 (s, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6) δ 20.58 (CH_3), 115.90, 120.22, 123.92, 126.50, 126.62, 126.69, 126.70, 128.90, 129.36, 129.43, 134.08, 134.62, 138.54, 141.24 (aromat.), 153.18 (C=N), 162.19 (C=N) ppm. Anal. ($C_{19}H_{13}ClN_4O_2S_2$) C, H, N.

*4.1.3.2. 8-Chloro-7-methyl-2-(3,5-dichlorophenylamino)-5,5-dioxo[1,2,4]triazolo[2,3-b][1,4,2]benzodithiazine (**19**)*. Yield: 1.05 g, 47%, m.p. 252–253 °C; IR (KBr) 3297, 3136 (NH), 1605 (C=N), 1595, 1555 (aromat.), 1370, 1185 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.59 (s, 3H, CH_3), 7.14 (t, J = 1.71 Hz, 1H, phenyl.), 7.58 (d, J = 1.71 Hz, 2H, phenyl), 8.26 (s, 1H, H-9), 8.39 (s, 1H, H-6), 10.57 (s, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6) δ 19.52 (CH_3), 115.33, 120.57, 125.87, 127.94, 129.87, 130.48, 134.62, 138.45, 140.19, 142.43 (aromat.), 153.21 (C=N), 160.57 (C=N) ppm. Anal. ($C_{15}H_9Cl_3N_4O_2S_2$) C, H, N.

*4.1.3.3. 8-Chloro-2-(4-methoxyphenylamino)-7-methyl-5,5-dioxo[1,2,4]triazolo[2,3-b][1,4,2]benzodithiazine (**20**)*. Yield:

0.99 g, 49%, m.p. 207–208 °C; IR (KBr) 3317 (NH), 1608 (C=N), 1368, 1184 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.50 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 6.94 (d, $J = 8.88$ Hz, 2H, aromat.), 7.49 (d, $J = 8.88$ Hz, 2H, aromat.), 8.26 (s, 1H, H-9), 8.39 (s, 1H, H-6), 9.88 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.54 (CH_3), 55.50 (OCH_3), 114.53, 118.88, 125.83, 127.94, 129.79, 130.59, 133.37, 138.37, 140.09, 153.26 (aromat.), 154.37 (C=N), 161.86 (C=N) ppm. Anal. ($C_{16}H_{13}ClN_4O_3S_2$) C, H, N.

*4.1.3.4. Methyl 8-chloro-2-(4-chlorophenylamino)-5,5-dioxo[1,2,4] triazolo[2,3-b][1,4,2]benzodithiazine-7-carboxylate (**21**)*. Yield: 0.94 g, 41%, m.p. 228–229 °C; IR (KBr) 3411 (NH), 1738 (CO), 1600 (C=N), 1370, 1140 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.92 (s, 3H, OCH_3), 7.34–7.62 (m, 4H, aromat.), 8.46 (s, 1H, H-9), 8.64 (s, 1H, H-6), 10.33 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 53.59 (OCH_3), 102.20, 118.81, 125.23, 128.46, 129.19, 130.32, 130.62, 132.09, 132.84, 138.19, 139.95 (aromat.), 152.56, 161.21 (C=N), 163.28 (CO) ppm. Anal. ($C_{16}H_{10}Cl_2N_4O_4S_2$) C, H, N.

*4.1.3.5. Methyl 8-chloro-2-(4-methoxyphenylamino)-5,5-dioxo [1,2,4]triazolo[2,3-b][1,4,2]benzodithiazine-7-carboxylate (**22**)*. Yield: 0.86 g, 38%, m.p. 205–207 °C; IR (KBr) 3340 (NH), 1738 (CO), 1610 (C=N), 1363, 1180 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.71 (s, 3H, OCH_3), 3.92 (s, 3H, $COOCH_3$), 6.93 (d, $J = 9.0$ Hz, 2H, aromat.), 7.47 (d, $J = 9.0$ Hz, 2H, aromat.), 8.44 (s, 1H, H-9), 8.63 (s, 1H, H-6), 9.93 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 53.56, 55.48 (OCH_3), 114.52, 118.89, 128.45, 130.21, 130.59, 132.02, 132.60, 133.24, 138.18, 154.41 (aromat.), 152.601, 161.86 (C=N), 163.26 (CO) ppm. Anal. ($C_{17}H_{13}ClN_4O_5S_2$) C, H, N.

*4.1.3.6. 8-Chloro-2-(4-methylphenylamino)-5,5-dioxo[1,2,4] triazolo[2,3-b][1,4,2]benzodithiazine. (**23**).* Yield: 0.55 g, 29%, m.p. 216–217 °C; IR (KBr) 3381 (NH), 1603 (C=N), 1367, 1180 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.25 (s, 3H, CH_3Ph), 7.09–7.51 (m, 4H, aromat.), 7.85 (dd, $J_{7,9} = 1.71$ Hz, $J_{7,6} = 8.6$ Hz, 1H, H-7), 8.27–8.39 (m, 2H, H-9, H-6), 10.0 (s, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6) δ 22.15 (CH_3), 118.89, 129.31, 130.93, 131.14, 131.25, 131.94, 132.29, 139.07, 141.21 (aromat.), 154.44, 163.15 (C=N) ppm. Anal. ($C_{15}H_{11}ClN_4O_2S_2$) C, H, N.

*4.1.3.7. 8-Chloro-2-phenylamino-5,5-dioxo[1,2,4]triazolo[2,3-b][1,4,2]benzodithiazine (**24**)*. Yield: 0.56 g, 31%, m.p. 218–220 °C; IR (KBr) 3382 (NH), 1601 (C=N), 1370, 1180 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.90–7.62 (m, 5H, aromat.), 7.86 (dd, $J_{7,9} = 1.46$ Hz, $J_{7,6} = 8.8$ Hz, 1H, H-7), 8.28–8.40 (m, 2H, H-9, H-6), 10.12 (s, 1H, NH) ppm. Anal. ($C_{14}H_9ClN_4O_2S_2$) C, H, N.

*4.1.3.8. 8-Chloro-2-(4-methoxyphenylamino)-5,5-dioxo[1,2,4] triazolo[2,3-b][1,4,2]benzodithiazine (**25**)*. Yield: 0.59 g, 30%, m.p. 199–201 °C; IR (KBr) 3388 (NH), 1609 (C=N), 1360, 1180 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.72 (s, 3H, OCH_3), 6.93 (d, $J = 8.8$ Hz, 2H, aromat.), 7.48 (d, $J = 8.8$ Hz, 2H, aromat.), 7.80–7.90 (m, 1H, H-7), 8.26–8.38 (m, 2H, H-9, H-6), 9.90 (s, 1H, NH) ppm. Anal. ($C_{15}H_{11}ClN_4O_3S_2$) C, H, N.

*4.1.3.9. 8-Chloro-2-(4-chlorophenylamino)-5,5-dioxo[1,2,4] triazolo[2,3-b][1,4,2]benzodithiazine (**26**)*. Yield: 1.0 g, 50%, m.p. 250–251 °C; IR (KBr) 3378 (NH), 1602 (C=N), 1362, 1190 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.36–7.62 (m, 4H, aromat.), 7.86 (dd, $J_{7,9} = 1.91$ Hz, $J_{7,6} = 8.5$ Hz, 1H, H-7), 8.30–8.38 (m, 2H, H-9, H-6), 10.29 (s, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6) δ 118.48, 124.85, 127.54, 128.84, 129.32, 129.43, 129.48, 130.46, 138.72, 139.44 (aromat.), 152.10, 160.88 (C=N) ppm. Anal. ($C_{14}H_8Cl_2N_4O_2S_2$) C, H, N.

4.2. Biology

4.2.1. Cytotoxicity studies

All reagents were purchased from Sigma (Deisenhofen, Germany) and the cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) (Brauschweig, Germany). Cell lines used here were: human bladder cancer RT-4, RT-112 and 5637; human esophagus cancer KYSE-70, KYSE-510 and KYSE-520; human pancreatic cancer YAPC and DAN-G; human cervix cancer SISO; human non-small cell lung cancer LCLC-103H and A-427 and human breast cancer MCF-7. The culture medium was RPMI-1640 medium containing 2 g l^{-1} HCO_3, and 10% FCS. Cells were grown in 75 cm^2 plastic culture flasks (Sarstedt, Nümbrecht, Germany) in a humid atmosphere of 5% CO_2 at 37 °C and passaged shortly before becoming confluent. For the cytotoxicity studies, 100 μl of a cell suspension were seeded into 96-well microtiter plates (Sarstedt) at a density of 1000 cells per well, except for the LCLC-103H cell line, which was plated out at 250 cells per well. One day after plating cells were treated with test substance at five concentrations per compound: 1000-fold concentrated stock solutions in DMF were serially diluted by 50% in DMF to give the feed solutions, which were diluted 500-fold into culture medium. The controls received just DMF. Each concentration was tested in eight wells, with each well receiving 100 μl of the medium containing the test substance. The concentration ranges were chosen to bracket the expected IC_{50} values as best as possible. Cells were then incubated for 96 h, after which time the medium was removed and replaced with a 1% glutaraldehyde/PBS solution for 20 min. Cells were stored at 4 °C under PBS. Staining with crystal violet was done as previously described in [26]. O.D. was measured at $\lambda = 570$ nm with an Anthos 2010 plate reader (Salzburg, Austria). Corrected T/C values were calculated by the equation: $(T/C)_{corr}$ (%) = $(O.D._T - O.D._{c,\ 0})/(O.D._c - O.D._{c,\ 0}) \times 100$ where $O.D._T$ is the mean absorbance of the treated cells, $O.D._c$ the mean absorbance of the controls and $O.D._{c,\ 0}$ the mean absorbance at the time drug was added. The IC_{50} values were estimated by a linear least-squares regression of the T/C_{corr} values versus the logarithm of the substance concentra-

tion; only concentrations that yielded T/C_{corr} values between 10% and 90% were used in the calculation. The reported IC_{50} values are the average of two to three independent experiments and these varied less than 20% from the individual values.

4.2.2. Antibacterial assay

Antibacterial activity was investigated in vitro on bacterial strains: *E. coli* (NCTC 8196), *S. aureus* (NCTC 4163) and *P. aeruginosa* (NCTC 6749). Overnight bacterial culture was diluted with Mueller–Hinton broth to the density of 105 CFU ml^{-1}. Tested compounds were dissolved in dimethyl sulfoxide and diluted (in geometric progression) to the concentration 62.5–1.9 μg ml^{-1} using Mueller–Hinton broth. Each tube was then inoculated with the bacterial suspension and incubated at 37 °C for 24 h. The lowest concentration at which there was no visible growth was taken as the minimal inhibitory concentration (MIC). In addition 100 μl of suspension from each tube without growth were inoculated in Mueller–Hinton agar plate to control bacterial viability. The minimal bactericidal concentration (MBC) was defined as the minimal concentration of compounds required to kill of the organisms in the medium after 24 h incubation [29].

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