Full Paper

Synthesis and Cytotoxicity Testing of Novel 2-(3-Substituted-6-chloro-1,1-dioxo-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3*H*)-quinazolinones

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A new series of thirteen 2-[3-(substituted amino)-6-chloro-1,1-dioxo-1,4,2-benzodithiazin-7-yl]-3-phenyl-4(3*H*)-quinazolinones **4-16** were prepared in order to evaluate their cytotoxic activity against 12 human cancer cell lines. The bioassay indicated that the quinazolinone derivatives **5**, **8**–**12**, **15**, and **16** possess cancer-cell growth-inhibitory properties. Compounds **5** and **12** showed a high level of selectivity for certain cell lines. The most active compounds **9**, **10**, **15**, and **16** showed moderate antiproliferative activity and were approximately 4-fold less potent than cisplatin.

Keywords: Cytotoxic activity / 2-Substituted-3-phenyl-4(3*H*)-quinazolinones / Synthesis

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Introduction

Quinazolinone derivatives have drawn attention due to their broad range of pharmacological activities, among others antifungal, anti-Parkinsonism, antimalarial, anti-inflammatory, anticonvulsant, antibacterial, antihypertensive [1–5], and anticancer activities [6–8]. Moreover, quinazolinone drugs with antiproliferative activity are known to bind to tubulin and interfere with its polymerization [9–11]. Our systematic studies on the synthesis of 1,4,2-benzodithiazine 1,1-dioxides and their subsequent transformation into *N*-(azolyl or azinyl)-2-mercaptobenzenesulfonamides have resulted in promising anticancer agents [12–14] or potent inhibitors of HIV-1 integrase (MBSAs) [15]. We also found that cyclic sulfonamide derivatives of 2-amino-8-chloro-5,5-dioxo[1,2,4]triazolo[2,3-*b*][1,4,2]benzodithiazine (I, Fig. 1) [16] or of type II–IV (Fig. 1) possess interesting *in-vitro* anticancer properties [17–21]. The putative mechanism of antitumor action appears to be involved in cell cycle arrest in the G0/G1 phase [22]. Therefore, the antiproliferative activity associated with both, quinazolinone and cyclic 2-mercaptosulfonamide moieties prompted us to synthesize new deriv-

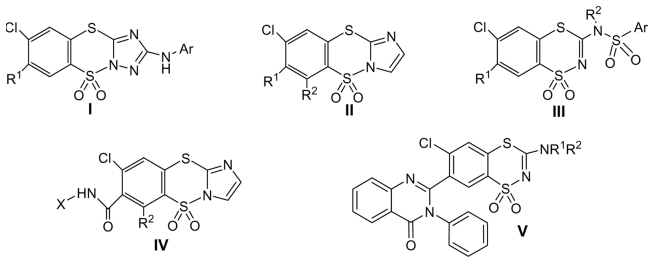

Figure 1. Structures of cytotoxic cyclic 2-mercaptobenzenesulfonamides.

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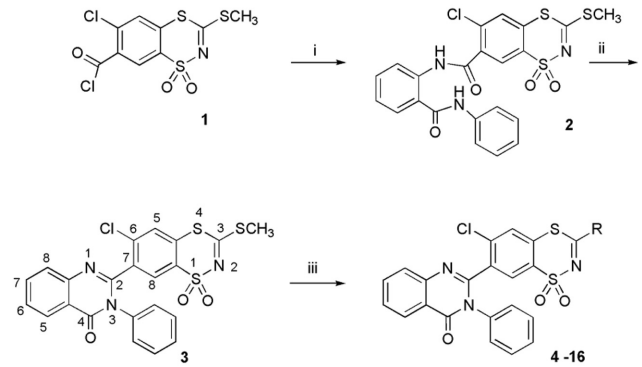
Abbreviation: relative standard deviation (RSD)



Compound	R	Compound	R
4	–N⟨ ⟩	**11**	$-NHCH_2C{\equiv}CH$
5	–N⟨ ⟩O	**12**	–N(H)–CH_2–⟨ ⟩
6	–N⟨ ⟩	**13**	–N(H)–$(CH_2)_2$–⟨ ⟩
7	–N⟨ ⟩N–⟨ ⟩	**14**	–NH–⟨ ⟩–CH_3
8	$-NHCH(CH_3)_2$	**15**	–N(H)–CH_2–⟨N⟩
9	$-NH(CH_2)_3OH$	**16**	–N(H)–CH_2–CH_2–⟨N⟩
10	$-NHCH_2CH{=}CH_2$		

Reagents: (i) 2-aminobenzanilide, pyridine, toluene; (ii) excess of thionyl chloride; (iii) the appropriate amine, dry benzene, or dry methanol.

Scheme 1. Synthesis of 2-(3-substituted amino-6-chloro-1,1-dioxo-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3*H*)-quinazolinones **4**–**16**.

atives with the objective of obtaining bi-heterocycles of type V (Fig. 1). Here, we report the synthesis of 13 new compounds of type V and the results of their *in-vitro* evaluation for cytotoxic activity.

Results and discussion

Chemistry

The previously described methods were employed for the synthesis of 6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazin-7-carbonyl chloride **1** [23, 24]. The reaction of **1** with the 2-aminobenzanilide was carried out in boiling toluene in the presence of pyridine and afforded the expected *N*-[2-(phenylcarbamoyl)phenyl]-6-chloro-1,1-dioxo-3-methylthio-1,4,2-benzodithiazin-7-carboxamide **2** in 87% yield. Treatment of **2** with an excess of thionyl chloride under reflux gave rise to the novel 2-(6-chloro-1,1-dioxo-3-methylthio-1,4,2-benzodithiazin-7-yl)-3-phenyl-3*H*-quinazolin-4-one **3** (Scheme 1).

In turn, nucleophilic displacement of the 3-methylthiol group of **3** by the appropriate amine in boiling methanol proceeded with elimination of methyl mercaptan, leading to the target quinazolinones **4**–**16** in 40–65% yields (Scheme 1). All the final products were characterized by IR and ^{1}H-NMR spectroscopy, and all active compounds were further characterized by ^{13}C-NMR as detailed in the experimental protocols. Elemental analyses of all products were in accordance with the proposed structures.

Biology

A microtiter based assay on the staining of adherent cells with crystal violet was used to quantify the antiproliferative potential of the new compounds on human cancer cell lines. Details of this test have been published elsewhere [25, 26]. Primary screening of compounds **4**–**16** for antiproliferative activity took place on six human cancer cell lines. Compounds that showed inhibition of cell growth by more than 50% at 20 μM in one or more of the cell lines were investigated further. 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, one cervical cancer line: SISO, and the breast cancer cell line: MCF-7. 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 [25].

The compounds **4**, **6**, **7**, **13**, and **14** were inactive up to a concentration of 20 μM. Generally, the IC_{50} values for the active compounds were all greater than those of cisplatin (Table 1). However, some compounds showed similar (**12** in KYSE-520) or even greater (**9** – YAPC; **15** - KYSE-520) activity than cisplatin in specific cell lines (Table 1).

The following conclusion may be drawn from the structure-activity relationship study. No correlations were found between the calculated logP values (ClogP ranged between 3.65 for **9** and 6.67 for **13**, calculated with Pallas 3.0, prolog P 6.0 module) and the IC_{50} values of the compounds in any of the cell lines. In the series of quinazolinone derivatives, the electronic character of the benzodithiazine ring substituent at position-3 appears to be an important factor influencing cytotoxicity. The most active compounds are **9**, **10**, **15**, and **16**, which maybe due to the moderate electron-donating nature of substituent at position-3. It was found that incorporation of phenylethylamino group (ionization constant of ß-phenylethylamine $K_a = 6.75 \times 10^{-5}$) into

Table 1. IC_{50} values (μM) for the inhibition of *in-vitro* cell growth of human cancer cell lines by compounds **5**, **8–12**, **15**, and **16**[a].

Tumor cell line	IC_{50} (μM) for compounds								
	5	**8**	**9**	**10**	**11**	**12**	**15**	**16**	Cisplatin[b]
RT-4	28.1 ± 6.5	10.5 ± .2	7.8 ± 1.0	8.9 ± 3.3	11.2 ± 4.0	4.1 ± 1.9	6.3 ± 3.7	6.2 ± 1.0	1.61
RT-112	58.6 ± 13.0	13.9 ± 2.9	7.2 ± 3.5	7.6 ± 0.4	10.7 ± 2.0	5.1 ± 1.9	4.8 ± 1.2	N[c]	1.22
5637	7.3 ± 1.9	7.8 ± 2.4	4.4 ± 0.4	5.2 ± 1.9	12.1 ± 7.6	7.7 ± 0.5	6.5 ± 0.3	6.5 ± 1.4	0.35
KYSE-70	2.6 ± 1.4	2.8 ± 0.9	5.3 ± 0.4	7.4 ± 4.8	4.9 ± 2.7	9.3 ± 2.8	8.8 ± 2.1	6.2 ± 2.3	0.63
KYSE-510	N[c]	12.3 ± 0.3	5.7 ± 0.9	8.2 ± 2.2	12.3 ± 1.6	N[c]	11.5 ± 3.2	8.4 ± 0.3	0.44
KYSE-520	9.2 ± 1.7	28.8 ± 1.8	7.2 ± 5.1	8.7 ± 1.3	13.3 ± 1.5	4.7 ± 0.6	3.1 ± 0.1	N[c]	3.61
YAPC	29.6 ± 9.4	13.3 ± 4.6	3.7 ± 0.1	10.5 ± 3.8	16.6 ± 2.3	7.7 ± 7.7	13.6 ± 4.5	N[c]	4.09
DAN-G	10.1 ± 2.7	5.6 ± 0.8	4.7 ± 2.1	4.2 ± 0.5	6.0 ± 0.3	4.6 ± 2.8	5.4 ± 1.6	5.9 ± 0.04	0.73
LCLC-103H	30.2 ± 9.3	13.3 ± 0.8	5.8 ± 1.5	3.9 ± 1.8	9.7 ± 4.3	4.8 ± 0.7	3.5 ± 0.8	3.3 ± 0.2	0.90
A-427	8.0 ± 3.4	6.8 ± 2.5	4.2 ± 0.1	3.1 ± 1.7	7.3 ± 2.8	20.6 ± 9.8	7.7 ± 0.7	5.2 ± 0.3	1.96
MCF-7	6.4 ± 3.3	6.9 ± 1.4	4.31.4	7.7 ± 1.4	8.1 ± 1.1	5.1 ± 0.4	2.9 ± 0.5	6.4 ± 0.7	1.38
SISO	N[c]	9.2 ± 4.9	5.33.1	5.7 ± 2.6	12.7 ± 6.5	40.0 ± 12.7	17.2 ± 13.6	4.5 ± 0.7	0.24
Average[d]	19.0	10.9	5.48	6.79	10.43	10.38	7.64	5.84	1.43
RSD[e] (%)	92	61	24	34	32	105	59	24	83

[a] Values were averages of three independent determinations ± 1 SD.
[b] Values were from ref. [25].
[c] Not determined.
[d] Averaged IC_{50} values over all tested cancer cell lines.
[e] Relative standard deviation.

position-3 resulted in inactive **13**. On the other hand, introduction of a benzylamino group (ionization constant of benzylamine, $K_a = 2.0 \times 10^{-5}$) led to **12** with moderate activity. Similarly, replacement of the allylamino group in **10** by propargylamino in **11** caused a reasonable decrease of sensitivity towards all cell lines.

A characteristic of anticancer drugs (*e.g.*, cisplatin) tested in our panel of cancer cell lines is that the relative standard deviation (RSD) of the IC_{50} values tend to be large (*i.e.* >80%). Thus, we calculated the RSD of the new compounds (Table 1). Both compounds **5** and **12** showed RSD greater than 80%, indicating that these compounds have a characteristic spectrum of activity, with some cell lines intrinsically resistant and other cell lines more sensitive to the compounds. For example, compound **12** had a large RSD because two cell lines, A-427 and SISO, are insensitive to the compound while the other cell lines show modest activity. By replacing the phenyl for a pyridinyl ring, in**15**, an overall increase in activity results, but a decrease in the RSD was observed; this is because the A-427 and SISO cell lines are more sensitive to **15** than **12**. Nevertheless, the increased cell selectivity, as evidenced by the larger RSD for **5** and **12**, stands in contrast to the relatively low RSDs found with the very same cell lines for the compounds of the series types I [21] and III [20].

Conclusion

The above data demonstrate the usefulness of connecting quinazolinone and benzodithiazine rings to form a scaffold, which promises to be useful in the search for more selective antineoplastic agents. It can be stated that the electronic character of substituents at position-3 of the benzodithiazine ring system influences the cytotoxicity of compounds **4–16**. Moreover, further structural modification of compounds **9**, **10**, and **15** may lead to the discovery of even more active quinazolinones with good selectivity against various cancer types.

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The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points are uncorrected and were determined on a B(chi SMP-20 apparatus (Büchi Labortechnik, Flawil, Switzerland). The IR spectra were recorded on 1600 FTIR Perkin Elmer (Perkin Elmer, Norwalk, CT, USA) spectrometer using potassium bromide pellets and frequencies were expressed in cm^{-1}. The ^{13}C-NMR and ^{1}H-NMR spectra were obtained on a Varian Gemini (50 MHz and 200 MHz) or Varian Unity Plus (125 MHz and 500 MHz) spectrometers (Varian Inc., Palo Alto, CA, USA). The chemical shift values δ were expressed in ppm relative to the residual solvent signal at 2.50 or 7.26 ppm and 39.5 or 77 ppm, respectively. MS spectra were recorded on a Finnigan MAT-95 spectrometer at 70 eV (Finnigan MAT, Bremen, Germany). The analytical results for C, H, and N were within ± 0.4% of the theoretical values. The starting 6-chloro-3-methylthio-1,1-dioxo-1,4,2-

benzodithiazin-7-carboxylic acid [23] and 6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazin-7-carbonyl chloride **1** [24] were obtained by the previously described methods.

N-[2-(Phenylcarbamoyl)phenyl]-6-chloro-1,1-dioxo-3-methylthio-1,4,2-benzodithiazin-7-carboxamide ***2***

To a suspension of compound **1** (3.42 g, 10 mmol) and 2-aminobenzanilide (2.33 g, 11 mmol) in anhydrous toluene (120 mL), pyridine (0.8 mL, 10 mmol) in anhydrous toluene (50 mL) was added. The reaction mixture was stirred at reflux for 48 h and then left overnight at room temperature. The precipitate was filtered off, washed with toluene (5 mL), dried, and suspended in 0.5% aqueous K_2CO_3 (200 mL). The mixture was stirred for 1 h, filtered off, washed successively with water (2 × 50 mL), methanol (20 mL) and dried. Yield: 4.5 g, 87%, mp. 248–250°C; IR (KBr, cm^{-1}) 3300, 3230 (NH), 1665, 1645 (CONH), 1605 (C=N), 1330, 1170 (SO_2); ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.72 (s, 3H, SCH_3), 7.07 (t, *J* = 7.3 Hz, 1H, aromat.), 7.25-7.42 (m, 3H, aromat.), 7.59 (ddd, *J* = 6.6 Hz, *J* = 1.3 Hz, *J* = 7.7 Hz, 1H, aromat.), 7.67–7.82 (m, 3H, aromat.), 7.99 (d, *J* = 8 Hz, 1H, aromat.), 8.15 (s, 1H, H-5 benzodit.), 8.34 (s, 1H, H-8 benzodit.), 10.46 (s, 1H, NH), 11.03 (s, 1H, NH). Anal. ($C_{22}H_{16}ClN_3O_4S_3$) C, H, N.

2-(6-Chloro-1,1-dioxo-3-methylthio-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***3***

A mixture of carboxamide **2** (5.0 g, 10 mmol) and thionyl chloride (50 mL) was refluxed for 20 h. The thionyl chloride was distilled off (80°C), then toluene was added to the residue (2 × 40 mL) and distilled off (111°C). The dry residue was recrystallized from anhydrous toluene (200 mL) to give compound **3**. Yield: 3.0 g, 60%, mp. 254–256°C. IR (KBr, cm^{-1}) 1670 (CO), 1628 (C=N), 1350, 1170 (SO_2); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.72 (s, 3H, CH_3), 7.09–7.44 (m, 5H, phenyl), 7.47 (s, 1H, H-5 benzodit.), 7.53–7.8 (m, 3H, aromat.), 8.44 (d, *J* = 7.5 Hz, 1H, aromat), 8.64 (s, 1H, H-8 benzodit.). ^{13}C-NMR ($CDCl_3$): δ 16.92 (SCH_3), 122.77, 125.34, 127.56, 127.89, 129.32, 129.4 (two overlapping signals), 129.77, 129.98, 130.09, 130.22, 130.26, 132.30, 134.68, 134.80, 134.96, 138.20, 142.54 (aromat.), 152.47, 152.56 (C=N), 180.17 (C=O). Anal. ($C_{22}H_{14}ClN_3O_3S_3$) C, H, N.

General procedure for preparation of 2-[6-chloro-3-(substituted amino)-1,1-dioxo-1,4,2-benzodithiazin-7-yl]-3-phenyl-4(3H)-quinazolinones ***4–16***

A mixture of **3** (0.5 g, 1 mmol) and the appropriate amine (1.1 mmol) in the appropriate solvent (20 mL) was stirred at room temperature for 20 h and then refluxed until the formation of MeSH had ceased (20–30 h) [Caution: because of high toxicity, MeSH should be trapped in an aqueous NaOH solution]. The precipitate was filtered off, washed successively with solvent (5 mL), chloroform (5 mL), and dried. In this manner, the following compounds were obtained.

2-(6-Chloro-1,1-dioxo-3-piperidin-1-yl-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***4***

Starting from piperidine (0.094 g) in benzene, the title **4** was obtained (0.31 g, 58%); mp. 213–214°C. IR (KBr, cm^{-1}) 1670 (CO), 1625 (C=N), 1310, 1160 (SO_2). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.74 (s, 6H, 3 × CH_2 piper.), 3.73 (s, 2H, H_2C-N-piper.), 3.94 (s, 2H, H_2C-N-piper.), 7.05–7.44 (m, 5H, phenyl), 7.49 (s, 1H, H-5 benzodit.) 7.50–7.78 (m, 3H, aromat.), 8.36 (ddd, *J* = 7 Hz, *J* = 3.3 Hz, *J* = 7.6 Hz, 1H, aromat.), 8.62 (s, 1H, H-8 benzodit.) ppm. Anal. ($C_{26}H_{21}ClN_4O_3S_2$) C, H, N.

2-(6-Chloro-1,1-dioxo-3-morpholin-4-yl-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***5***

Starting from morpholine (0.096 g) in benzene, the title **5** was obtained (0.30 g, 55%); mp. 223–225°C. IR (KBr, cm^{-1}) 1675 (CO), 1620 (C=N), 1320, 1160 (SO_2). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.66–4.10 (m, 8H, CH_2), 7.05–7.44 (m, 5H, phenyl), 7.51 (s, 1H, H-5 benzodit.), 7.53–7.65 (m, 2H, aromat.), 7.68–7.78 (m, 1H, aromat.), 8.30–8.55 (m, 1H, aromat.), 8.62 (s, 1H, H-8 benzodit.). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 48.38 (2 C, H_2C-N-CH_2), 66.56 (2 C, H_2C-O-CH_2), 122.75, 124.24, 125.21, 127.23, 127.42, 127.83, 129.03, 129.43 (two overlapping signals), 130.05, 130.47, 130.52, 131.71, 132.12, 132.20, 134.74, 137.12, 142.60 (18 C, aromat.), 152.86, 152.03 (C=N), 162.73 (CO) ppm. Anal. ($C_{25}H_{19}ClN_4O_4S_2$) C, H, N.

2-(6-Chloro-1,1-dioxo-3-pyrrolidin-1-yl-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***6***

Starting from pyrrolidine (0.078 g) in benzene, the title **6** was obtained (0.25 g, 48%); mp. 250–251°C. IR (KBr, cm^{-1}) 1677 (CO), 1625 (C=N), 1315, 1160 (SO_2). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.99–2.18 (m, 4H, 2 × CH_2), 3.60 (s, 2H, N-CH_2), 3.80 (s, 2H, N-CH_2), 7.1–7.42 (m, 5H, phenyl), 7.49 (s, 1H, H-5 benzodit.), 7.5–7.62 (m, 2H, aromat.), 7.68–7.76 (m, 1H, aromat.), 8.28–8.48 (m, 1H, aromat.), 8.64 (s, 1H, H-8 benzodit.) ppm. Anal. ($C_{25}H_{19}ClN_4O_3S_2$) C, H, N.

2-[6-Chloro-3-(4-phenylpiperazin-1-yl)-1,1-dioxo-1,4,2-benzodithiazin-7-yl]-3-phenyl-4(3H)-quinazolinone ***7***

Starting from 1-phenylpiperazine (0.18 g) in benzene, the title **7** was obtained (0.25 g, 40%); mp. 229–231°C. IR (KBr, cm^{-1}) 1675 (CO), 1625 (C=N), 1325, 1160 (SO_2). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.15–3.40 (m, 4H, 2 × CH_2), 3.85–4.28 (m, 4H, 2 × CH_2), 6.82–7.45 (m, 10H, 2 × phenyl), 7.53 (s, 1H, H-5 benzodit.), 7.54–7.65 (m, 2H, aromat.), 7.68 (d, *J* = 6.7 Hz, 1H, aromat.), 8.32 (d, *J* = 7.5 Hz, 1H, aromat.), 8.63 (s, 1H, H-8 benzodit.) ppm. Anal. ($C_{31}H_{24}ClN_5O_3S_2$) C, H, N.

2-(6-Chloro-3-isopropylamino-1,1-dioxo-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***8***

Starting from 2-aminopropane (0.07 g) in methanol, the title **8** was obtained (0.30 g, 59%); mp. 275-277°C. IR (KBr, cm^{-1}) 3265 (NH), 1676 (CO), 1628 (C=N), 1317, 1150 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.17 (d, *J* = 6.2 Hz, 6H, 2 × CH_3), 4.0–4.22 (m, 1H, CH), 7.0–7.38 (m, 5H, phenyl), 7.52–7.68 (m, 2H, aromat.), 7.72–7.84 (m, 1H, aromat.), 8.05 (s, 1H, H-5 benzodit.), 8.23 (d, *J* = 7.6 Hz, 1H, aromat.), 8.39 (s, 1H, H-8 benzodit.), 9.76 (s, 1H, NH) ppm. ^{13}C-NMR (50 MHz, DMSO-d_6): δ 21.39 (2 C, 2 × CH_3), 46.13 (CH), 119.15, 122.11 (two overlapping signals), 124.03, 126.08, 126.86, 127.16, 128.68 (two overlapping signals), 129.42, 129.97, 130.12, 131.47, 133.47, 134.23, 134.98, 141.80, 144.97 (18 C, aromat.), 145.03, 152.37 (C=N), 160.38 (CO) ppm. Anal. ($C_{24}H_{19}ClN_4O_3S_2$) C, H, N.

2-[6-Chloro-3-(3-hydroxypropylamino)-1,1-dioxo-1,4,2-benzodithiazin-7-yl]-3-phenyl-4(3H)-quinazolinone ***9***

Starting from 3-amino-1-propanol (0.083 g) in methanol, the title **9** was obtained (0.30 g, 57%); mp. 210–212°C. IR (KBr, cm^{-1})

3380 (NH), 1675 (CO), 1625 (C=N), 1320, 1155 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.69 (q, *J* = 6.6 Hz, 2H, CH_2), 3.38 – 3.49 (m, 4H, CH_2N, CH_2OH), 4.57 (t, *J* = 4.8 Hz, 1H, OH), 7.08 (t, *J* = 7.2 Hz, 1H, phenyl), 7.19 (d, *J* = 7.2 Hz, 2H, phenyl), 7.26-7.39 (m, 2H, phenyl), 7.55-7.66 (m, 2H, aromat.), 7.79 (ddd, *J* = 1.5 Hz, *J* = 7.6 Hz, *J* = 6.4 Hz, 1H, aromat.), 8.05 (s, 1H, H-5 benzodit.), 8.25 (dd, *J* = 1.5 Hz, *J* = 6.8 Hz, 1H, aromat.), 8.39 (s, 1H, H-8 benzodit.), 9.83 (s, 1H, NH) ppm. ^{13}C-NMR (50 MHz, DMSO-d_6): δ 31.37 (-CH_2-), 41.2 (CH_2N), 58.32 (CH_2OH), 119.47, 122.43 (two overlapping signals), 124.35, 126.39, 127.17, 127.49, 129.0 (two overlapping signals), 129.74, 130.29, 130.39, 131.76, 133.74, 134.55, 134.83, 135.30, 142.12 (18 C, aromat.), 145.29, 152.69 (C=N), 161.70 (CO) ppm. Anal. ($C_{24}H_{19}ClN_4O_4S_2$) C, H, N.

2-(3-Allylamino-6-chloro-1,1-dioxo-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***10***

Starting from allylamine (0.063 g) in benzene, the title **10** was obtained (0.34 g, 68%); mp. 236 – 238°C. IR (KBr, cm^{-1}) 3300 (NH), 1674 (CO), 1628 (C=N), 1320, 1150 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 4.06 (br s, 2H, NCH_2), 5.20 (dd, *J* = 1.3 Hz, *J* = 6.0 Hz, 1H, =CH_2), 5.27 (dd, *J* = 1.3 Hz, *J* = 13.1 Hz, 1H, =CH_2), 5.8 – 6.0 (m, 1H, CH=), 7.12 (t, *J* = 7.2 Hz, 1H, phenyl), 7.23 (d, *J* = 7.2 Hz, 2H, phenyl), 7.31 – 7.44 (m, 2H, phenyl), 7.58 – 7.70 (m, 2H, aromat.), 7.83 (ddd, *J* = 1.4 Hz, *J* = 7.0 Hz, *J* = 7.6 Hz, 1H, aromat.), 8.11 (s, 1H, H-5 benzodit.), 8.27 (d, *J* = 7.0 Hz, 1H, aromat), 8.44 (s, 1H, H-8 benzodit.), 10.07 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 45.36 ($NHCH_2$), 117.29 (=CH_2), 119.16, 122.11 (two overlapping signals), 124.03, 126.08, 126.86, 127.22, 127.66, 128.69 (two overlapping signals), 129.43, 130.06, 130.13, 131.31, 132.42, 133.32, 134.23, 135.05 (aromat.), 141.80 (CH=), 144.98, 152.36 (C=N), 161.65 (C=O) ppm. EIMS m/z 508.1 [M^+] (65.7). Anal. ($C_{24}H_{17}ClN_4O_3S_2$) C, H, N.

2-(6-Chloro-1,1-dioxo-3-prop-2-ynylamino-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***11***

Starting from propargylamine (0.06 g) in benzene, the title **11** was obtained (0.32 g, 63%); mp. 240 – 242°C. IR (KBr, cm^{-1}): 3300, 3260 (NH), 1674 (CO), 1628 (C=N), 1320, 1150 (SO_2). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.36 (s, 1H, CH), 4.21 (d, *J* = 1.9 Hz, 2H, CH_2), 7.09 (t, *J* = 7.3 Hz, 1H, phenyl), 7.20 (d, *J* = 7.8 Hz, 2H, phenyl), 7.33 (t, *J* = 7.8 Hz, 2H, phenyl), 7.58 – 7.64 (m, 2H, aromat.), 7.79 (t, *J* = 7.8 Hz, 1H, aromat.), 8.07 (s, 1H, H-5 benzodit.), 8.23 (d, *J* = 7.8 Hz, 1H, aromat), 8.42 (s, 1H, H-8 benzodit.), 10.30 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 33.14 (CH_2), 75.84 (CH), 79.21 (C), 119.90, 122.86 (two overlapping signals), 124.79, 126.83, 127.61, 128.03, 129.22, 129.44 (two overlapping signals), 130.19, 130.93, 131.81, 133.94, 134.98, 135.88, 142.53, 145.72 (aromat.), 145.78, 153.06 (C=N), 162.46 (C=O) ppm. EIMS m/z 506.1 [M^+] (77.8). Anal. ($C_{24}H_{15}ClN_4O_3S_2$) C, H, N.

2-(6-Chloro-3-benzylamino-1,1-dioxo-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***12***

Starting from benzylamine (0.12 g) in methanol, the title **12** was obtained (0.38 g, 67%); mp. 236 – 238°C. IR (KBr, cm^{-1}) 3290 (NH), 1674 (CO), 1628 (C=N), 1315, 1140 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 4.6 (s, 2H, CH_2), 7.03 – 7.42 (m, 10H, 2 × phenyl), 7.54 – 7.65 (m, 2H, aromat.), 7.73 – 7.85 (m, 1H, aromat.), 8.07 (s, 1H, H-5 benzodit.), 8.23 (d, *J* = 8.0 Hz, 1H, aromat), 8.41 (s, 1H, H-8 benzodit.), 10.33 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 46.85 ($NHCH_2$), 119.47, 122.42 (two overlapping signals), 124.35, 126.39, 127.18, 127.55, 127.88, 128.04 (two overlapping signals), 128.86 (two overlapping signals), 129.01 (two overlapping signals), 129.74, 130.37, 130.49, 131.64, 133.65, 134.55, 135.38, 136.78, 142.11, 145.28 (aromat.), 145.39, 152.67 (C=N), 162.21 (C=O) ppm. Anal. ($C_{28}H_{19}ClN_4O_3S_2$) C, H, N.

2-(6-Chloro-1,1-dioxo-3-phenethylamino-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***13***

Starting from phenethylamine (0.13 g) in methanol, the title **13** was obtained (0.36 g, 63%); mp. 175 – 177°C. IR (KBr, cm^{-1}) 3370, 3270 (NH), 1674 (CO), 1628 (C=N), 1320, 1160 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.87 (t, *J* = 6.3 Hz, 2H, CH_2), 3.49 – 3.69 (m, 2H, CH_2), 7.02 – 7.42 (m, 10H, 2 × phenyl), 7.52 – 7.86 (m, 3H, aromat.), 8.03 (s, 1H, H-5 benzodit.), 8.22 (d, *J* = 7.3 Hz, 1H, aromat.), 8.40 (s, 1H, H-8 benzodit.), 9.91 (s, 1H, NH) ppm. Anal. ($C_{29}H_{21}ClN_4O_3S_2$) C, H, N.

2-(6-Chloro-1,1-dioxo-3-p-tolylamino-1,4,2-benzodithiazin-7-yl)-3-phenyl-4(3H)-quinazolinone ***14***

Starting from tolylamine (0.11 g) in benzene, the title **14** was obtained (0.15 g, 27%); mp. 251 – 253°C. IR (KBr, cm^{-1}) 3270, 3210 (NH), 1676 (CO), 1622 (C=N), 1320, 1140 (SO_2) cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 7.02 – 7.40 (m, 7H, phenyl), 7.42 – 7.70 (m, 4H, 2H phenyl and 2H aromat.), 7.71 – 7.85 (m, 1H, aromat.), 8.14 (s, 1H, H-5 benzodit.), 8.23 (d, *J* = 7.6 Hz, 1H, aromat.), 8.44 (s, 1H, H-8 benzodit.), 11.50 (br s, 1H, NH) ppm. Anal. ($C_{28}H_{19}ClN_4O_3S_2$) C, H, N.

2-{6-Chloro-1,1-dioxo-3-[(pyridin-3-ylmethyl)amino]-1,4,2-benzodithiazin-7-yl}-3-phenyl-4(3H)-quinazolinone ***15***

Starting from 3-(aminomethyl)pyridine (0.12 g) in methanol, the title **15** was obtained (0.21 g, 37%), mp. 228 – 230°C. IR (KBr, cm^{-1}): 3240 (NH), 1670 (CO), 1628 (C=N), 1325, 1155 (SO_2); ^{1}H-NMR (200 MHz, DMSO-d_6): δ 4.63 (s, 2H, NCH_2), 7.08 (t, *J* = 7.1 Hz, 1H, phenyl), 7.19 (d, *J* = 7.3 Hz, 2H, phenyl), 7.31 (d, *J* = 7.3 Hz, 2H, phenyl), 7.33 – 7.41 (m, 1H, H-5 pyrid.), 7.52 – 7.68 (m, 3H, aromat.), 7.71 – 7.85 (m, 1H, H-4 pyrid.), 8.08 (s, 1H, H-5 benzodit.), 8.23 (d, *J* = 7.3 Hz, 1H, aromat), 8.41 (s, 1H, H-8 benzodit.), 8.50 (d, *J* = 3.6 Hz, 1H, H-6 pyrid.), 8.55 (s, 1H, H-2 pyrid.), 10.33 (s,1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 44.53 (NCH_2), 119.46, 122.42 (two overlapping signals), 126.39, 127.18, 127.58, 129.01 (two overlapping signals), 129.76, 130.40, 130.51, 131.52, 132.49, 133.59, 134.55, 135.42, 135.60, 142.10 (aromat.), 123.93, 124.35 (C-3 and 5 pyrid.), 135.96 (C-4 pyrid), 149.11, 149.45 (C-2 and 6 pyrid.), 145.32, 152.65 (C=N), 162.37 (C=O) ppm. Anal. ($C_{27}H_{18}ClN_5O_3S_2$) C, H, N.

2-[6-Chloro-1,1-dioxo-3-(2-pyridin-2-ylethylamino)-1,4,2-benzodithiazin-7-yl]-3-phenyl-4(3H)-quinazolinone ***16***

Starting from 2-(2-aminoethyl)pyridine (0.11 g) in methanol, the title **16** was obtained (0.30 g, 52%), mp. 237 – 238°C. IR (KBr, cm^{-1}): 3190 (NH) 1676 (CO), 1626 (C=N), 1325, 1155 (SO_2) cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 3.03 (t, *J* = 7 Hz, 2H, CH_2), 3.75 (t, *J* = 7 Hz, 2H, CH_2), 7.08 (t, *J* = 7.3 Hz, 1H, phenyl), 7.15 – 7.38 (m, 6H, 4H phenyl, H-3 and H-5 pyrid.), 7.54 – 7.64 (m, 2H, aromat), 7.65 (m, 2H, aromat and H-4 pyrid.), 8.03 (s, 1H, H-5 benzodit.), 8.22 (d, *J* = 7.3 Hz, 1H, aromat.), 8.40 (s, 1H, H-8 benzodit.), 8.50 (d, *J* = 4.4 Hz, 1H, H-6 pyrid.), 9.93 (br s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 35.71 (CH_2), 42.87 (NCH_2), 119.53, 121.75, 122.11 (two over-

lapping signals), 124.03, 126.08, 126.86, 127.21, 128.69 (two overlapping signals), 129.43, 130.0, 130.10, 131.35, 134.23, 135.01, 141.79, 144.97 (aromat.), 122.06, 123.93, (C-3 and 5 pyrid.), 136.57 (C-4 pyrid), 149.10, 149.22 (C-2 and 6 pyrid.), 145.02, 157.89 (C=N), 161.56 (C=O) ppm. Anal. ($C_{28}H_{20}ClN_5O_3S_2$) C, H, N.

In-vitro cytotoxicity studies

The method used for cytotoxicity testing has been described in detail elsewhere [25, 26]. All cells were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Stock solutions of compounds were prepared in DMSO and diluted 1000-fold with cell culture medium for testing. For IC_{50} determinations, all substances were tested at five serially diluted concentrations.

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