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Synthesis of 1-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)semicarbazides and their transformation into 4-chloro-2-mercapto-*N*-(4,5-dihydro-5-oxo-4-phenyl-1*H*-1,2,4-triazol-3-yl)benzenesulfonamides as potential anticancer and anti-HIV agents

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Abstract

Synthesis of a series of 1-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)semicarbazides (**6**–**16**) and 4-chloro-2-mercapto-*N*-(4,5-dihydro-5-oxo-4-phenyl-1*H*-1,2,4-triazol-3-yl)benzenesulfonamides (**17**–**22**) were reported. Compounds **7**–**9**, **17**, **19**–**22** were tested at the US National Cancer Institute for their in vitro anticancer and anti-HIV activities. Results of anticancer screening showed moderate activity of **21** and **22**, while **19** was found to have encouraging anti-HIV activity at $EC_{50} = 28.8$ μM.

Keywords: 1-(6-Chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)semicarbazides; 2-Mercapto-*N*-(4,5-dihydro-5-oxo-4-phenyl-1*H*-1,2,4-triazol-3-yl)benzenesulfonamides; Anticancer activity; Anti-HIV activity

1. Introduction

Aryl and heteroarylsulfonamides are an important class of therapeutic agents in current medicinal science. In recent years much attention has been devoted to the synthesis of novel arylsulfonamides due to their anticancer and anti-HIV activities [1–5]. We reported previously the synthesis of 1,1-dioxo-1,4,2-benzodithiazines, and their subsequent transformation into 4-chloro-2-mercaptobenzenesulfonamide derivatives **I** (MBSAs) with the nitrogen atom of sulfonamide moiety attached to variety of heterocyclic ring systems (Fig. 1). These compounds, depending on structure displayed either anticancer [6–9,12,13,15] or/and anti-HIV [6–11,14] activities and have been described by Neamati et al. [16–18] as a novel class of potent HIV-1 integrase inhibitors.

These results promoted us to design new analogues with further modification of the triazole ring. Since, 4-(2-mercaptobenzenesulfonyl)perhydro-1,2,4-triazin-3-ones (**II**) exhibited relatively high anti-HIV activity, we anticipated that the introducing carbonyl group into the triazole ring (structure **III**) might bring about significant biological consequence. Moreover, semicarbazides as well as hydrazine itself, have proved mutagenic and carcinogenic activities [19], so that activity of new 1-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)semicarbazides could be interesting. Thus, some new 1-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)semicarbazides and 4-chloro-2-mercapto-*N*-(4,5-dihydro-5-oxo-4-phenyl-1*H*-1,2,4-triazol-3-yl)benzenesulfonamides were prepared in order to further explore the structure–activity relationships of 2-mercaptobenzenesulfonamides.

2. Results and discussion

The key intermediates for the syntheses were 6-chloro-1,1-dioxo-7-(methyl or arylcarbamoyl)-3-methylthio-1,4,2,-benzodithiazines (1-5) previously prepared in our laboratory [14,20,21]. The reactions of the appropriate benzodithiazine with semicarbazide hydrochloride proceeded smoothly in methanol at the presence triethyla-

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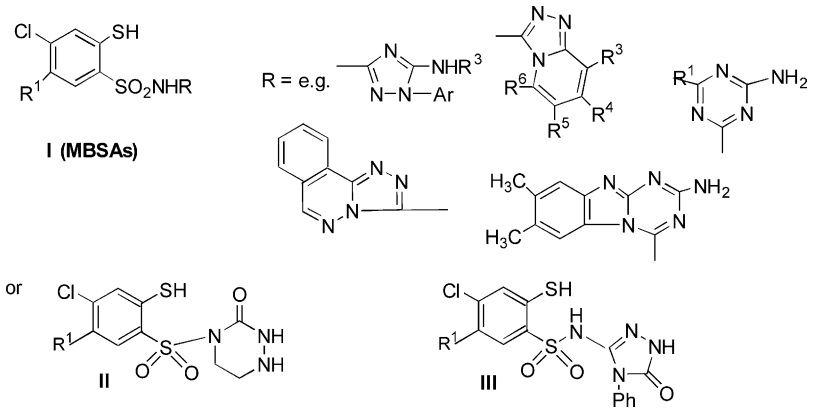
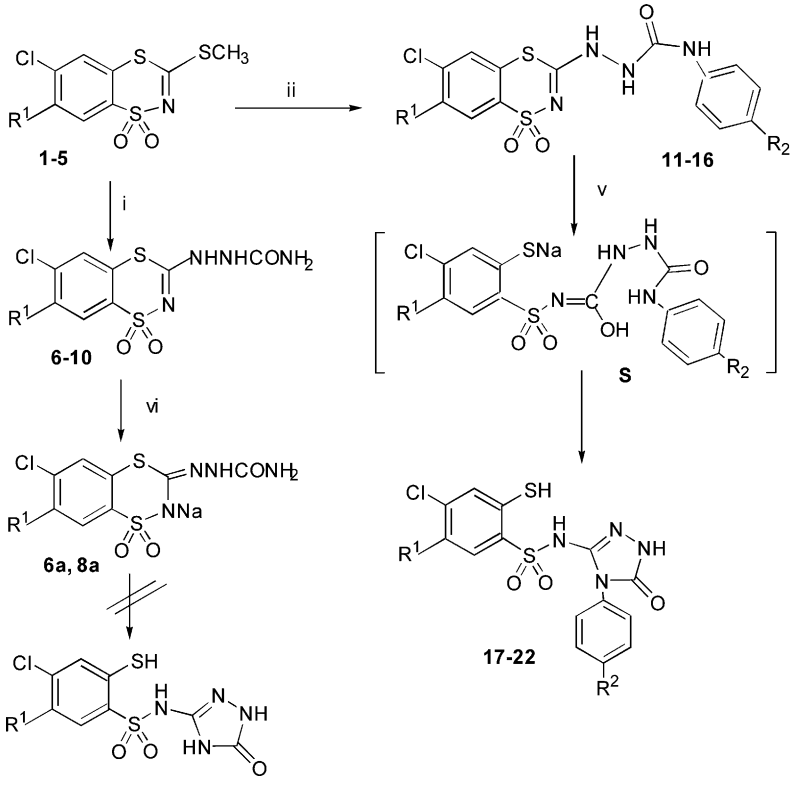


Fig. 1.

mine to give 1-(6-chloro-1,1-dioxo-7-R^2-1,4,2-benzodithiazin-3-yl)semicarbazides (**6**–**10**). As outlined in Scheme 1, similar reaction with 4-arylsemicarbazides gave rise to the formation of corresponding 1-(6-chloro-1,1-dioxo-7-R^1-1,4,2-benzodithiazin-3-yl)-4-arylsemicarbazides (**11**–**16**), which upon treatment with aqueous sodium hydroxide at 95 °C afforded the expected 4-chloro-2-mercapto-7-R^2-*N*-(4,5-dihydro-5-oxo-4-phenyl-1*H*-1,2,4-triazol-3-yl)benzenesulfonamides (**17**–**22**). A possible mechanism of the formation of **17**–**22** can be rationalized by invoking the initial nucleophilic ring opening of benzodithiazine with the formation of

Compound	R^1	R^2
1, 6, 11, 17	CH_3	H
1, 12, 18	CH_3	F
2, 7, 13, 19	PhNHCO	H
3, 8, 14, 20	4-CH_3PhNHCO	H
4, 9, 15, 21	4-ClPhNHCO	H
5, 10, 16, 22	2-ClPhNHCO	H



Scheme 1. (i) $NH_2NHCONH_2 \cdot HCl$, NEt_3, MeOH, reflux 30 h; (ii) $NH_2NHCONHPhR^2$, MeOH, reflux 30 h; (v) (1) NaOH/H_2O 95 °C, 1 h, (2) H_2O/HCl; (vi) NaOH/H_2O 95 °C, 1 h or NaOH/H_2O, reflux 5 min.

Table 1
Physico-chemical properties, IR and ^{1}H NMR spectroscopic data of semicarbazides **6**–**10**

No.	M.p. (°C)	Yield (%)	Formula index (M_W)	IR (KBr, λ cm^{-1})	^{1}H NMR (δ, ppm)
6	231–232	92	$C_9H_9ClN_4O_3S_2$ (320.78)	3430, 3325, 1662, 1568, 1340, 1160	2.43 (s, 3H, CH_3), 6.48 (s, 2H, NH_2), 7.95 (s, 1H, H-5), 7.99 (s, 1H, H-8), 8.65 (s, 1H, HN-2), 11.05 (s, 1H, HN-1)
6a	294–296	76	$C_9H_8ClN_4O_3S_2Na$ (342.76)	3471, 3342, 1669, 1560, 1300, 1146	2.35 (s, 3H, CH_3), 5.93 (s, 2H, NH_2), 7.46 (s, 1H, H-5), 7.71 (s, 1H, H-8), 8.01 (br.s, 1H, HN-2)
7	241–242	69	$C_{15}H_{12}ClN_5O_4S_2$ (425.87)	3442, 3354, 1666, 1584, 1307, 1163	6.53 (s, 2H, NH_2), 7.14–7.71 (m, 5H, phenyl), 8.11 (s, 1H, H-5), 8.14 (s, 1H, H-8), 8.73 (s, 1H, HN-2), 10.69 (s, 1H, NHCO), 11.21 (s, 1H, HN-1)
8	247–248	74	$C_{16}H_{14}ClN_5O_4S_2$ (439.89)	3442, 3336, 1663, 1584, 1307, 1153	2.30 (s, 3H, CH_3), 6.53 (s, 2H, NH_2), 7.18 (d, $J = 8$ Hz, 2H, Ph), 7.60 (d, $J = 8$ Hz, 2H, Ph), 8.13 (s, 1H, H-5), 8.17 (s, 1H, H-8), 8.77 (s, 1H, HN-2), 10.65 (s, 1H, NHCO), 11.25 (s, 1H, HN-1)*
8a	289–291	77	$C_{16}H_{13}ClN_5O_4S_2Na$ (460.87)	3472, 3343, 1659, 1584, 1316, 1151	2.29 (s, 3H, CH_3), 5.97 (s, 2H, NH_2), 7.17 (d, $J = 8.4$ Hz, 2H, Ph), 7.59 (d, $J = 8.3$ Hz, 2H, Ph), 7.65 (s, 1H, H-5); 7.79 (s, 1H, H-8), 8.10 (s, 1H, HN-2); 10.53 (s, 1H, NHCO)
9	249–250	81	$C_{15}H_{11}Cl_2N_5O_4S_2$ (460.32)	3442, 3348, 1669, 1587, 1307, 1163	6.51 (s, 1H, NH_2), 7.72 (d, $J = 9$ Hz, 2H, Ph), 7.42 (d, $J = 9$ Hz, 2H, Ph), 8.15 (s, 2H, H-5 and 8), 8.73 (s, 1H, HN-2), 10.82 (s, 1H, NHCO), 11.22 (s, 1H, HN-1)
10	248–249	75	$C_{15}H_{11}Cl_2N_5O_4S_2$ (460.32)	3442, 3336, 1672, 1590, 1307, 1116	6.56 (s, 1H, NH_2), 7.29-7.83 (m, 4-H, Ph), 8.17 (s, 1H, H-5), 8.18 (s, 1H, H-8), 8.76 (s, 1H, HN-2), 10.53 (s, 1H, NHCO), 11.25 (s, 1H, HN-1)

isourea intermediate **S**, and its subsequent cyclocondensation leading to the target triazolone derivatives. However, attempted reaction of semicarbazide **6** or **8** with sodium hydroxide under analogous conditions failed, and only corresponding sodium salts **6a** and **8a** were obtained. Hence, it is assumed that the presence of an electron-withdrawing aromatic substituent at the N-4 nitrogen atom of the semicarbazide moiety is necessary for the formation of the triazole ring. Analytical and spectral data (IR, ^{1}H NMR and ^{13}C NMR) confirmed the proposed structures of all the new compounds **6**–**22**.

It is worth noting that the 1-(1,1-dioxo-1,4,2-benzodithiazin-3-yl)semicarbazides (**6**–**16**) represent an interesting example of possible side chain tautomerism [22,23]. We studied this phenomenon both in solution by NMR spectroscopy and by ab initio quantum chemical calculations for isolated molecules [24,25].[1] In general, all of the obtained semicarbazides can exist in form of two possible tautomers **A** and **B** as depicted in Fig. 2.

For example, ^{1}HNMR spectrum run in DMSO-d_6 of compound **11** displayed doubled signals of the semicarbazide moiety protons, indicating the presence of two

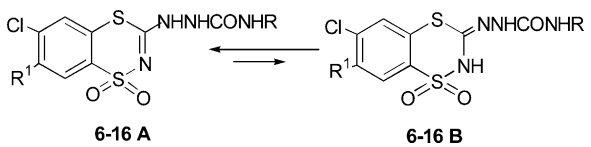


Fig. 2. The probable tautomeric forms of semicarbazides **6**–**16**.

[1] Structures **A** and **B** were fully optimized without any symmetry restrictions until the gradient was smaller than 0.0001 au. The molecular orbital calculations were carried out with ab initio and density functional modules as implemented into SPARTAN program.

tautomers **11A** and **11B** in a ratio 2.7:1, respectively. The calculated energies of the tautomers **11A** and **11B** presented in Table 2 indicate that the amino-tautomer **11A** is more stable by 1.76 kcal/mol than imino-tautomer **11B**. However, based upon their calculated dipole moments, tautomer **11B** (2.60 Debye) would be predicted to predominate over tautomer **11A** (2.02 Debye) in polar solvents.

The minimum energy structures of both tautomers are presented in Fig. 3. In case of semicarbazide **11**, the imino-tautomer **11B** is stabilized by intramolecular N^1–H···O=C bonding, as shown in Fig. 3.

Some of the newly synthesized compounds were submitted to the US National Cancer Institute (Bethesda, MD) for in vitro anticancer (**7**–**9**, **17**, **19**–**22**) and anti-HIV (**17**, **19**, **20**) evaluations. Semicarbazides **7**–**9** showed weak anticancer activity against single cell line (**8**—Melanoma UACC-62, $GI_{50} = 0.83$ μM, **7**—Leukemia K-562, $GI_{50} = 55.5$ μM; **9**—CNS cancer SNB-75, $GI_{50} = 88.0$ μM). Triazolones **17**, **19** and **20** were inactive, whereas **21** and **22** exhibited moderate antineoplastic activity against some of the human cell lines (Table 3). The substitution of the phenyl of the carbamyl group by the electron-withdrawing chlorine atom leads to an increase in activity of the triazolone **21**. The compounds **17** and **20** tested for their in vitro anti-

Table 2
Calculated energies (E, hartrees), relative energies (ΔE, kcal/mol) and dipole moments (μ, Debye) of tautomers **A** and **B** of compound **11**

Comp.	E	ΔE	μ
11A	−2281.29362	0	2.02
11B	−2281.29082	1.76	2.60

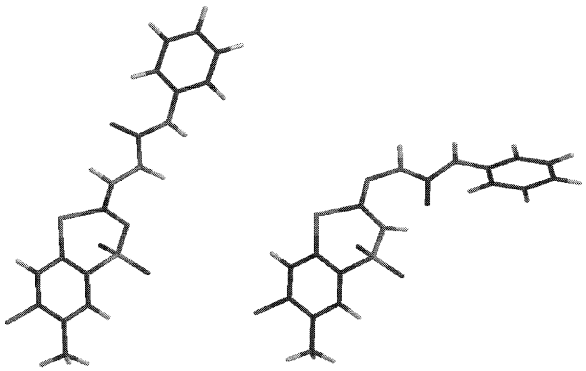


Fig. 3. The minimum energy structures of the tautomeric form **11A** and **11B**.

HIV activity were essentially inactive, while **19** (R^1 = NHCOPh, R^2 = H) displayed moderate activity (IC_{50} > 200 μM, EC_{50} = 28.8 μM, TI_{50} > 6.94).

In summary we have successfully synthesized new 1-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl)semicarbazide derivatives and some of them were transformed into suitable triazolones. Generally, new semicarbazides and triazolones did not improve their anticancer and anti-HIV activity compared to the derivatives reported previously [9,10], which exhibited moderate or high potency. The introduction of the carbonyl group into the triazole ring did not increase the pharmacological activity. However, these results provide useful information about structure–activity relationships.

3. Experimental

3.1. 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 (200MHz) or Varian Unity Plus (500 MHz) spectrometers in dimethyl sulfoxide-d_6. The chemical shift values δ are expressed in ppm relative to tetramethylsilane as internal standard and coupling constants (J) are in Hertz. Abbreviation are as follows: s, singlet; d, doublet; br, broad; m, multiplet. The analytical results for C, H, and N were within ±0.4% of the theoretical values. The starting compounds **1**–**5** were obtained by the previously described methods [14,20,21].

Table 3
In vitro anticancer data for compounds **21** and **22**

Panel	Cell line	GI50 μM	
		21	**22**
Leukemia	Molt-4	6.22	
CNS cancer	SF-295	15.8	46.2
Non-small cell lung cancer	NCI-H226	24.3	60.8
Colon cancer	KM12	24.7	
	COLO205	41.6	48.3
Ovarian cancer	OVCAR-3	6.28	
Prostate cancer	DU-145	20.7	
Breast cancer	BT-549	37.6	37.7
	T-47D	30.0	
	MDA-MB-231/ATCC	48.1	43.4

TGI and LC50 > 100 μM.

3.1.1. General procedure for preparation of 1-(6-chloro-1,1-dioxo-7-R^1-1,4,2-benzodithiazin-3-yl)semicarbazides (**6**–**10**)

A stirred mixture of the appropriate 3-methylthiobenzodithiazine (**1**–**5**) (15 mmol), semicarbazide hydrochloride (2.01 g, 18 mmol), triethylamine (1.82 g, 18 mmol, 2.5 ml) and methanol (60 ml) were refluxed for 20 h (until the evolution of MeSH had ceased). The solid product was collected by filtration, washed with methanol and dried. Yields, melting points, analytical and spectroscopic data of the semicarbazides **6**–**10** are reported in Table 1. Compound **7**: ^{13}C NMR (DMSO-d_6, δ ppm): 121.8, 124.8, 128.2, 129.2, 129.7, 130.5, 132.6, 134.0, 137.0, 137.8 (C arom.), 157.9 (C=N), 163.2 (C=O amide), 169.7 (C=O semicarb.). Compound **8**: ^{13}C NMR (DMSO-d_6, δ ppm): 20.54 (CH_3), 119.8, 124.3, 129.2, 129.3, 130.0, 131.9, 133.2, 133.6, 136.0, 137.1 (C arom.), 157.4 (C=N), 162.5 (C=O amide), 169.8 (C=O semicarb.). Compound **9**: ^{13}C NMR (DMSO-d_6, δ ppm): 121.4, 124.5, 127.8, 128.3, 129.4, 130.1, 132.2, 133.6,136.6, 137.5 (C arom.), 157.5 (C=N), 162.8 (C=O amide), 169.4 (C=O semicarb.).

3.1.2. Attempts for preparation of 4-chloro-5-(methyl or 4-R^2-phenylcarbamoyl)-2-mercapto-N-(4,5-dihydro-5-oxo-1H-1,2,4-triazol-3-yl)benzenesulfonamides

A suspension of the appropriate semicarbazide **6** or **8** (2 mmol) in 2.5% aqueous sodium hydroxide (20 ml) was stirred at 95 °C for 1 h. The resulting solution was diluted with hot water (70 ml) and 2% hydrochloric acid was added dropwise to pH 5. After cooling, the mixture was filtered off, and the filtrate was acidified with 2% hydrochloric acid to pH 2. The precipitate was filtered, washed with water and dried. Yields, melting points, analytical and spectroscopic data of the obtained **6a**, **8a** are reported in Table 1.

*3.1.2.1. Sodium salts of compounds **6** and **8** (**6a**, **8a**).* A suspension of compound **6** or **8** (1 mmol) in 1.5% aqueous sodium hydroxide (10 ml) was refluxed for 2 min. After cooling, the precipitated solid was collected by suction, washed with water and dried. The physical

Table 4
Physico-chemical properties, analytical and spectroscopic (IR, ^{1}H NMR) data of semicarbazides **11**–**16**

No.	M.p. (°C)	Yield (%)	Formula index (M_W)	IR (KBr, λ cm^{-1})	^{1}H NMR (δ, ppm)
11A	216–218	85	$C_{15}H_{13}ClN_4O_3S_2$ (396.88)	3324, 3284, 3218, 1695, 1595, 1349, 1296, 1155	2.42 (s, 3H, 7-CH_3), 6.97–7.53 (m, 5H, Ph), 7.94 (s, 1H, H-5), 8.01 (s, 1H, H-8), 8.94 (s, 1H, HN-2), 9.39 (s, 1H, HN-4), 11.23 (s, 1H, HN-1)[a]
11B	216–218	85	$C_{15}H_{13}ClN_4O_3S_2$ (396.88)	3324, 3284, 3218, 1695, 1595, 1349, 1296, 1155	2.42 (s, 3H, 7-CH_3), 6.92–7.47(m, 5H, Ph), 7.97 (s, 1H, H-5), 8.0 (s, 1H, H-8), 8.85 (s, 1H, HN-1), 8.94 (s, 1H, HN-2), 9.03 (s, 1H, HN-4)[a]
12	229–232	91	$C_{15}H_{12}ClFN_4O_3S_2$ (414.87)	3359, 3207, 3077, 1681, 1584, 1340, 1310, 1161	2.51 (s, 3H, 7-CH_3), 6.9–7.75 (m, 4H, Ph), 7.97 (s, 1H, H-5), 8.02 (s, 1H, H-8), 8.98 (s, 1H, HN-2), 9.42 (s, 1H, HN-4), 11,23 (s, 1H, HN-1)
13	267–269	79	$C_{21}H_{16}ClN_5O_4S_2$ (501.97)	3354, 3271, 3215, 1684, 1658, 1595, 1314, 1138	6.98–7.71 (m, 10H, 2 × Ph), 8.13 (s, 1H, H-5), 8.16 (s, 1H, H-8), 9.04 (s, 1H, HN-2), 9.44 (s, 1H, HN-4), 10.69 (s, 1H, NHCO), 11.39 (s, 1H, HN-1)
14	276–278	95	$C_{22}H_{18}ClN_5O_4S_2$ (515.99)	3359, 3275, 3207, 1684, 1654, 1592, 1313, 1165	2.28 (s, 3H, CH_3Ph), 6.98–7.61 (m, 9H, 2 × Ph), 8.11 (s, 1H, H-5), 8.15 (s, 1H, H-8), 8.99 (s, 1H, HN-2), 9.44 (s, 1H, HN-4), 10.60 (s, 1H, NHCO), 11.39 (s, 1H, HN-1)
15	267–269	93	$C_{21}H_{15}Cl_2N_5O_4S_2$ (536.42)	3359, 3256, 3201, 1684, 1657, 1592, 1352, 1305, 1163	6.97–7.77 (m, 9H, 2 × Ph), 8.17 (s, 2H, H-5, H-8), 9.0 (s, 1H, HN-2), 9.44 (s, 1H, HN-4), 10.83 (s, 1H, NHCO), 11.40 (s, 1H, HN-1)
16	255–257	93	$C_{21}H_{15}Cl_2N_5O_4S_2$ (536.42)	3354, 3260, 3207, 1681, 1660, 1595, 1349, 1314, 1163	6.96–7.79 (m, 9H, 2 × Ph), 8.16 (s, 2H, H-5, H-8), 9.03 (s, 1H, HN-2), 9.45 (s, 1H, HN-4), 10.49 (s, 1H, NHCO), 11.40 (s, 1H, HN-1)

[1]HNMR spectra in DMSO-d_6: spectrometers: 500 MHz[a], 200 MHz.

and analytical data were in accordance with those reported in Table 1.

3.1.3. General procedure for preparation of 1-(6-chloro-1,1-dioxo-7-R^1-1,4,2-benzodithiazin-3-yl)-4-(R^2-phenyl)semicarbazides (**11**–**16**)

A stirred mixture of the appropriate benzodithiazine (**1**–**5**) (15 mmol), the proper 4-arylsemicarbazide (15 mmol), and methanol (80 ml) were refluxed for 30 h (until the evolution of CH_3SH had ceased). The solid product was filtered off, washed with methanol and dried. Yields, melting points, analytical and spectroscopic data of the semicarbazides **11**–**16** are reported in Table 4.

Compound **13**: ^{13}C NMR (DMSO-d_6, δ ppm): 119.3, 120.1, 124.7, 129.4, 129.5, 129.6, 130.3, 131.8, 132.0, 133.5, 134.0, 136.3, 136.6, 137.4 (C arom.), 154.7 (C=N), 162.8 (C=O amide), 169.7 (C=O semicarb.). Compound **14**: ^{13}C NMR (DMSO-d_6, δ ppm): 20.64 (CH_3), 119.3, 120.0, 124.5, 124.7, 129.1, 129.4, 129.6, 130.3, 131.8, 132.1, 134.0, 136.6, 137.3, 138.8 (C arom.), 154.7 (C=N), 163.0 (C=O amide), 169.7 (C=O semicarb.).

3.1.4. General procedure for preparation of 4-chloro-5-(methyl or 4-R^2-phenylcarbamoyl)-2-mercapto-N-(4,5-dihydro-5-oxo-4-phenyl-1H-1,2,4-triazol-3-yl)benzenesulfonamides (**17**–**22**)

A suspension of the appropriate semicarbazide (**11**–**16**) (2 mmol) in 2.5% aqueous sodium hydroxide (20 ml)

Table 5
Physico-chemical properties, IR and [1]HNMR spectroscopic data of compounds **17**–**22**

No.	M.p. (°C)	Yield (%)	Formula index (M_W)	IR (KBr, λ cm^{-1})	^{1}H NMR (δ, ppm)
17	145–147	85	$C_{15}H_{13}ClN_4O_3S_2$ (396.88)	3183, 3077, 2554, 1704, 1581, 1357, 1163	2.26 (s, 3H, 5-CH_3), 7.25–7.50 (m, 5H, Ph), 7.62 (s, 1H, H-3), 7.64 (s, 1H, H-6), 11.87 (br.s, 1H, NH)
18	203–205	48	$C_{15}H_{12}ClFN_4O_3S_2$ (414.87)	3213, 3083, 2560, 1704, 1613, 1587, 1302, 1163	2.35 (s, 3H, 5-CH_3), 7.16–7.50 (m, 4H, Ph), 7.76 (s, 1H, H-3) 7.88 (s, 1H, H-6), 10.5 (br s, 1H, NH)
19	181–183	41	$C_{21}H_{16}ClN_5O_4S_2$ (501.97)	3260, 3195, 3072, 2560, 1715, 1657, 1598, 1315, 1163	7.2–7.78(m, 10H, 2 × Ph), 7.91 (s, 1H, H-3), 8.07 (s, 1H, H-3), 10.68 (s, 1H, NHCO)
20	184–186	71	$C_{22}H_{18}ClN_5O_4S_2$ (515.99)	3254, 3195, 3072, 2554, 1713, 1654, 1584, 1313, 1163	2.30 (s, 3H, CH_3), 7.12–7.75 (m, 10H, 2 × Ph, H-3), 8.05 (s, 1H, H-6), 9.56 (br.s, 1H, NHCO)
21	172–175	40	$C_{21}H_{15}Cl_2N_5O_4S_2$ (536.42)	3254, 3195, 3054, 2554, 1716, 1660, 1595, 1307, 1163	7.08–7.87 (m, 9H, 2 × Ph), 7.93 (s, 1H, H-3), 8.04 (s, 1H, H-6), 8.92 (s, 1H, NH), 9.85 (s, 1H, NHCO)
22	160-164	43	$C_{21}H_{15}Cl_2N_5O_4S_2$ (536.42)	3365, 3236, 3083, 2566, 1707, 1672, 1587, 1302, 1163	7.08–7.80 (m, 9H, 2 × Ph); 8.18 (s, 1H, H-3); 8.28 (s, 1H, H-6); 9.32 (s, 1H, NHCO)

was stirred at 95 °C for 1 h. The resulting solution was diluted with hot water (70 ml) and 2% hydrochloric acid was added dropwise to pH 5.

After cooling, the mixture was filtered off, and the filtrate was acidified with 2% hydrochloric acid to pH 2. The precipitate was filtered, washed with water and dried. Yields, melting points, analytical and spectroscopic data of the triazoles **17**–**22** are reported in Table 5.

3.2. Pharmacology

The tests of anti-HIV activity of compounds **20**, **22** and **23** were performed on T-4 lymphocytes (CEM-SS cell line) uninfected or infected with HIV-1. The viability of the cells was determined spectrophotometrically using the tetrazolium assay procedure [26]. The anticancer activity of the compounds was evaluated by using total 61 human tumor. Cell Lines, derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. The compounds were tested at five concentrations at tenfold dilution. A 48-h continuous drug exposure protocol was used and sulphorhodamine B (SRB) protein assay was used to estimate cell growth. The antitumor activity of a test compound is given by three parameters for each cell line: GI_{50}, molar concentration of the compound that inhibits 50% net cell growth; TGI molar concentration of the compound leading to total inhibition; and LC_{50}, molar concentration of the compound leading to 50% net cell death [27].

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