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Original article

Synthesis, anti-HIV-1 integrase, and cytotoxic activities of 4-chloro-*N*-(4-oxopyrimidin-2-yl)-2-mercaptobenzenesulfonamide derivatives

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Abstract

Several 4-chloro-*N*-(4-oxopyrimidin-2-yl)-2-mercaptobenzenesulfonamide derivatives **13**–**28** and **35**–**44** have been synthesized and tested as potential HIV-1 integrase (IN) inhibitors. Compounds **15**–**17**, **19**, **21**–**28**, **36** and **41** inhibited IN with IC_{50} values in the range of 3.3–63.0 μM. The compounds **13**, **15**, **16**, **21**–**24** and **26**–**28** were further tested at the US National Cancer Institute for their in vitro activity against a panel of 53–57 human tumor cell lines. The compounds **26**–**28** were inactive, whereas the other compounds exhibited high or reasonable activity ($GI_{50} < 0.01$–20.0 μM) against one or more human tumor cell lines.

Keywords: 4-Chloro-*N*-(3,4-dihydro-4-oxopyrimidin-2-yl)-2-mercaptobenzenesulfonamide derivatives; Synthesis; Anti-HIV activity; Cytotoxic activity

1. Introduction

The arylsulfonamides constitute an important class of compounds with several types of biological activities and well-established safety profile [1]. Currently, there is significant interest in the discovery and development of novel arylsulfonamides for the treatment of cancer and HIV infections [2–5]. As part of a broad investigation of structures containing 4-chloro-2-mercaptobenzenesulfonamide scaffold, several series of novel sulfonamides with remarkable antitumor activity (Fig. 1, structures: **A** [6–14], **B** [15–17], **C** [18–20] and **D** [21]) or anti-HIV activity (Fig. 1, structures: **A** [6–8, 22–25], **B** [26], **C** [20] **E** and **F** [27]) and HIV-1 integrase inhibitory activity (Fig. 1, structures: **A** [22,24] and **B** [26]) were discovered in our laboratories. In order to better understand the nature of their cytotoxic versus antiviral properties, we extended our studies on the synthesis of a new series of 4-chloro-2-mercaptobenzenesulfonamide derivatives of type **G** (Fig. 1) with potential anti-HIV-1 integrase and cytotoxic activities.

2. Results and discussion

2.1. Chemistry

The previously described methods were employed for the synthesis of compounds **2**–**9** and **13**–**20** [25]. Analogously, we prepared novel methyl 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)thiophene-2-carboxylates **10**–**12** (Scheme 1).

The synthesis of the target 2-mercaptobenzenesulfonamides **21**–**28** were achieved by reacting corresponding methyl *o*-(1,4,2-benzodithiazin-3-ylamino)aryl carboxylates **2**, **4**, **5**, **6**, **11** and **12** with benzyl- or furfurylamine in boiling toluene. We propose a reaction sequence for the transformations as shown in Scheme 2. Nucleophilic attack of amine at the

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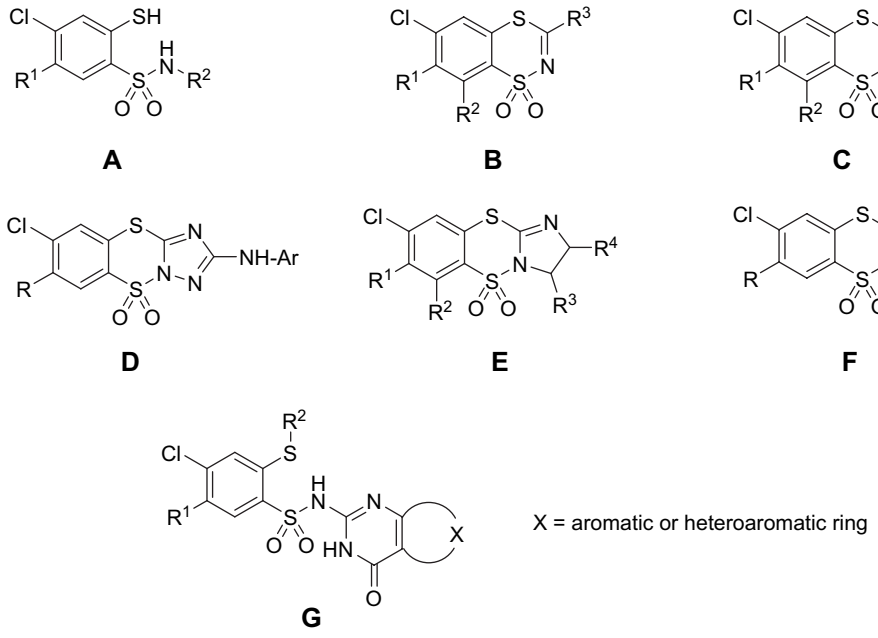


Fig. 1.

carbon C-3 atom of benzodithiazine ring results in guanidine intermediate **A**, which undergoes a cyclocondensation reaction leading to the formation of aminium salt of the final products **B**, which after acidification with boiling glacial acetic acid gave free 2-mercaptobenzenesulfonamides **21**–**28** in 67–83% yields.

As shown in Scheme 3, the desired 2-benzylthiobenzenesulfonamides **35**–**44** were obtained by reacting the previously described [13] *N*-(2-benzylthio-4-chlorobenzenesulfonyl)cyanamide potassium salts **29**–**34** with the corresponding methyl *o*-aminoaryl carboxylates in glacial acetic acid.

The structures of the newly obtained compounds **10**–**12**, **21**–**28** and **35**–**44** were confirmed by elemental analyses (C, H, N) and spectroscopic data presented in Section 4.

2.2. Biology

All the final sulfonamides **13**–**28** and **35**–**44** were tested as potential HIV-1 integrase inhibitors. On the basis of data presented in Table 1, several pertinent features emerge to be important for IN inhibition. Compounds **13**–**20** with a free mercapto group and the NH_2 substituent at N-3 nitrogen atom of oxopyrimidine moiety possess rather weak activity (IC_{50} values above 55 μM for 3′-processing and above 35 μM for strand transfer inhibition). Similarly, compounds **35**–**43** with benzyl-protected mercapto group and no substituent at N-3 nitrogen atom are either inactive (compound **37**, IC_{50} values above 100 μM) or possess weak activity (IC_{50} values above 47 and 33 μM for 3′-processing and strand transfer inhibition, respectively).

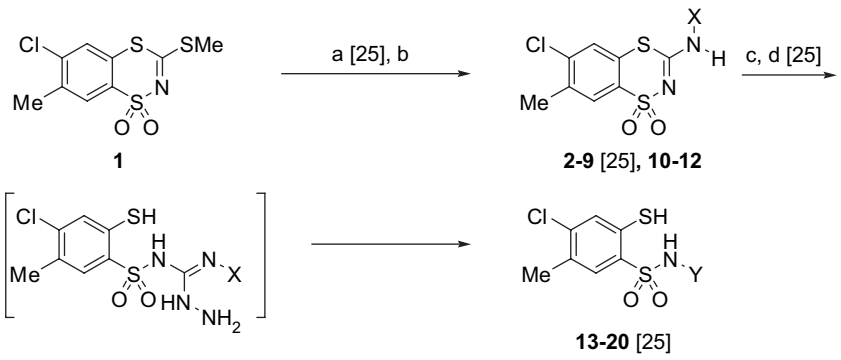
On the other hand, the series of 2-mercaptobenzenesulfonamides **21**–**28** with benzyl or furfuryl substituent at N-3 nitrogen atom of oxopyrimidine moiety showed very promising activity against purified IN, indicating that bulkier group at this position is responsible for enhanced potency. Moreover, the most active compounds **25**–**28** incorporate thieno[2,3-*e*] pyrimidine ring with lipophylic *tert*-butyl, phenyl or thienyl group at position 5 (IC_{50} values in the range of 6–22 μM for 3′-processing and 4–6 μM for strand transfer). These latter compounds show potency comparable to those exhibited by the well known 2-mercaptobenzenesulfonamide NSC 661073 [24].

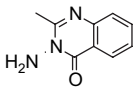
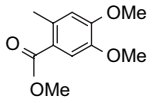
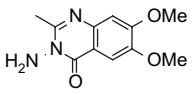
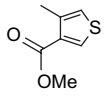
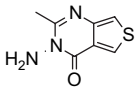
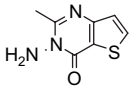
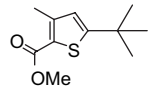
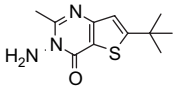
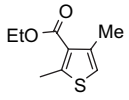
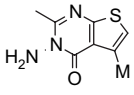
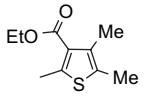
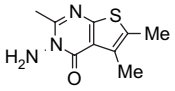
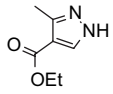
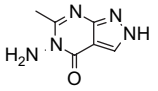
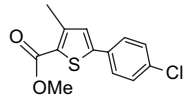
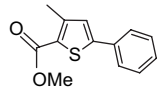
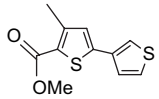
The sulfonamides **13**, **15**, **16**, **21**–**24** and **26**–**28** were further tested at the National Cancer Institute (Bethesda, MD) against a panel of approximately 60 human tumor cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast (NCI 60) [28–30].

First of all, it should be pointed out that the compounds **26**–**28**, which exhibited highest HIV-1 integrase inhibitory activity, proved to be inactive in cytotoxicity tests ($GI_{50} >$ 100 μM), and therefore, they can be considered for further development as anti-HIV agents.

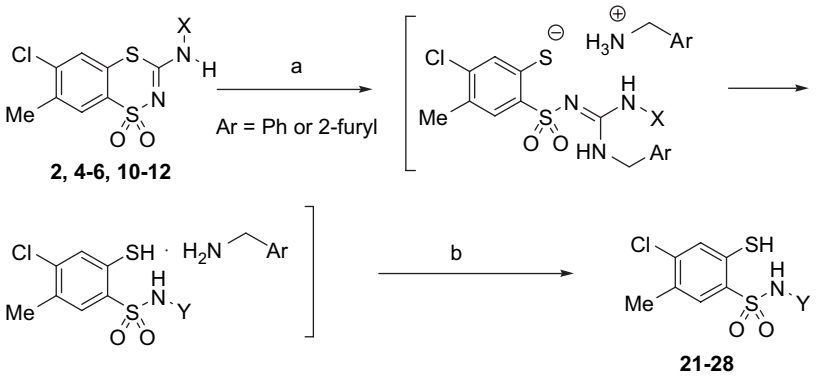
On the other hand, the other tested compounds exhibited structure dependent high, reasonable or weak activity ($GI_{50} < 0.01$–99.8 μM) against 25–96% of the 53–57 human tumor cell lines (Table 2). The data of the most susceptible cell lines ($GI_{50} < 20.0$ μM) recorded in Table 2 indicate the following rank order of potency: **22** (against nine cell lines, $GI_{50} = 3.9$–19.9 μM), **23** (against nine cell lines, $GI_{50} = 14.2$–19.9 μM), **21** (six cell lines, $GI_{50} < 0.01$–19.5 μM), **13** (six cell lines, $GI_{50} = 7.2$–19.7 μM) **24**, (two cell lines, $GI_{50} = 9.4 = 19.4$ μM), **15** (against two cell lines, $GI_{50} = 15.0$–17.7 μM) and **16** ($GI_{50} > 20.0$ μM for all cell lines).

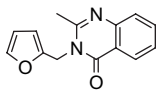
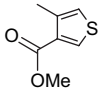
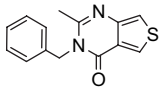
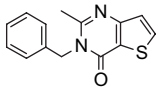
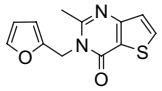
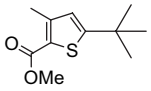
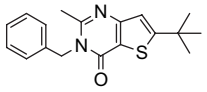
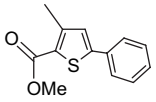
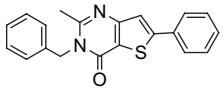
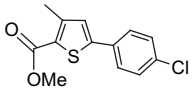
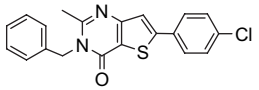
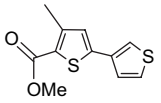
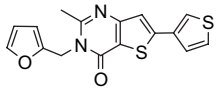
Analysis of the structure–activity relationship suggests that electronic effects of substituents Y affect considerably their



Compd	X	Y
2, 13		
3, 14		
4, 15		
5, 16		
6, 17		
7, 18		
8, 19		
9,20		
10		—
11		—
12		—

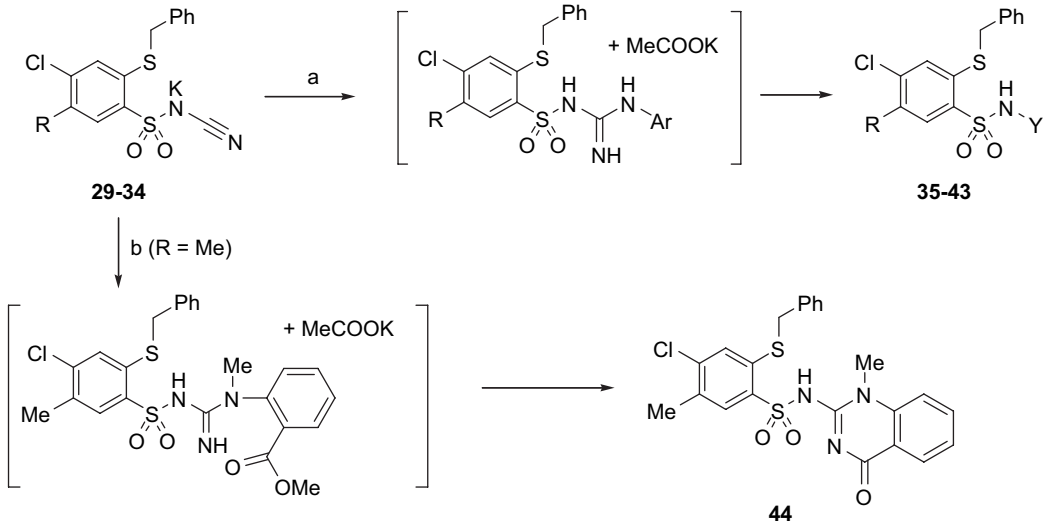
Scheme 1. Synthesis of alkyl *o*-(6-chloro-7-methy-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)arylcarboxylates **2**–**12** and 4-chloro-2-mercapto-5-methylbenzene-sulfonamides **13**–**20**. Reagents, conditions and yields: (a, for **9**, **10**) alkyl *o*-aminoaryl carboxylate (1 molar equiv), pyridine (1.33 molar equiv), toluene, reflux, 55–91% (**1**–**9**); (b) 3-amino-5-(phenyl-, 4-chlorophenyl- or 3-thienyl)thiophene-2-carboxylate (1 molar equiv), pyridine (1.33 molar equiv), toluene, reflux 45–60 h, 44–72%; (c, for **13**–**17**) hydrazine hydrate (3.4 molar equiv), methanol, room temperature, 60–64 h, 90–96%; (d, for **18**–**20**) hydrazine hydrate (2.2 molar equiv), toluene, room temperature 12 h, reflux 8 h, 41–81%.

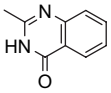
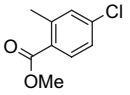
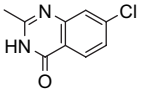
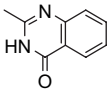
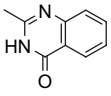
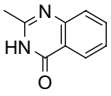
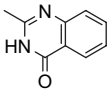
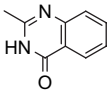
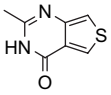
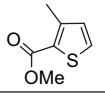
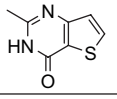


Compd	X	Y
2, 21		
4, 22		
5, 23		
5, 24		
6, 25		
10, 26		
11, 27		
12, 28		

Scheme 2. Synthesis of 4-chloro-2-mercapto-5-methylbenzenesulfonamides **21**–**28**. Reagents, conditions and yields: (a) benzyl or furfurylamine (2.3 molar equiv), toluene, reflux 6 h; (b) glacial acetic acid, reflux 3 min, 67–83%.

cytotoxic potency, specificity and selectivity. Thus, compound **13** (Y = 3-amino-3,4-dihydro-4-oxoquinazolin-2-yl) exhibited the highest inhibitory activity ($GI_{50} = 7.2–19.7$ μM) against six cell lines derived from three different human cancer types (Table 2). Replacement of substituent Y in compound **13** by even stronger electron-withdrawing 3-amino-3,4-dihydro-4-oxothieno[3,4-*e*]pyrimidin-2-yl moiety yielded analogue **15** with antitumor activity limited to SF-268 and SNB-75 cell lines of CNS cancer ($GI_{50} = 15.0–17.7$ μM). Similarly, replacement of Y = 3-benzyl-3,4-dihydro-4-oxothieno[2,3-*e*] pyrimidin-2-yl in one of the most active **23** (nine cell lines from four different human cancer types, $GI_{50} = 14.2–19.9$ μM) by stronger electron-withdrawing substituents (Y = 3-furfuryl-3,4-dihydro-4-oxothieno[2,3-*e*]pyrimidin-2-yl or 3-benzyl-3,4-dihydro-4-oxo-6-phenylthieno[2,3-*e*]pyrimidin-2-yl) resulted in distinguished decrease of cytotoxic potency (**24**: two cell lines from leukemia) till the complete loss of activity in case of **26** (Table 2).



Compd	R	Ar	Y
29, 35	Me		
29, 36	Me		
30, 37	H_2NCO		
31, 38	PhNHCO		
32, 39	4-MePhNHCO		
33, 40	4-MeOPhNHCO		
34, 41	4-ClPhNHCO		
29, 42	Me		
29, 43	Me		

Scheme 3. Synthesis of 2-benzylthio-4-chlorobenzenesulfonamides **35**–**44**. Reagents, conditions and yields: (a) methyl *o*-aminoaryl carboxylate (1.0 molar equiv), glacial acetic acid, reflux, 15 h, 18–63%; (b) methyl 2-methylaminobenzoate (1.0 molar equiv), glacial acetic acid, reflux, 15 h, 43%.

Table 1
Anti-HIV integrase activity of novel 4-chloro-2-mercaptobenzenesulfonamide derivatives **13**–**28** and **35**–**44** and the reference 4-chloro-*N*-(3-amino-1*H*-1,2,4-triazol-5-yl)-2-mercapto-5-methylbenzenesulfoamide (NSC 661073) [24]

Compd	IC_{50}[a] (μM)	
	3′-procesing	Strand transfer
13	>100	>100
14	>100	>100
15	69 ± 6	43 ± 2
16	65 ± 2	53 ± 3
17	60 ± 12	35 ± 2
18	>100	81 ± 9
19	55 ± 5	39 ± 2
20	>100	>100
21	78 ± 15	42 ± 8
22	80 ± 35	31 ± 10
23	53 ± 7	29 ± 13
24	62 ± 4	53 ± 3
25	22 ± 11	4 ± 1
26	9 ± 2	6 ± 1
27	6 ± 3	4 ± 2
28	8 ± 2	6 ± 2
35	>100	83 ± 30
36	47 ± 10	33 ± 14
37	>100	>100
38	73 ± 28	36 ± 3
39	>100	77 ± 23
40	>100	93 ± 7
41	100	52 ± 27
42	>100	78 ± 6
43	>100	70 ± 9
44	>100	90 ± 10
NSC 661073	4.79	4.90

[a] IC_{50}: Inhibitory concentration 50% (inhibition of purified integrase).

3. Conclusions

We have developed methods for the synthesis of various 4-chloro-*N*-(4-oxopyrimidin-2-yl)-2-mercaptobenzenesulfonamide derivatives containing the pyrimidine moiety fused with an aromatic or heteroaromatic ring. Some of these sulfonamides depending on their structure exhibited either HIV-1 integrase inhibitory activity (**25**–**28**) or cytotoxic activity (**13**, **15** and **21**–**24**). Moreover, the sulfonamide **21** acting as potent inhibitor toward CNS cancer SNB-19 cell line ($GI_{50} < 0.01$ μM) may serve as useful lead compound for the search of more powerful selective antineoplastic agents.

4. Experimental protocols

4.1. Synthesis

The following instruments and parameters were used: (melting points) Büchi 535 apparatus; (IR spectra) KBr pellets, 400–4000 cm^{-1} Perkin–Elmer 1600 FTIR spectrophotometer; (^1H and ^{13}C NMR spectra) Varian Gemini 200 apparatus at 200 and 50 MHz, respectively (chemical shifts are expressed at δ values relative to Me_4Si as standard).

4.1.1. General procedure for the preparation of methyl 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)thiophene-2-carboxylates (**10**–**12**)

To a solution of 6-chloro-7-methyl-3-methylthio-1,4,2-benzodithiazine 1,1-dioxide **1** (4.4 g, 0.015 mol) and the appropriate methyl 3-aminothiophene-2-carboxylate (0.015 mol) in dry toluene (15 ml) was added anhydrous pyridine (1.6 g, 0.02 mol). The reaction mixture was refluxed with stirring until the evolution of MeSH had ceased (45–60 h) (CAUTION: because of high toxicity, MeSH should be trapped in an aqueous NaOH solution). After cooling to room temperature the suspension was left overnight. The precipitate was collected by filtration, washed successively with toluene (4 × 2.5 ml) and methanol (4 × 2.5 ml), dried and recrystallized from DMF.

In this manner, the following carboxylates were obtained.

*4.1.1.1. Methyl 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)-5-phenylthiophene-2-carboxylate (**10**).* Starting from methyl 3-amino-5-phenylthiophene-2-carboxylate (3.5 g), the title compound **10** was obtained (5.2 g, 72%); m.p. 261–262 °C. IR (KBr) 3210 (NH), 1675 (C=O), 1325, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.82 (s, 3H, CH_3O), 7.47–7.51 (m, 3H, Ph), 7.73–7.78 (m, 2H, Ph), 7.84 (s, 1H, H-5, benzodithiazine), 8.03 (s, 1H, H-8, benzodithiazine), 8.05 (s, 1H, H-4, thiophene), 11.42 (s, 1H, NH) ppm. Anal. Calcd. for $C_{20}H_{15}ClN_2O_4S_3$ (478.98): C, 50.14; H, 3.15; N, 5.85. Found: C, 50.10; H, 3.19; N, 6.01.

*4.1.1.2. Methyl 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)-5-(4-chlorophenyl)thiophene-2-carboxylate (**11**).* Starting from methyl 3-amino-5-(4-chlorophenyl)thiophene-2-carboxylate (4.02 g), the title compound **11** was obtained (5.4 g, 70%); m.p. 280–282 °C dec. IR (KBr) 3205 (NH), 1675 (C=O), 1325, 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.49 (s, 3H, CH_3), 3.96 (s, 3H, CH_3O), 7.44 (d, $J = 8.7$ Hz, 2H, 4-ClPh), 7.49 (s, 1H, H-5, benzodithiazine), 7.62 (d, $J = 8.7$ Hz, 2H, 4-ClPh), 8.07 (s, 1H, H-8, benzdithiazine), 8.42 (s, 1H, H-4, thiophene), 10.83 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 22.09, 54.58, 113.30, 120.72, 128.78, 129.60, 129.72, 129.80, 131.44, 131.92, 132.93, 127.79, 140.61, 140.91, 145.36, 151.03, 161.37, 166.87 ppm. Anal. Calcd. for $C_{20}H_{14}Cl_2N_2O_4S_3$ (513.43): C, 46.78; H, 2.75; N, 5.45. Found: C, 46.71; H, 2.86; N, 5.40.

*4.1.1.3. Methyl 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)-5-(3-thienyl)thiophene-2-carboxylate (**12**).* Starting from methyl 3-amino-5-(3-thienyl)thiophene-2-carboxylate (3.6 g), the title compound **12** was obtained (3.2 g, 44%); m.p. 245–247 °C. IR (KBr) 3215 (NH), 1670 (C=O), 1325, 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.49 (s, 3H, CH_3), 3.94 (s, 3H, CH_3O), 7.36–7.44 (m, 2H, 3-thienyl), 7.49 (s, 1H, H-5, benzodithiazine), 7.64 (dd, $J_{2-4} = 1.3$ Hz, $J_{2-5} = 2.7$ Hz, 1H, H-2, 3-thienyl), 8.07 (s, 1H, H-8, benzodithiazine), 8.32 (s, 1H, H-thiophene), 10.86 (s, 1H, NH) ppm. Anal. Calcd. for

Table 2
Inhibition of in vitro human cancer cell lines growth by 4-chloro-2-mercaptobenzenesulfonamide derivatives **13**, **15**, **16**, **21**–**24** and **26**–**28**[a]

Compd	Number of the cell lines					Most sensitive cell lines ($GI_{50} < 20.0$ μM)			
	Investigated	Giving positive GI_{50} and TGI				Panel	Cell line	GI_{50}[b] (μM)	TGI[c] (μM)
		GI_{50}[b] (μM)		TGI[c] (μM)					
		No	Range	No	Range				
13	53	51	7.2–71.1	10	41.1–92.7	Non-small cell lung cancer	EKVX	14.8	29.2
							NCI-H522	19.0	50.8
						CNS cancer	SF-268	7.2	40.1
							SNB-19	15.7	47.2
							SNB-75	13.3	32.7
						Melanoma	UACC-62	19.7	49.2
15	54	48	8.1–96.6	6	48.5–90.4	CNS cancer	SF-268	17.7	68.1
							SNB-75	15.0	58.5
16	53	29	31.3–94.9	1	88.0	–	–	–	–
21	57	53	<0.01–99.8	8	40.7–88.8	Leukemia	MOLT-4	18.6	70.6
							SR	19.5	40.7
						CNS cancer	SNB-19	<0.01	88.8
						Melanoma	LOXIMVI	16.1	59.3
						Breast cancer	BT-549	15.7	51.9
							T-47D	15.0	81.4
22	56	51	3.9–81.6	8	32.5–90.8	Leukemia	CCRF-CEM	14.0	47.6
							MOLT-4	3.9	32.5
						Non-small cell lung cancer	HOP-62	16.5	39.3
							NCI-H322 M	18.2	45.9
						Colon cancer	HCC-2998	18.9	68.5
							KM 12	19.9	74.7
						Melanoma	LOXIMVI	18.2	51.6
						Renal cancer	SN12C	16.6	54.5
						Breast cancer	T-47D	4.5	40.8
23	55	53	14.2–87.6	25	34.2–98.7	Non-small cell lung cancer	HOP-62	19.7	46.6
							NCI-H322 M	19.5	48.8
						Colon cancer	HCT-116	19.2	37.7
							KM 12	19.9	48.2
						Melanoma	MALME-3M	19.9	56.1
							M14	19.8	49.6
							SK-MEL-5	12.5	34.2
							UACC-62	19.5	44.4
						Renal cancer	A498	14.2	49.3
24	57	14	9.9–99.5	2	29.7–84.2	Leukemia	MOLT-4	19.4	84.2
							SR	9.9	29.7

[a] Data obtained from the in vitro cancer cell lines screen performed at the NCI [28–30]. Compounds **26**–**28** were inactive ($GI_{50} > 100.0$ μM).
[b] GI_{50}: molar concentration that inhibits 50% net cell growth.
[c] TGI: molar concentration giving total growth inhibition.

$C_{18}H_{13}ClN_2O_4S_4$ (485.03): C, 44.57; H, 2.70; N, 5.77. Found: C, 44.62; H, 2.79; N, 5.81.

4.1.2. General procedure for the preparation of 4-chloro-N-(3,4-dihydro-4-oxopyrimidin-2-yl)-2-mercapto-5-methylbenzenesulfonamide derivatives (*21*–*28*)

To a solution of benzylamine (1.24 g, 0.0115 mol) or furfurylamine (1.12 g, 0.0115 mol) in dry toluene (30 ml), the corresponding methyl *o*-(1,4,2-benzodithiazin-3-ylamino)arylcarboxylate **2**, **4**, **5**, **6**, **10**, **11** or **12** (0.005 mol) was added and stirred at reflux for 6 h. After cooling to room temperature, the aminium salt of the appropriate product that precipitated was separated by suction, washed with toluene (2 × 2 ml) and immediately acidified in boiling glacial acetic acid (30 ml) for 3 min. After cooling to room temperature, the precipitate of the adequate sulfonamide obtained was collected by filtration, washed successively with acetic acid (2 × 2 ml) and toluene (4 × 2 ml), and dried at temperature gradually increasing to 90 °C.

*4.1.2.1. 4-Chloro-N-(3-furfuryl-3,4-dihydro-4-oxoquinazolin-2-yl)-2-mercapto-5-methylbenzenesulfonamide (**21**).* Starting from methyl 2-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)benzoate **2** (1.98 g) and furfurylamine, the title compound **21** was obtained (1.7 g, 74%); m.p. 169–170 °C. IR (KBr) 3275 (NH), 2540 (SH), 1710 (C=O), 1350, 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 4.39 (s, 1H, SH), 5.43 (s, 2H, CH_2), 6.19

(s, 2H, furan), 7.28 (s, 1H, furan), 7.36 (t, $J = 7.8$ Hz, 2H, quinazoline), 7.39 (s, 1H, H-3, $PhSO_2$), 7.71 (t, $J = 7.8$ Hz, 1H, quinazoline), 7.97 (s, 1H, H-6, $PhSO_2$), 8.21 (d, $J = 8.3$ Hz, 1H, quinazoline), 10.89 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 19.50, 38.31, 109.87, 110.31, 15.28, 115.97, 125.23, 128.63, 130.66, 130.74, 131.82, 133.79, 135.79, 136.55, 137.37, 138.47, 142.15, 148.75, 148.93, 159.95 ppm. Anal. Calcd. for $C_{20}H_{16}ClN_3O_4S_2$ (461.92): C, 52.00; H, 3.49; N, 9.09. Found: C, 51.98; H, 3.53; N, 9.12.

*4.1.2.2. N-(3-Benzyl-3,4-dihydro-4-oxothieno[3,4-e]pyrimidin-2-yl)-4-chloro-2-mercapto-5-methylbenzenesulfonamide (**22**).* Starting from 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)thiophene-4-carboxylate **4** (2.01 g) and benzylamine, the title compound **22** was obtained (1.6 g, 67%); m.p. 168–170 °C dec. IR (KBr) 3270 (NH), 2545 (SH), 1690 (C=O), 1370, 1340, 1140 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 4.19 (s, 1H, SH), 5.26 (s, 2H, CH_2), 6.95 (d, $J = 3.3$ Hz, 1H, H-7, thienopyrimidine), 7.05–7.29 (m, 5H, Ph), 7.31 (s, 1H, H-3, $PhSO_2$), 7.89 (s, 1H, H-6, $PhSO_2$), 8.29 (d, $J = 3.3$ Hz, 1H, H-5, thienopyrimidine), 10.88 (s, 1H, NH) ppm. Anal. Calcd. for $C_{20}H_{16}ClN_3O_3S_3$ (477.99): C, 50.25; H, 3.37; N, 8.79. Found: C, 50.19; H, 3.44; N, 8.73.

*4.1.2.3. N-(3-Benzyl-3,4-dihydro-4-oxothieno[2,3-e]pyrimidin-2-yl)-4-chloro-2-mercapto-5-methylbenzenesulfonamide (**23**).* Starting from 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)thiophene-2-carboxylate **5** (2.01 g) and benzylamine, the title compound **23** was obtained (1.8 g, 75%); m.p. 193–195 °C dec. IR (KBr) 3245 (NH), 2548 (SH), 1680 (C=O), 1355, 1335, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3), 4.14 (s, 1H, SH), 5.29 (s, 2H, CH_2), 7.03 (d, $J = 5.2$ Hz, 1H, H-7, thienopyridine), 7.06–7.32 (m, 6H, Ph and H-3, $PhSO_2$), 7.79 (d, $J = 5.2$ Hz, 1H, H-6, thienopyridine), 7.89 (s, 1H, H-6, $PhSO_2$), 11.30 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 19.46, 45.32, 117.03, 127.62, 128.10, 128.88, 129.01, 130.37, 130.62, 131.95, 133.81, 135.69, 136.57, 137.30, 138.38, 141.91, 149.80, 156.55 ppm. Anal. Calcd. for $C_{20}H_{16}ClN_3O_3S_3$ (477.99): C, 50.25; H, 3.37; N, 8.79. Found: C, 50.22; H, 3.36; N, 8.84.

*4.1.2.4. 4-Chloro-N-(3-furfuryl-3,4-dihydro-4-oxothieno[2,3-e]-pyrimidin-2-yl)-2-mercapto-5-methylbenzenesulfonamide (**24**).* Starting from 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)thiophene-2-carboxylate **5** (2.01 g) and furfurylamine, the title compound **24** was obtained (1.9 g, 82%); m.p. 212–213 °C dec. IR (KBr) 3270 (NH), 2545 (SH), 1690 (C=O), 1355, 1340, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 4.35 (s, 1H, SH), 5.32 (s, 2H, CH_2), 6.18 (s, 2H, H-3 and H-4, furan), 7.03 (d, $J = 5.0$ Hz, 1H, H-7, thienopyrimidine), 7.28 (s, 1H, H-5, furan), 7.39 (s, 1H, H-3, $PhSO_2$), 7.81 (d, $J = 5.0$ Hz, 1H, H-6, thienopyrimidine), 7.96 (s, 1H, H-6, $PhSO_2$), 11.29 (s, 1H, NH) ppm. Anal. Calcd. for $C_{18}H_{14}ClN_3O_4S_3$ (467.98): C, 46.19; H, 3.02; N, 8.98. Found: C, 46.27; H, 3.20; N, 9.07.

*4.1.2.5. N-(3-Benzyl-6-(tert-butyl)-3,4-dihydro-4-oxothieno[2,3-e]pyrimidin-2-yl)-4-chloro-2-mercapto-5-methylbenzenesulfonamide (**25**).* Starting from 5-(*tert*-butyl)-3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)thiophene-2-carboxylate **6** (2.29 g) and benzylamine, the title compound **25** was obtained (2.0 g, 75%); m.p. 216–217 °C dec. IR (KBr) 3270 (NH), 2560 (SH), 1690 (C=O), 1360, 1340, 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H, *tert*-butyl), 2.36 (s, 3H, CH_3-5, $PhSO_2$), 4.13 (s, 1H, SH), 5.27 (s, 2H, CH_2), 6.79 (s, 1H, H-3, $PhSO_2$), 7.03–7.26 (m, 5H, Ph), 7.30 (s, 1H, H-6, $PhSO_2$), 7.88 (s, 1H, H-7, thienopyrimidine), 11.18 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 21.54, 33.86, 37.86, 47.25, 114.04, 129.58, 130.12, 131.02, 132.41, 132.66, 134.04, 135.83, 137.98, 139.58, 140.32, 143.87, 144.02, 151.93, 158.47, 172.66 ppm. Anal. Calcd. for $C_{24}H_{24}ClN_3O_3S_3$ (534.11): C, 53.96; H, 4.53; N, 7.86. Found: C, 53.87; H, 4.68; N, 7.88.

*4.1.2.6. N-(3-Benzyl-3,4-dihydro-4-oxo-6-phenylthieno[2,3-e]pyrimidin-2-yl)-4-chloro-2-mercapto-5-methylbenzenesulfonamide (**26**).* Starting from 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)-5-phenylthiophene-2-carboxylate **10** (2.39 g) and benzylamine, the title compound **26** was obtained (2.3 g, 83%); m.p. 267–268 °C dec. IR (KBr) 3290 (NH), 2555 (SH), 1675 (C=O), 1355, 1340, 1155 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 4.12 (s, 1H, SH), 5.30 (s, 2H, CH_2), 7.11–748 (m, 10H, Ph-2 and $PhCH_2$), 7.64 (s, 1H, H-3, $PhSO_2$), 7.66 (s, 1H, H-6, $PhSO_2$), 7.91 (s, 1H, H-7, thienopyrimidine), 11.29 (s, 1H, NH) ppm. Anal. Calcd. for $C_{26}H_{20}ClN_3O_3S_3$ (554.08): C, 56.35; H, 3.64; N, 7.58. Found: C, 56.42; H, 3.71; N, 7.51.

*4.1.2.7. N-[3-Benzyl-6-(4-chlorophenyl)-3,4-dihydro-4-oxothieno[2,3-e]pyrimidin-2-yl]-4-chloro-2-mercapto-5-methylbenzenesulfonamide (**27**).* Starting from 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)-5-(4-chlorophenyl)-thiophene-2-carboxylate **11** (2.57 g) and benzylamine, the title compound **27** was obtained (2.4 g, 81%); m.p. 267–268 °C dec. IR (KBr) 3280 (NH), 2560 (SH), 1700 (C=O), 1350, 1165 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 4.09 (s, 1H, SH), 5.29 (s, 2H, CH_2), 7.08–7.20 (m, 5H, Ph), 7.27 (s, 1H, H-3, $PhSO_2$), 7.32 (s, 1H, H-6, $PhSO_2$), 7.45 (d, $J = 8.1$ Hz, 2H, 4-ClPh), 7.58 (d, $J = 8.1$ Hz, 2H, 4-ClPh), 7.91 (s, 1H, H-7, thienopyrimidine), 11.29 (s, 1H, NH) ppm. Anal. Calcd. for $C_{26}H_{19}Cl_2N_3O_3S_3$ (588.52): C, 53.05; H, 3.25; N, 7.14. Found: C, 53.14; H, 3.28; N, 7.07.

*4.1.2.8. 4-Chloro-N-[3-furfuryl-3,4-dihydro-4-oxo-6-(3-thienyl)thieno[2,3-e]pyrimidin-2-yl]-2-mercapto-5-methylbenzenesulfonamide (**28**).* Starting from 3-(6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazin-3-ylamino)-5-(3-thienyl)thiophene-2-carboxylate **12** (2.42 g) and furfurylamine, the title compound **28** was obtained (2.1 g, 76%); m.p. 259–261 °C. IR (KBr) 3250 (NH), 2552 (SH), 1670 (C=O), 1350, 1320, 1155 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.32 (s, 3H, CH_3), 3.50 (s, 1H, SH), 5.16 (s, 2H, CH_2), 6.08 (d, $J = 3.2$ Hz, 1H, H-3, furan), 6.24 (d, $J = 3.2$ Hz, 1H, H-4, furan), 7.44

(s, 1H, H-3, $PhSO_2$), 7.50–7.77 (m, 5H, arom.), 7.89 (s, 1H, H-7, thienopyrimidine), 8.13 (s, 1H, NH) ppm. Anal. Calcd. for $C_{22}H_{16}ClN_3O_4S_4$ (550.08): C, 48.03; H, 2.93; N, 7.64. Found: C, 48.00; H, 3.11; N, 7.72.

*4.1.3. General procedure for the preparation of 2-benzylthio-4-chloro-N-(3,4-dihydro-4-oxopyrimidin-2-yl)benzenesulfonamide derivatives (**35–43**)*

A suspension of the corresponding *N*-(2-benylthio-4-chlorobenzenesulfonyl)cyanamide potassium salt **29–34** (3 mmol) and appropriate methyl *o*-aminoaryl carboxylate (3 mmol) in glacial acetic acid (15 ml) was refluxed with stirring for 15–20 h. After cooling the reaction mixture was left to stand overnight at room temperature. The precipitate was collected by filtration, washed with glacial acetic acid (2×0.5 ml) and dried. The crude reaction product was purified by recrystallization from ethanol.

In this manner, the following compounds were obtained.

*4.1.3.1. 2-Benzylthio-4-chloro-N-(3,4-dihydro-4-oxoquinazolin-2-yl)-5-methylbenzenesulfonamide (**35**).* Starting from cyanamide potassium salt **29** (1.17 g) and methyl 2-aminobenzoate (0.45 g), the title compound **35** was obtained (0.6 g, 46%); m.p. 191–192 °C. IR (KBr) 3283, 3227 (NH), 1701 (C=O), 1302, 1130 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 4.30 (s, 2H, SCH_2), 6.99–7.17 (m, 5H, Ph), 7.38 (t, 1H, H-6, quinazoline), 7.51 (d, $J = 8.3$ Hz, 1H, H-8, quinazoline), 7.65 (s, 1H, H-3, $PhSO_2$), 7.78 (t, $J = 8.3$ Hz, 1H, H-7, quinazoline), 7.95 (s, 1H, H-6, $PhSO_2$), 7.96–8.00 (m, 1H, H-5, quinazoline), 11.33 (s, 1H, NH), 11.86 (s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.25, 36.98, 115.68, 117.46, 124.94, 127.18, 127.45, 128.51, 128.96, 129.17, 130.61, 133.26, 135.64, 136.07, 136.22, 137.61, 139.16, 139.43, 149.38, 159.95 ppm. Anal. Calcd. for $C_{22}H_{18}ClN_3O_3S_2$ (471.99): C, 55.98; H, 3.84; N, 8.90. Found: C, 56.08; H, 3.98; N, 8.92.

*4.1.3.2. 2-Benzylthio-4-chloro-N-(7-chloro-3,4-dihydro-4-oxoquinazolin-2-yl)-5-methylbenzenesulfonamide (**36**).* Starting from cyanamide potassium salt **29** (1.17 g) and methyl 2-amino-4-chlorobenzoate (0.56 g), the title compound **36** was obtained (0.3 g, 18%); m.p. 230–232 °C. IR (KBr) 3304, 3265, 3135 (NH), 1692 (C=O), 1306, 1130 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 4.34 (s, 2H, SCH_2), 7.07–7.23 (m, 5H, Ph), 7.42–7.47 (m, 1H, quinazoline), 7.68 (s, 2H, H-3 and H-6, $PhSO_2$), 7.97–8.02 (m, 2H, quinazoline), 11.52 (s, 1H, NH), 11.83 (s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.26, 37.04, 114.68, 117.09, 125.08, 127.49, 128.52, 129.01, 129.18, 130.63, 133.28, 135.80, 136.21, 137.72, 138.96, 140.39, 140.48, 149.49, 159.46 ppm. Anal. Calcd. for $C_{22}H_{17}Cl_2N_3O_3S_2$ (506.43): C, 52.18; H, 3.38; N, 8.29. Found: C, 52.25; H, 3.44; N, 8.29.

*4.1.3.3. 4-Benzylthio-2-chloro-5-(3,4-dihydro-4-oxoquinazolin-2-ylaminosulfonyl)benzamide (**37**).* Starting from cyanamide potassium salt **30** (1.26 g) and methyl 2-aminobenzoate (0.45 g), the title compound **37** was obtained (0.7 g, 48%); m.p. 310–311 °C dec. IR (KBr) 3371, 3195 (NH), 1710 (C=O), 1685 (C=O), 1307, 1147 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.38 (s, 2H, SCH_2), 7.06–7.09 (m, 2H, H–Ar), 7.12–7.14 (m, 1H, H–Ar), 7.18–7.21 (m, 2H, H–Ar), 7.35–7.39 (m, 1H, H–Ar), 7.52–7.53 (m, 1H, H–Ar), 7.69 (s, 1H, H-3, $PhSO_2$), 7.73 (s, 1H, $CONH_a$), 7.76–7.79 (m, 1H, H–Ar), 7.98 (br s, 2H, $CONH_b$, H–Ar), 8.03 (s, 1H, H-6, $PhSO_2$), 11.39 (s, 1H, NH), 11.86 (s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 36.40, 107.38, 115.83, 117.69, 124.98, 127.15, 127.58, 128.51, 128.61, 129.00, 133.28, 134.06, 135.94, 136.02, 138.55, 139.55, 139.56, 149.57, 160.12, 166.88 ppm. Anal. Calcd. for $C_{22}H_{17}ClN_4O_4S_2$ (500.99): C, 52.74; H, 3.42; N, 11.18. Found: C, 52.88; H, 3.32; N, 10.91.

*4.1.3.4. 4-Benzylothio-2-chloro-5-(3,4-dihydro-4-oxoquinazolin-2-ylaminosulfonyl)-N-(phenyl)benzamide (**38**).* Starting from cyanamide potassium salt **31** (1.46 g) and methyl 2-aminobenzoate (0.45 g) the title compound **38** was obtained (0.8 g, 47%); m.p. 282–283 °C. IR (KBr) 3289, 3236 (NH), 1726 (C=O), 1684 (C=O), 1303, 1143 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.47 (s, 2H, S–CH_2), 7.17–7.27 (m, 5H, H–Ar), 7.37–7.44 (m, 3H, H–Ar), 7.59–7.62 (m, 2H, H–Ar), 7.72–7.75 (m, 3H, H–Ar), 7.83 (s, 1H, H-3, $PhSO_2$), 8.02–8.05 (m, 1H, H–Ar), 8.15 (s, 1H, H-6, $PhSO_2$), 10.66 (s, 1H, CONH), 11.50 (s, 1H, NH), 11.88 (s, 1H, SO_2NH) ppm. Anal. Calcd. for $C_{28}H_{21}ClN_4O_4S_2$ (577.09): C, 58.27; H, 3.67; N, 9.70. Found: C, 58.32; H, 3.75; N, 9.57.

*4.1.3.5. 4-Benzylothio-2-chloro-5-(3,4-dihydro-4-oxoquinazolin-2-ylaminosulfonyl)-N-(4-methylphenyl)benzamide (**39**).* Starting from cyanamide potassium salt **32** (1.53 g) and methyl 2-aminobenzoate (0.45 g), the title compound **39** was obtained (0.8 g, 46%); m.p. 271–274 °C. IR (KBr) 3278, 3242 (NH), 1726 (C=O), 1685 (C=O), 1303, 1143 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.29 (s, 3H, CH_3), 4.44 (s, 2H, SCH_2), 7.15–7.24 (m, 7H, H–Ar), 7.36–7.44 (m, 2H, H–Ar), 7.57–7.60 (m, 3H, H–Ar), 7.79 (m, 1H, H-3, $PhSO_2$), 7.99–8.02 (m, 1H, H–Ar), 8.11 (s, 1H, H-6, $PhSO_2$), 10.55 (s, 1H, CONH), 11.46 (s, 1H, NH), 11.86 (s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 20.81, 36.36, 115.86, 117.72, 120.00, 125.05, 127.15, 127.61, 128.64, 128.80, 129.02, 129.48, 133.16, 133.35, 134.34, 135.97, 136.10, 136.44, 138.66, 139.47, 140.49, 149.56, 160.12, 163.41 ppm. Anal. Calcd. for $C_{29}H_{23}ClN_4O_4S_2$ (591.11): C, 58.93; H, 3.92; N, 9.48. Found: C, 59.03; H, 3.99; N, 9.62.

*4.1.3.6. 4-Benzylothio-2-chloro-5-(3,4-dihydro-4-oxoquinazolin-2-ylaminosulfonyl)-N-(4-methoxyphenyl)benzamide (**40**).* Starting from cyanamide potassium salt **33** (1.58 g) and methyl 2-aminobenzoate (0.45 g) the title compound **40** was obtained (1.0 g, 55%); m.p. 295–297 °C dec. IR (KBr) 3283, 3242 (NH), 1725 (C=O), 1681 (C=O), 1303, 1143 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.74 (s, 3H, OCH_3), 4.42 (s, 2H, SCH_2), 6.91–6.95 (m, 2H, H–Ar), 7.12–7.22 (m, 5H, H–Ar), 7.32–7.48 (m, 1H, H–Ar), 7.58–7.61 (m, 3H, H–Ar), 7.73 (br s, 2H, H-3, $PhSO_2$ and H–Ar), 7.97–8.01 (m, 1H, H–Ar), 8.09

(s, 1H, H-6, $PhSO_2$), 10.47 (s, 1H, CONH), 11.46 (s, 1H, NH), 11.84 (s, 1H, SO_2NH) ppm. Anal. Calcd. for $C_{29}H_{23}ClN_4O_5S_2$ (607.11): C, 57.37; H, 3.82; N, 9.23. Found: C, 57.44; H, 3.90; N, 9.37.

*4.1.3.7. 4-Benzylothio-2-chloro-N-(4-chlorophenyl)-5-(3,4-dihydro-4-oxoquinazolin-2-ylaminosulfonyl)benzamide (**41**).* Starting from cyanamide potassium salt **41** (1.59 g) and methyl 2-aminobenzoate (0.45 g), the title compound **41** was obtained (0.7 g, 41%); m.p. 263–264 °C. IR (KBr) 3236 (NH), 1727 (C=O), 1681 (C=O), 1304, 1143 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.46 (s, 2H, S–CH_2), 7.14–7.39 (m, 5H, H–Ar), 7.43–7.48 (m, 3H, H–Ar), 7.57–7.62 (m, 1H, H–Ar), 7.73–7.85 (m, 4H, H-3, H–Ar), 8.00–8.05 (m, 1H, H–Ar), 8.16 (s, 1H, H-6, $PhSO_2$), 10.79 (s, 1H, CONH), 11.48 (s, 1H, NH), 11.87 (s, 1H, SO_2NH) ppm. Anal. Calcd. for $C_{28}H_{20}Cl_2N_4O_4S_2$ (611.53): C, 54.99; H, 3.30; N, 9.16. Found: C, 55.10; H, 3.36; N, 9.18.

*4.1.3.8. 2-Benzylthio-4-chloro-N-(3,4-dihydro-4-oxothieno[3,4-e]pyrimidin-2-yl)-5-methylbenzenesulfonamide (**42**).* Starting from cyanamide potassium salt **29** (1.17 g) and methyl 3-aminothiophene-4-carboxylate (0.47 g) the title compound **42** was obtained (0.9 g, 63%); m.p. 237–239 °C dec. IR (KBr) 3294, 3228, 3191 (NH), 1692 (C=O), 1363, 1341, 1132 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.30 (s, 2H, SCH_2), 7.12–7.17 (m, 3H, Ph), 7.21–7.23 (m, 2H, Ph), 7.39–7.40 (d, $J = 3.4$ Hz, 1H, H-7, thienopyrimidine), 7.61 (s, 1H, H-3, $PhSO_2$), 7.96 (s, 1H, H-6, $PhSO_2$), 8.50 (d, $J = 2.9$ Hz, 1H, H-5, thienopyrimidine), 11.35 (s, 1H, NH), 11.58 (s, 1H, SO_2NH) ppm. Anal. Calcd. for $C_{20}H_{16}ClN_3O_3S_3$ (487.01): C, 50.25; H, 3.37; N, 8.79. Found: C, 52.40; H, 3.43; N, 8.64.

*4.1.3.9. 2-Benzylthio-4-chloro-N-(3,4-dihydro-4-oxothieno[2,3-e]pyrimidin-2-yl)-5-methylbenzenesulfonamide (**43**).* Starting from cyanamide potassium salt **29** (1.17 g) and methyl 3-aminothiophene-2-carboxylate (0.47 g), the title compound **43** was obtained (0.5 g, 35%); m.p. 212–214 °C. IR (KBr) 3303, 3208, 3173, 3113, 3102 (NH), 1684 (C=O), 1340, 1127, 1105 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.29 (s, 2H, SCH_2), 7.07–7.22 (m, 5H, Ph), 7.26 (d, $J = 5.2$ Hz, 1H, H-7, thienopyrimidine), 7.59 (s, 1H, H-3, $PhSO_2$), 7.95 (s, 1H, H-6, $PhSO_2$), 8.13–8.16 (d, $J = 5.2$ Hz, 1H, H-6, thienopyrimidine), 11.59 (br s, 1H, NH), 12.70 (br s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.25, 36.99, 114.35, 119.72, 127.49, 128.56, 128.92, 129.08, 130.79, 132.95, 135.73, 136.28, 137.06, 137.32, 139.46, 146.56, 150.69, 156.81 ppm. Anal. Calcd. for $C_{20}H_{16}ClN_3O_3S_3$ (487.01): C, 50.25; H, 3.37; N, 8.79. Found: C, 52.20; H, 3.48; N, 8.91.

*4.1.3.10. 2-Benzylthio-4-chloro-N-(1,4-dihydro-1-methyl-4-oxoquinazolin-2-yl)-5-methylbenzenesulfonamide (**44**).* Starting from cyanamide potassium salt **29** (1.17 g) and methyl 2-(methylamino)benzoate (0.49 g), the title compound **44** was obtained (0.6 g, 43%); m.p. 224–226 °C. IR (KBr) 3232 (NH), 1704 (C=O), 1330, 1124, 1105 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 3.55 (s, 3H, NCH_3), 4.37 (s, 2H, SCH_2), 7.06–7.16 (m, 5H, Ph), 7.47–7.55 (m, 1H, H–Ar), 7.68–7.75 (m, 2H, H-3 $PhSO_2$ and H–Ar), 7.88–8.11 (m, 3H, H-6, $PhSO_2$ and 2H–Ar), 11.03 (s, 1H, NH) ppm. Anal. Calcd. for $C_{23}H_{20}ClN_3O_3S_2$ (486.01): C, 56.84; H, 4.15; N, 8.65. Found: C, 56.95; H, 4.21; N, 8.84.

4.2. Integrase assay

To determine the extent of 3′-processing and strand transfer, HIV-1 IN was preincubated at a final concentration of 200 nM with inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothretiol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/ml bovine serum albumin, 10 mM 2-mercaptoethanol, 10% DMSO and 25 mM MOPS, pH 7.2) at 30 °C for 30 min. Then, 20 nM of the 5′-end ^{32}P-labeled linear oligonucleotide substrate was added and incubation was continued for an additional 1 h. Reactions were quenched by an addition of (8 μl) loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol and 0.025% bromophenol blue). An aliquot (5 μl) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M tris–borate pH 8.3, 2 mM EDTA, 20% acrylamide, 8 M urea). Gels were dried, exposed on a PhosphorImager cassette, read on a Typhoon 8610 Variable mode Imager (Amersham Biosciences), and quantitated using ImageQuant 5.2 (Amersham Biosciences).

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