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Bioorganic & Medicinal Chemistry 12 (2004) 3663–3672
Bioorganic & Medicinal Chemistry

Synthesis, antiviral, and anti-HIV-1 integrase activities of 3-aroyl-1,1-dioxo-1,4,2-benzodithiazines ☆

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Received 26 November 2003; revised 12 April 2004; accepted 15 April 2004
Available online 18 May 2004

Abstract—HIV-1 integrase (IN) is an essential enzyme for effective viral replication and is an attractive target for selective blockade of viral infection. Previously, we identified a series of sulfones, sulfonamides, and mercaptosalicylhydrazides (MBSAs) as IN inhibitors with antiviral activities in cell-based assays. In an effort to optimize a series of our active site directed lead compounds, we designed and synthesized novel benzodithiazines starting from MBSAs. In contrast to all reported IN inhibitors benzodithiazines are essentially nontoxic. Significant antiviral potency was only observed at concentration exceedingly higher than that required to inhibit purified IN.

1. Introduction

Integration of viral DNA into the host cell chromosomal DNA to form a provirus is an essential step in the viral life cycle. This process is mediated by IN, a 32 kDa viral enzyme.[1,2] During the past 10 years a plethora of inhibitors have been identified and some were shown to be selective against IN and block viral replication (for recent reviews see Refs. 3–5). The two most predominant classes of inhibitors have been the catechol containing hydroxylated aromatics[6] and more recently the diketoacid containing aromatics.[7] Discovery of a *bona fide* IN inhibitor has continuously been challenging and is of paramount importance because currently there are no FDA approved drugs targeting this enzyme. Inclusion of IN inhibitors with the highly active antiretroviral cocktails is expected to improve the efficacy of existing drugs by lowering the viral load to undetectable level.

In our continuous efforts in developing IN inhibitors we focused our efforts in developing compounds that (1) lack hydroxylated aromatic moieties, which in most cases has been associated with their notorious lack of selectivity and toxicity, (2) represent novel chemotypes that are easily amenable to derivatization, (3) exhibit antiviral activity in cell-based assays. Among a series of lead compounds we identified that satisfy all these requirements are the mercaptobenzenesulfonamides (MBSAs)[8,9] and mercaptobenzenesalicylhydrazides (MSHs).[10] More recently a dipyrimidine class of compound was identified as selective IN inhibitor and among all the tested compounds the mercaptodipyrimidine analog was found to be the most potent.[11] A common theme in these leads is the requirement for a free mercaptoaryl group for antiviral activity as well as anti-IN potency (Fig. 1).[8,10,12] It was recently demonstrated that compound **MSH** could possibly form a ternary complex with magnesium and binds to the Cysteine 65 on the active site of IN.[10] A major problem with these molecules in general is, their lack of in vitro and in vivo stability due to dimerization and metabolism that could potentially lead to their toxicity. Therefore, optimization of these lead compounds requires significant modification to enhance their stability under physiological conditions.

Keyword: HIV-1 integrase inhibitors.
☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.04.024
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doi:10.1016/j.bmc.2004.04.024

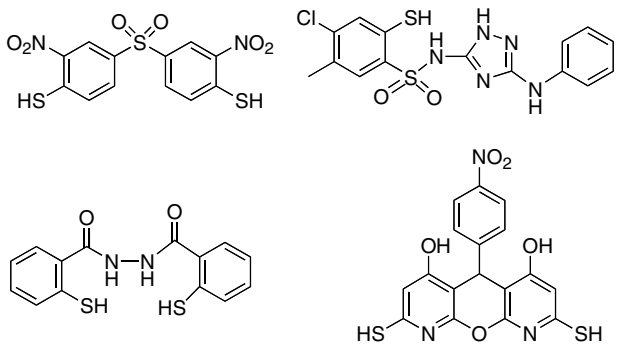


Figure 1. Structures of mercaptoaryl lead compounds where an SH group is believed to be important for both antiviral and anti-integrase activities.

Because arylsulfonamides represent a class of drugs with a well-established safety profile (for a recent review see Ref. 13) we developed a new method for the synthesis of cyclic analogs of MBSAs (Scheme 1) incorporating aroyl group in their structure. In this study we prepared a set of closely related analogs in order to determine minimum structural features that are required for anti-IN and antiviral activity.

2. Results and discussion

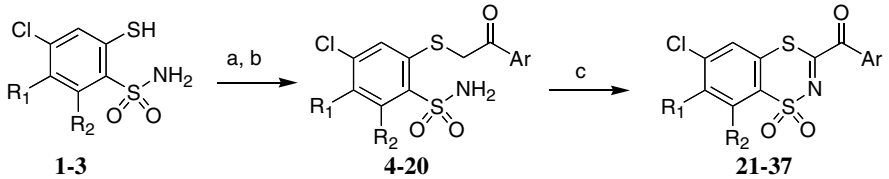
2.1. Synthesis

The synthesis of a series of novel benzodithiazine compounds is presented in Scheme 1. First, the 4-chloro-2-mercaptobenzenesulfonamides **1**–**3** prepared according to the previously described procedure[14] were subjected to the reaction with bromomethyl ketones in the presence of triethylamine to give the corresponding S-substituted derivatives **4**–**20**. The later compounds were reacted with thionyl chloride to give the target products **21**–**37** in 39–75% yield. The ring closure observed in the reaction of benzenesulfonamides containing an aroylmethylthio group at *ortho*-position with thionyl chloride represents a new type of reaction leading to 3-substituted 1,1-dioxo-1,4,2-benzodithiazine derivatives.

2.2. Single-crystal X-ray structure of 24

In order, to design more potent analogs using high throughput structure-based drug design we solved the X-ray crystal structure of a representative compound (Fig. 2). Compound **24** was found to crystallize in monoclinic space group $P2_1/n$, $a = 7.2102(5)$, $b = 13.6632(7)$, $c = 15.6326(8)$ Å, $\beta = 91.406(5)°$, $V = 1539.57(15)$ Å^3, $Z = 4$, $d_x = 1.666\,g\,cm^{-3}$, $T = 155$ K (Table 1). Final R indices for 2808 reflections with $I > 2\sigma(I)$ and 244 refined parameters are: $R_1 = 0.0388$, $wR_2 = 0.0921$ ($R_1 = 0.0455$, $wR_2 = 0.0964$ for all 3112 data). Atom labeling is shown in Figure 2. Compound **24** is virtually flat due to a nearly planar conformation of the 1,4,2-dithiazine ring and only small twists (ca. 7°) about the C_{sp^2}–C_{sp^2} bonds to the carbonyl group. This molecular conformation is stabilized by weak intramolecular C–H···N interaction between the phenyl ring and dithiazine moiety (H···N 2.26 Å, <C20–H20···N13 124°).

It has been shown that 1,1-dioxo-1,2,4-dithiazine ring can adopt planar[15] and boat[14] conformations whereas 1,4,2-dithiazine ring with the lone pairs of electrons on S atom prefers the boat conformation; the planar conformation of this 8π-electron system is destabilized by anti-aromaticity.[15] The geometry of the 1,1-dioxo-1,2,4-dithiazine system in **24** is very similar to that observed in 5,6-dimethyl-3-(4-bromophenyl)-1,4,2-dithiazine 1,1-dioxide[15] with the only discrepancy in the C–C bond



1-3 **4-20** **21-37**

Compd.	R^1	R^2	Ar	Compd.	R^1	R^2	Ar
1, 4, 21	Me	H	Ph	**1, 13, 30**	Me	H	2-naphthyl
1, 5, 22	Me	H	4-MeO Ph	**2, 14, 31**	H	Me	4-ClPh
1, 6, 23	Me	H	4-BrPh	**2, 15, 32**	H	Me	3,4-diClPh
1, 7, 24	Me	H	4-Cl-Ph	**2, 16, 33**	H	Me	2-naphthyl
1, 8, 25	Me	H	4-FPh	**3, 17, 34**	H	H	4-ClPh
1, 9, 26	Me	H	4-O_2NPh	**3, 18, 35**	H	H	3-O_2NPh
1, 10, 27	Me	H	3-O_2NPh	**3, 19, 36**	H	H	3,4-diClPh
1, 11, 28	Me	H	3,4-diClPh	**3, 20, 37**	H	H	2-naphthyl
1, 12, 29	Me	H	4-Ph-Ph				

Scheme 1. Synthesis of the benzenesulfonamides **4**–**20** and their transformation into benzodithiazines **21**–**37**. Reagents, conditions, and yields: (a) Et_3N (1.1 M equiv), CH_2Cl_2, 18–24 °C; (b) $ArCOCH_2Br$ (1.1 M equiv), 18–24 °C, 1 h, reflux, 6 h, 59–85%; (c) $SOCl_2$ (excess), reflux, 46 h, 39–75%.

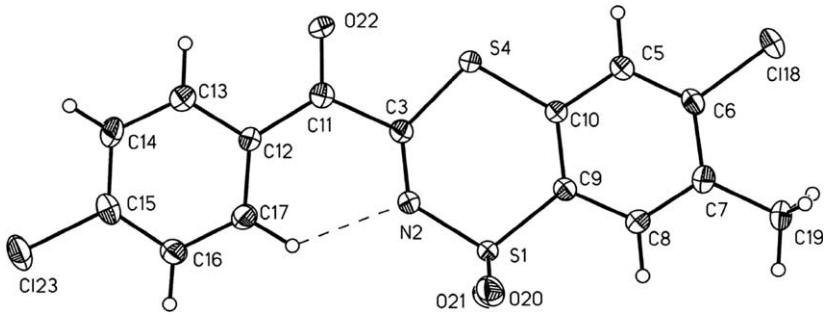


Figure 2. Ortep drawing of compound **24**. Thermal ellipsoids were drawn at 50% probability level.

Table 1. Crystal data and structure refinement for compound **24**

Empirical formula	$C_{15}H_9C_{12}NO_3S_2$
Formula weight	386.25
Temperature	155(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell dimensions	$a = 7.2102(5)$ Å $\alpha = 90°$
	$b = 13.6632(7)$ Å $\beta = 91.406(5)°$
	$c = 15.6326(8)$ Å $\gamma = 90°$
Volume	1539.57(15) Å^3
Z	4
Density (calculated)	1.666 Mg/m^3
Absorption coefficient	0.705 mm^{-1}
$F(000)$	784
Crystal size	$0.80 \times 0.15 \times 0.08$ mm^3
θ range for data collection	3.48–26.37°
Index ranges	$-9 \leqslant h \leqslant 6$, $-17 \leqslant k \leqslant 16$,
	$-17 \leqslant l \leqslant 19$
Reflections collected	8422
Independent reflections	3112 [R(int) = 0.0283]
Completeness to $\theta = 26.37°$	99.1%
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3112/0/244
Goodness-of-fit on F^2	1.118
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0388$, $wR_2 = 0.0921$
R indices (all data)	$R_1 = 0.0455$, $wR_2 = 0.0964$
Largest diff. peak and hole	0.290 and −0.431 e Å^{-3}

length as a result of a difference in the C–C bond character (aromatic vs double) in both compounds. Interestingly, despite the planar molecular conformation of **24** the C_{sp^2}–C_{sp^2} bond length of 1.533 Å between the benzoyl and heterocyclic moieties indicates no π-electron conjugation for this bond.

2.3. In vitro biology

Previously, we discovered that MBSAs inhibit purified IN and protected HIV-1 infected CEM cells.[8,9] In that study it was shown that aromatic mercapto group was important for activity.[8] More recently we provided evidence that the MSHs class of compounds selectively inhibit IN, chelate magnesium ion, and bind to the C65 residue on the active site of IN.[10] In an effort to improve upon the lack of stability of aforementioned compounds we prepared a series of cyclic derivatives and tested their inhibitory potency against wild-type IN as well as the C65S mutant. The C65 residue located in close proximity of the highly conserved D64, D116, and E152 triad once mutated to a serine residue retains all it's catalytic activity similar to those of the wild-type IN. We have used this mutant enzyme previously to demonstrate that some compounds can potentially bind to this residue and that this amino acid might be important for drug resistance viral strains.[10,16] In the current study we observed significant differential potencies only with compounds **21**–**23** and **35**–**37** against C65S mutant versus those of the wild type. This may suggest that these latter compounds could potentially interact with C65.

We also observed that all compounds inhibited IN with IC_{50} values below 100 μM (Fig. 3 and Table 2) while their antiviral activities varied greatly. In general the antiviral activities were observed only with concentrations 1–10 folds higher than those against purified IN (Table 2). Except for compound **33** all other compounds showed cytotoxcity well above 100 μM ranges. It is also important to note that although high concentration was required for antiviral activity but in many instances 100% protection of HIV-1 infected CEM cells was achieved (compounds designated as 'A', Table 2).

In conclusion, we have demonstrated that the cyclic analogs of MBSAs or the benzodithiazines are novel class of IN inhibitors, they exhibit antiviral activity albeit at high concentrations, and are essentially nontoxic. Further structural modifications are underway to optimize their potency.

3. Experimental

3.1. Chemistry. General

Melting points: Buchi 535 apparatus; IR spectra KBr pellets, 400–4000 cm^{-1} Perkin–Elmer 1600 FTIR spectrometer; ^{1}H NMR spectra: Varian Gemini 200 apparatus (chemical shifts are expressed as δ values relative to Me_4Si as standard). ^{13}C NMR spectra were taken on a Varian Unity 500 spectrometer. Analyses of C, H, N, were within ±0.4% of the theoretical values.

3.2. General procedure for the preparation of 2-[(2-aryl-oxoethyl) sulfanyl]benzenesulfonamides 4–20

To a suspension of the corresponding 2-mercaptobenzenesulfonamide **1**, **2**, or **3** (0.02 mol) in dichloromethane

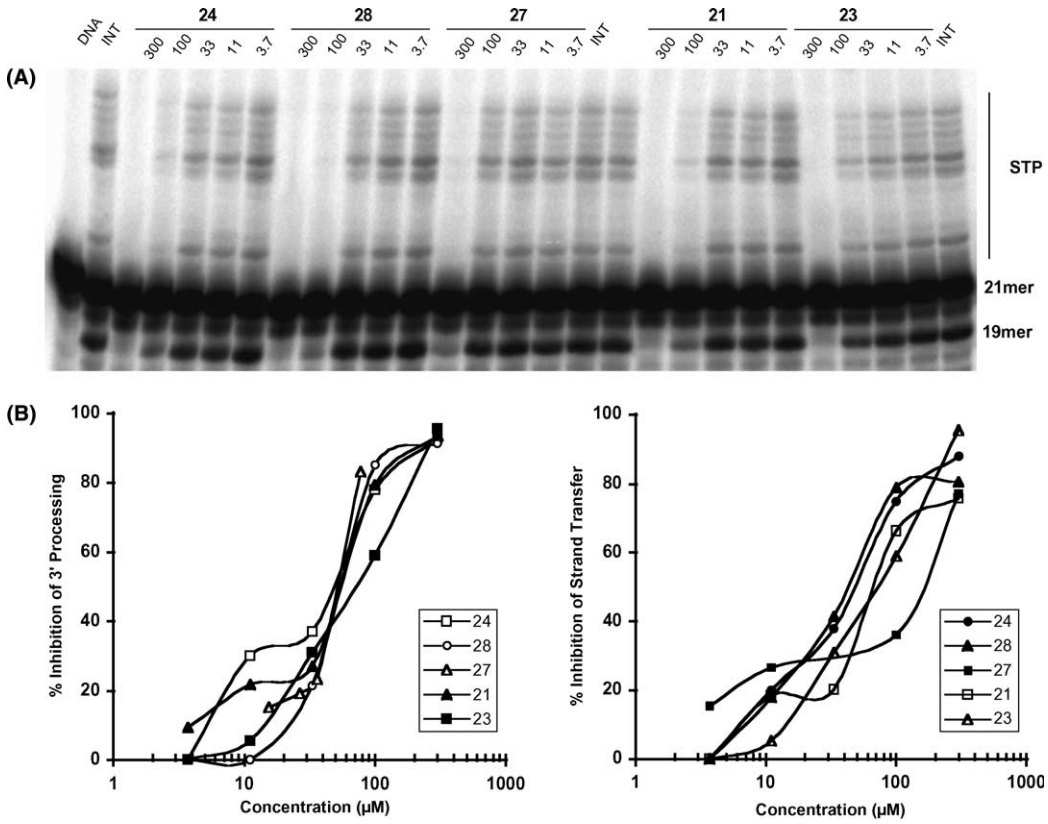


Figure 3. (A) A representative gel showing inhibition of purified IN by selected compounds. A 21-mer blunt-end oligonucleotide corresponding to the U5 end of the HIV-1 LTR, 5′ end-labeled with ^{32}P, is reacted with purified IN. The initial step involves nucleolytic cleavage of two bases from the 3′-end, resulting in a 19-mer oligonucleotide. Subsequently, 3′ ends are covalently joined at several sites to another identical oligonucleotide that serves as the target DNA. This reaction is referred to as strand transfer and the products formed migrate slower than the original substrate (shown in the figure as STP for strand transfer products). Drug concentrations in μM are indicated above each lane. (B) Quantitation of panel A.

(30 mL), triethylamine (2.23 g, 0.022 mol) was added with stirring. The solution thus obtained was treated portionwise with the appropriate aryl bromomethyl ketone (0.022 mol) at 18–24 °C (water–ice bath). Then, the reaction mixture was stirred at room temperature for 1 h followed by reflux for 6 h. After cooling to room temperature and standing overnight the precipitate of the adequate S-substituted 2-mercaptobenzenesulfonamide was filtered off and washed successively with dichloromethane (3×3 mL), 2-propanol (2× 1.5 mL), water (4×5 mL), and 2-propanol (2×2 mL).

In this manner the following sulfonamide were obtained:

3.2.1. 4-Chloro-2-[(2-phenyl-2-oxoethyl)sulfanyl]-5-methylbenzenesulfonamide (4). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and phenacyl bromide (4.4 g); yield: 4.2 g (59%); mp 159–161 °C; IR (KBr) 3300, 3200 (NH_2), 1685 (C=O), 1355, 1330, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.91 (s, 2H, CH_2), 7.40 (s, 2H, NH_2), 7.53–7.68 (m, 4H, arom.), 7.83 (s, 1H, H-6, $PhSO_2$), 8.06 (d, $J = 7.5$ Hz, 2H, PhCO) ppm. Anal. ($C_{15}H_{14}ClNO_3S_2$) C, H, N.

3.2.2. 4-Chloro-2{[2-(4-methoxyphenyl)-2-oxoethyl]sulfanyl}-5-methylbenzenesulfonamide (5). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and 4-methoxyphenacyl bromide (5.0 g); yield: 6.0 g (77%); mp 154–155 °C; IR (KBr) 3295, 3200 (NH_2), 1670 (C=O), 1350, 1325, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 3.89 (s, 3H, CH_3O), 4.85 (s, 2H, CH_2), 7.11 (d, $J = 8.9$ Hz, 2H, $4CH_3OPhCO$), 7.43 (s, 2H, NH_2H), 7.71 (s, 1H, H-3, $PhSO_2$), 7.85 (s, 1H, H-6, $PhSO_2$), 8.07 (d, $J = 8.9$ Hz, 2H, 4-CH_3OPhCO) ppm. Anal. ($C_{16}H_{16}ClNO_3S_2$) C, H, N.

3.2.3. 4-Chloro-2-{[2-(4-bromophenyl)-2-oxoethyl]sulfanyl}-5-methylbenzenesulfonamide (6). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and 4-bromophenacyl bromide (6.1 g); yield: 6.2 g (71%); mp 185–186 °C; IR (KBr) 3365, 3255 (NH_2), 1675 (C=O), 1350, 1330, 1160, (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 7.40 (s, 2H, NH_2), 7.66 (s, 1H, H-3, $PhSO_2$), 7.78 (d, $J = 8.3$ Hz, 2H, 4-BrPhCO), 7.80 (s, 1H, H-6, $PhSO_2$), 7.99 (d, $J = 8.3$ Hz, 2H, 4-BrPHCO) ppm. Anal. ($C_{15}H_{13}BrClNO_3S_2$) C, H, N.

3.2.4. 4-Chloro-2-{[2-(4-chlorophenyl)-2-oxoethyl]sulfanyl}-5-methylbenzenesulfonamide (7). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and 4-chlorophenacyl bromide (5.1 g); yield: 5.9 g (75%); mp 169–171 °C; IR (KBr) 3295, 3205 (NH_2), 1685 (C=O), 1350,

Table 2. Antiviral and anti-HIV-1 integrase activities of novel benzodithiazines **21**–**37**

Compd	Inhibition of integrase catalitic activities		Antiviral activities[a]					
	3′-process IC_{50} (μM)[b] WT[g]/C65S[h]	Integration IC_{50} (μM)[b] WT[g]/C65S[h]	EC_{50} (μM)[c]	CC_{50} (μM)[d]	TI[e]	Maximum of Protection Dose (μM)	%	Comments[f]
21	12	12	80.9	287.5	3.5	100.0	103	A
	90	90						
22	30	33	205.8	>1000.0	>4.8	1000.0	99	A
	>100	30						
23	30	30	NT[j]	NT[j]	[i]			
	65	65						
24	30	65	>159.0	159.0	NR	200.0	25	I
	33	33						
25	57	42	161.0	>200.0	>1.25	200.0	60	M
	75	17						
26	NT	NT	52.5	125.0	2.4	63.3	60	M
	11	11						
27	57	50	77.5	183.0	2.4	200.0	102	A
	95	80						
28	15	7	>200.0	143.0	NR[i]	200.0	31	I
	20	10						
29	11	10	>200.0	110.0	NR	200.0	15	I
	18	20						
30	28	3	>200.0	>200.0	NR	200.0	47	I
	30	15						
31	27	25	256.7	>1000.0	>3.9	1000.0	106	A
	60	65						
32	17	25	>200.0	>200.0	NR	200.0	12	I
	8	3						
33	22	7	>200.0	49.2	NR	200.0	18	I
	20	20						
34	100	90	72.7	>200.0	>2.7	100.0	113	A
	100	100						
35	25	20	49.0	230.7	4.7	100.0	104	A
	62	80						
36	19	19	61.2	124.0	2.0	63.3	52	M
	47	20						
37	8	8	54.5	170.0	3.1	100.0	83	M
	65	35						

[a] Data obtained from the NCI's in vitro anti-HIV primary screen.
[b] IC_{50}: Inhibitory concentration 50% (inhibition of purified integrase).
[c] EC_{50}: Effective concentration 50% (protection of HIV-1 infected CEM cells).
[d] CC_{50}: Cytotoxic concentration 50% (toxicity to uninfected CEM cells).
[e] TI: Therapeutic index = CC_{50}/EC_{50}.
[f] Comments: NCI designated activity: A (confirmed active); M (confirmed moderate); I (confirmed inactive).
[g] The top values in each row are samples tested against wild-type HIV-1 integrase.
[h] The bottom values in each row are samples tested against active-site mutant C65S. The values are presented as an average of two to four experiments with the standard error of less than 5%.
[i] NR: No therapeutic benefit reached due to cytotoxicity.
[j] NT: not tested.

1330, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.89 (s, 2H, CH_2), 7.40 (s, 2H, NH_2). 7.62 (d, $J = 8.6$ Hz, 2H, 4ClPhCO), 7.66 (s, 1H, H-3, $PhSO_2$), 7.83 (s, 1H, H-6, $PhSO_2$), 8.7 (d, $J = 8.6$ Hz, 2H, 4-ClPhCO) ppm. Anal. ($C_{15}H_{13}Cl_2NO_3S_2$) C, H, N.

3.2.5. 4-Chloro-2-{[2-(4-fluorophenyl)-2-oxoethyl]sulfanyl}-5-methylbenzenesulfonamide (8). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and 4-fluorophenacyl bromide (4.8 g); yield: 5.5 g (73%); mp 138–139 °C; IR (KBr) 3305, 3225 (NH_2), 1680 (C=O), 1345, 1325, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 7.35–7.44 (m, 4H, NH_2 and 4-FPhCO), 7.66 (s, 1H, H-3, $PhSO_2$), 7.83 (s, 1H, H-6, $PhSO_2$), 8.11–8.19 (m, 2H, 4-$FPhSO_2$) ppm; ^{13}C NMR (DMSO-d_6) δ 19.28, 115.91, 116.35, 129.67, 130.30, 131.84, 132.03, 132.33, 133.42, 134.03, 137.27, 140.48, 163.09, 168.12, 193.32 ppm. Anal. ($C_{15}H_{13}ClFNO_3S_2$) C, H, N.

3.2.6. 4-Chloro-2-{[2-(4-nitrophenyl)-2-oxoethyl]sulfanyl}-5-methylbenzenesulfonamide (9). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and 4-nitrophenacyl bromide (5.4 g); yield: 4.9 g (61%); mp

195–196 °C; IR (KBr) 3375, 3280 (NH_2), 1690 (C=O), 1350, 1150 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 7.41 (s, 2H, NH_2), 7.61 (s, 1H, H-3, $PhSO_2$), 7.84 (s, 1H, H-6, $PhSO_2$), 8.28 (d, $J = 8.7$ Hz, 2H, 4-O_2NPhCO), 8.38 (d, $J = 8.7$ Hz, 2H, 4-O_2NPhCO) ppm. Anal. ($C_{15}H_{13}ClN_2O_5S_2$) C, H, N.

3.2.7. 4-Chloro-2-[{2-(3-nitriphenyl)-2-oxoethyl]sulfanyl}-5-methylbenzenesulfonamide (10). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and 3-nitrophenacyl bromide (5.4 g); yield: 6.5 g (81%); mp 176–178 °C; IR (KBr) 3365, 3270 (NH_2), 1685 (C=O), 1350, 1150 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 5.01 (s, 2H, CH_2), 7.39 (s, 2H, NH_2), 7.70 (s, 1H, H-3, $PhSO_2$), 7.83–7.90 (m, 2H, arom.), 8.45–8.52 (m, 2H, arom.), 8.73 (t, $J = 1.8$ Hz, 1H, H-2, 3-O_2NPhCO) ppm: ^{13}C NMR (DMSO-d_6) δ 19.32, 123.21, 128.13, 129.93, 130.35, 130.87, 133.58, 135.04, 136.82, 137.26, 140.70, 148.31, 193.25 ppm. Anal, ($C_{15}H_{13}ClN_2O_5S_2$) C, H, N.

3.2.8. 4-Chloro-2-{[2-(3,4-dichlorophenyl)-2-oxoethyl]sulfanyl}-5-methylbenzenesulfonamide (11). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and 3,4-dichlorophenacyl bromide (5.8 g); yield: 5.6 g (66%); mp 179–180 °C; IR (KBr) 3290, 3200 (NH_2), 1695 (C=O), 1350, 1325, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.50 (s, 3H, CH_3), 5.05 (s, 2H, CH_2), 7.57 (s, 2H, NH_2), 7.82 (s, 1H, H-3, $PhSO_2$), 7.98–8.16 (m, 3H, arom.), 8.42 (s, 1H, H-6, $PhSO_2$) ppm; ^{13}C NMR (DMSO-d_6) δ 19.30, 128.76, 129.82, 130.32, 130.77, 131.39, 132.11, 133.54, 133.65, 135.77, 136.84, 137.26, 140.59, 192.91 ppm. Anal. ($C_{15}H_{12}Cl_3NO_3S_2$) C, H, N.

3.2.9. 4-Chloro-2-{[2-(4-biphenylyl)-2-oxoethyl]sulfanyl}-5-methylbenzenesulfonamide (12). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and 4-phenylphenacyl bromide (6.0 g); yield: 5.3 g (61%); mp 199–200 °C; IR (KBr) 3355, 3260 (NH_2), 1660 (C=O), 1355, 1330, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 4.93 (s, 2H, CH_2), 7.41 (s, 2H, NH_2), 7.44–7.57 (m, 3H, arom.), 7.70 (s, 1H, H-3, $PhSO_2$), 7.76–7.89 (m, 5H, arom.), 8.14 (d, $J = 8.4$ Hz, 2H, arom.) ppm. Anal. ($C_{21}H_{18}ClNO_3S_2$) C, H, N.

3.2.10. 4-Chloro-2-{[2-(2-naphthyl)-2-oxoethyl]sulfanyl}-5-methylbenzenesulfonamide (13). Starting from 2-mercaptobenzenesulfonamide **1** (4.76 g) and bromomethyl 2-naphthyl ketone (5.5 g); yield: 6.1 g (75%); mp 170–171 °C; IR (KBr) 3265, 3170 (NH_2), 1675 (C=O), 1350, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 5.02 (s, 2H, CH_2), 7.41 (s, 2H, NH_2), 7.64–7.71 (m, 2H, arom.), 7.73 (s, 1H, H-3, $PhSO_2$), 7.84 (s, 1H, H-6, $PhSO_2$), 8.00–8.16 (m, 4H, arom.), 8.81 (s, 1H, H-1, 2-naphthoyl) ppm. Anal. ($C_{19}H_{16}ClNO_3S_2$) C, H, N.

3.2.11. 4-Chloro-2-{[2-(4-chlorophenyl)-2-oxoethyl]sulfanyl}-6-methylbenzenesulfonamide (14). Starting from 2-mercaptobenzenesulfonamide **2** (4.76 g) and 4-chlorophenacyl bromide (5.1 g); yield: 5.4 g (69%); mp 154–156 °C; IR (KBr) 3280, 3185 (NH_2), 1680 (C=O), 1335, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.59 (s, 3H, CH_3), 4.84 (s, 2H, CH_2), 7.27 (s, 2H, NH_2), 7.48 (s, 1H, H-5, $PhSO_2$), 7.51 (s, 1H, H-3, $PhSO_2$), 7.64 (d, $J = 8.5$ Hz, 2H, 4-ClPhCO), 8.08 (d, $J = 8.5$ Hz, 2H, 4-ClPhCO) ppm. Anal. ($C_{15}H_{13}Cl_2NO_3S_2$) C, H, N.

3.2.12. 4-Chloro-2-{[2-(3,4-dichlorophenyl)-2-oxoethyl]-sulfanyl}-6-methylbenzenesulfonamide (15). Starting from 2-mercaptobenzenesulfonamide **2** (4.76 g) and 3,4-dichlorophenacyl bromide (5.8 g); yield: 6.6 g (78%); mp 189–190 °C; IR (KBr) 3290, 3190 (NH_2), 1680 (C=O), 1330, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.59 (s, 3H, CH_3), 4.86 (s, 2H, CH_2), 7.28 (d, $J = 1.8$ Hz, 1H, arom.), 7.49 (s, 3H, NH_2 and 1H arom.), 7.83–8.02 (m, 2H, arom.), 8.30 (d, $J = 1.8$ Hz, 1H, arom.) ppm. Anal. ($C_{15}H_{12}Cl_3NO_3S_2$) C, H, N.

3.2.13. 4-Chloro-2-{[2-(2-naphthyl)-2-oxoethyl]sulfanyl}-6-methylbenzenesulfonamide (16). Starting from 2-mercaptobenzenesulfonamide **2** (4.76 g) and bromomethyl 2-naphthyl ketone (5.5 g); yield: 6.9 g (85%); mp 186–187 °C; IR (KBr) 3290, 3190 (NH_2), 1680 (C=O), 1330, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.59 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 7.27 (d, $J = 2.0$ Hz, 1H, arom.), 7.51 (s, 2H, NH_2), 7.59 (d, $J = 2.0$ Hz, 1H, arom.), 7.62–7.74 (m, 2H, arom.), 8.00–8.17 (m, 4H, arom.), 8.83 (s, 1H, H-1, 2-naphthoyl) ppm. Anal. ($C_{19}H_{16}ClNO_3S_2$) C, H, N.

3.2.14. 4-Chloro-2-{[2-(4-chlorophenyl)-2-oxoethyl]sulfanyl}benzenesulfonamide (17). Starting from 2-mercaptobenzenesulfonamide **3** (4.47 g) and 4-chlorophenacyl bromide (5.1 g); yield: 4.9 g (65%); mp 161–162 °C; IR (KBr) 3280, 3200 (NH_2), 1690 (C=O), 1330, 1160 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.97 (s, 2H, CH_2), 7.43 (dd, $J_{ortho} = 8.5$ Hz, $J_{meta} = 1.9$ Hz, 1H, H-5, $PhSO_2$), 7.50 (s, 2H, NH_2), 7.62–7.68 (m, 3H, arom.), 7.87 (d, $J_{ortho} = 8.5$ Hz, 1H, H-6, $PhSO_2$), 8.11 (d, $J = 8.5$ Hz, 2H, arom.) ppm. Anal. ($C_{14}H_{11}Cl_2NO_3S_2$) C, H, N.

3.2.15. 4-Chloro-2-{[2-(3-nitrophenyl)-2-oxoethyl]sulfanyl}benzenesulfonamide (18). Starting from 2-mercaptobenzenesulfonamide **3** (4.47 g) and 3-nitrophenacyl bromide (5.4 g); yield: 4.6 g (59%); mp 196–197 °C; IR (KBr) 3300, 3210 (NH_2), 1705 (C=O), 1350, 1330, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.06 (s, 2H, CH_2), 7.41 (dd, $J_{ortho} = 8.5$ Hz, $J_{meta} = 1.9$ Hz, 1H, H-5, $PhSO_2$), 7.46 (s, 2H, NH_2), 7.69 (d, $J_{meta} = 1.9$ Hz, 1H, H-3, $PhSO_2$), 7.82–7.90 (m, 2H, arom.), 8.46–8.52 (m, 2H, arom.), 8.75 (d, $J = 1.9$ Hz, 1H arom.) ppm. Anal. ($C_{14}H_{11}ClN_2O_5S_2$) C, H, N.

3.2.16. 4-Chloro-2-{[2-(3,4-dichlorophenyl)-2-oxoethyl]-sulfanyl}benzenesulfonamide (19). Starting from 2-mercaptobenzenesulfonamide **3** (4.47 g, 0.02 mol) and 3,4-dichlorophenacyl bromide (5.8 g, 0.022 mol), the title

compound **19** was obtained (5.3 g, 64%): mp 171–173 °C; IR (KBr) 3390, 3240 (NH_2), 1685 (C=O), 1340, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.00 (s, 2H, CH_2), 7.45 (dd, $J_{ortho} = 8.5$ Hz,, $J_{meta} = 1.8$ Hz, 1H, H-5, $PhSO_2$), 7.53 (s, 2H, NH_2), 7.70 (d, $J_{meta} = 1.8$ Hz, 1H, H-3, $PhSO_2$), 7.89 (d, $J = 8.5$ Hz, 2H, arom.), 8.04 (dd, $J_{ortho} = 8.5$ Hz, $J_{meta} = 1.8$ Hz, 1H, H-5, PhCO), 8.34, d, $J_{meta} = 1.8$ Hz, 1H, H-2, PhCO) ppm. Anal. ($C_{14}H_{10}Cl_3NO_3S_2$) C, H, N.

3.2.17. 4-Chloro-2-{[2-(2-naphthyl)-2-oxoethyl]sulfanyl}-benzenesulfonamide (20). Starting from 2-mercaptobenzenesulfonamide **3** (4.47 g) and bromomethyl 2-naphthyl ketone (5.5 g); yield: 5.2 g (66%); mp 171–172 °C; IR (KBr) 3355, 3205 (NH_2), 1680 (C=O), 1345, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.08 (s, 2H, CH_2), 7.41 (dd, $J_{ortho} = 8.5$ Hz, $J_{meta} = 2.0$ Hz, 1H, H-5, $PhSO_2$), 7.49 (s, 2H, NH_2), 7.60–7.73 (m, 3H, arom.), 7.85 (d, $J_{ortho} = 8.5$ Hz, 1H, H-6, $PhSO_2$), 7.99–8.16 (m, 4H, arom.), 8.84 (s, 1H, H-1, 2-naphthoyl) ppm. Anal. ($C_{18}H_{14}ClNO_3S_2$), C, H, N.

3.3. General procedure for the preparation of 3-aroyl-1,1-dioxo-1,4,2-benzodithiazines 21–37

A solution of the corresponding benzenesulfonamide **4**–**20** (0.01 mol) in thionyl chloride (45 mL) was refluxed for 46 h. Excess thionyl chloride was evaporated under reduced pressure (toluene (10 mL) was added to facilitate the removal of thionyl chloride). Then, the resulting solid residue was refluxed in toluene (20–25 mL) for 0.5 h. The insoluble material was separated by filtration, and the pure product (**21**–**37**) that precipitated from the filtrate upon cooling to room temperature was collected by filtration, washed with toluene (3 × 3 mL), and dried.

In this manner the following benzodithiazines were obtained:

3.3.1. 3-Benzoyl-6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazine (21). Starting from benzenesulfonamide **4** (3.56 g); yield: 1.6 g (46%); mp 159–160 °C; IR (KBr) 1660 (C=O), 1590 (C=N), 1345, 1175 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3), 7.50–7.58 (m, 4H, arom.), 8.10 (s, 1H, H-8), 8.19–8.24 (m, 2H, arom.) ppm. Anal. ($C_{15}H_{10}ClNO_3S_2$) C, H, N.

3.3.2. 6-Chloro-7-methyl-3-(4-methoxybenzoyl)-1,1-dioxo-1,4,2-benzodithiazine (22). Starting from benzenesulfonamide **5** (3.86 g); yield: 1.5 g (39%); mp 202–203 °C; IR (KBr) 1665 (C=O), 1595 (C=N), 1345, 1340, 1180, 1165 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 3.91 (s, 3H, CH_3O), 6.99 (d, $J = 9.0$ Hz, 2H, 4-CH_3OPh), 7.54 (s, 1H, H-5), 8.09 (s, 1H, H-8), 8.26 (d, $J = 9.0$ Hz, 2H, 4CH_3OPh) ppm; ^{13}C NMR ($CDCl_3$): 20.79, 56.24, 114.90, 124.89, 125.74, 128.18, 128.52, 129.72, 134.57, 140.35, 140.84, 166.12, 170.06, 183.90 ppm. Anal. ($C_{16}H_{12}ClNO_4S_2$) C, H, N.

3.3.3. 3-(4-Bromobenzoyl)-6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazine (23). Starting from benzenesulfonamide **6** (4.35 g); yield: 2.5 g (58%); mp 257–258 °C; IR (KBr) 1660 (C=O), 1580 (C=N), 1330, 1165 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H, CH_3), 7.57 (s, 1H, H-5), 7.69 (d, $J = 8.8$ Hz, 2H, 4-BrPh), 8.08 (s, 1H, H-8), 8.12 (d, $J = 8.8$ Hz, 2H, 4BrPh) ppm; ^{13}C NMR ($CDCl_3$) δ 20.83, 112.90, 125.96, 128.25, 128.59, 130.86, 131.84, 132.86, 133.12, 140.68, 141.20, 169.95, 185.70 ppm. Anal. ($C_{15}H_9BrClNO_3S_2$) C, H, N.

3.3.4. 6-Chloro-3-(4-chlorobenzoyl)-7-methyl-1,1-dioxo-1,4,2-benzodithiazine (24). Starting from benzenesulfonamide **7** (3.9 g); yield: 2.2 g (57%); mp 241–242 °C; IR (KBr) 1655 (C=O), 1580 (C=N), 1330, 1170 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3), 7.52 (d, $J = 8.8$ Hz, 2H, 4-ClPh), 7.57 (s, 1H, H-5), 8.09 (s, 1H, H-8), 8.20 (d, $J = 8.8$ Hz, 2H, 4-ClPh) ppm; ^{13}C NMR ($CDCl_3$) δ 20.59, 125.34, 128.00, 128.33, 129.00, 129.62, 130.28 132.93, 140.31, 140.96, 142.66, 169.61, 184.98 ppm. Anal. ($C_{15}H_9Cl_2NO_3S_2$) C, H, N.

3.3.5. 6-Chloro-3-(4-fluorobenzoyl)-7-methyl-1,1-dioxo-1,4,2-benzodithiazine (25). Starting from benzenesulfonamide **8** (3.76 g); yield: 1.8 g (48%); mp 194–195 °C; IR (KBr) 1655 (C=O), 1590 (C=N), 1340,1170 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3), 7.18–7.27 (m, 2H, 4-FPh), 7.57 (s, 1H., H-5), 8.10 (s, 1H, H-8), 8.28–8.35 (m, 2H, 4-FPh) ppm; MS m/z 369 (M^+). Anal. ($C_{15}H_9ClFNO_3S_2$) C, H, N.

3.3.6. 6-Chloro-7-methyl-3-(4-nitrobenzoyl)-1,1-dioxo-1,4,2-benzodithiazine (26). Starting from benzenesulfonamide **9** (4.0 g); yield: 1.9 g (48%); mp 236–237 °C; IR (KBr) 1675 (C=O), 1613 (NO_2), 1580 (C=N), 1350, 1175 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 7.60 (s, 1H, H-5), 8.11 (s, 1H, H-8), 8.39 (s, 4H, 4-O_2NPhCO) ppm. Anal. ($C_{15}H_9ClN_2O_5S_2$) C, H, N.

3.3.7. 6-Chloro-7-methyl-3-(3-nitrobenzoyl)-1,1-dioxo-1,4,2-benzodithiazine (27). Starting from benzenesulfonamide **10** (4.0 g); yield: 2.0 g (50%); mp 226–227 °C; IR (KBr) 1670 (C=O), 1610 (NO_2), 1575 (C=N), 1345, 1170 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H, CH_3), 7.60 (s, 1H, H-5), 7.77 (t, $J = 8.0$ Hz, 1H, 3-O_2NPh), 8.11 (s, 1H, H-8), 8.52–8.63 (m, 2H, 3-O_2NPh), 9.00 (t, $J = 1.7$ Hz, 1H, 3-O_2NPh) ppm; ^{13}C NMR ($CDCl_3$) δ 20.86, 125.57, 126.40, 128.34, 128.68, 128.85, 129.62, 130.68, 133.64, 137.22, 140.75, 141.53, 148.95, 168.76, 184.95 ppm. Anal. ($C_{15}H_9ClN_2O_5S_2$) C, H, N.

3.3.8. 6-Chloro-3-(3,4-dichlorobenzoyl)-7-methyl-1,1-dioxo-1,4,2-benzodithiazine (28). Starting from benzenesulfonamide **11** (4.25 g); yield: 1.9 g (45%); mp 207–209 °C; IR (KBr) 1680 (C=O), 1580 (C=N), 1325, 1170 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H, CH_3), 7.57–7.56 (m, 2H, arom.), 8.10 (s, 1H, H-8), 8.11–8.16 (m, 1H, arom.), 8.30 (d, $J = 2.0$ Hz, 1H, arom.) ppm.

EIMS (70 eV): $m/z = 420$ (M^+). Anal. ($C_{15}H_8Cl_3NO_3S_2$) C, H, N.

3.3.9. 6-Chloro-7-methyl-3-biphenylyl-1,1-dioxo-1,4,2-benzodithiazine (29). Starting from benzenesulfonamide **12** (4.32 g); yield: 1.7 g (40%); mp 208–209 °C; IR (KBr) 1655 (C=O), 1600 (C=N), 1330, 1170 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H, CH_3), 7.44–7.55 (m, 3H, biPh), 7.58 (s, 1H, H-5), 7.62–7.69 (m, 2H, biPh), 7.76 (d, $J = 8.5$ Hz, 2H, biPH), 8.12 (s, 1H, H-8), 8.32 (d, $J = 8.5$ Hz, 2H, biPh) ppm. Anal. ($C_{21}H_{14}ClNO_3S_2$) C, H, N.

3.3.10. 6-Chloro-7-methyl-3-(2-naphthoyl)-1,1dioxo-1,4,2-benzodithiazine (30). Starting from benzenesulfonamide **13** (4.06 g); yield: 1.6 g (40%); mp 216–217 °C; IR (KBr) 1660 (C=O), 1620 (C=N), 1330, 1165 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 756–7.59 (m, 3H, arom.), 7.64–8.05 (m, 3H, arom.), 8.13–8.18 (m, 2H, arom.), 8.89 (s, 1H, H-1, naphthyl) ppm. Anal. ($C_{19}H_{12}ClNO_3S_2$) C, H, N.

3.3.11. 6-Chloro-3-(4-chlorobenzoyl)-8-methyl-1,1-dioxo-1,4,2-benzodithiazine (31). Starting from benzenesulfonamide **14** (3.9 g); yield: 2.9 g (75%); mp 229–230 °C; IR (KBr) 1660 (C=O), 1595 (C=N), 1330, 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.71 (s, 3H, CH_3), 7.71 (d, $J = 8.6$ Hz, 2H, 4-ClPh), 7.81 (d, $J = 1.8$ Hz, 1H, H-7), 8.04 (d, $J = 1.8$ Hz, 1H, H-5), 8.12 (d, $J = 8.6$ Hz, 2H, 4-ClPh) ppm. Anal. ($C_{15}H_9ClNO_3S_2$) C, H, N.

3.3.12. 6-Chloro-3-(3,4-dichlorobenzoyl)-8-methyl-1,1-dioxo-1,4,2-benzodithiazine (32). Starting from benzenesulfonamide **15** (4.25 g); yield: 2.2 g (52%); mp 248–250 °C; IR (KBr) 1655 (C=O), 1590 (C=N), 1335, 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.71 (s, 3H, CH_3), 7.82 (d, $J = 1.4$ Hz, 1H, arom.), 7.93 (d, $J = 8.4$ Hz, 1H, arom.), 8.02–8.08 (m, 2H, arom.), 8.29 (d, $J = 1.9$ Hz, 1H, arom.) ppm. Anal. ($C_{15}H_8Cl_3NO_3S_2$) C, H, N.

3.3.13. 6-Chloro-8-methyl-3-(2-naphthoyl)-1,1-dioxo-1,4,2-benzodithiazine (33). Starting from benzenesulfonamide **16** (4.06 g); yield: 2.6 g (65%); mp 198–200 °C; IR (KBr) 1655 (C=O), 1620 (C=N), 1335, 1170 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.86 (s, 3H, CH_3), 7.31 (d, $J = 1.4$ Hz, 1H, H-7, benzodithiaz.),7.42 (d, $J = 1$ Hz, 1H, H-5, benzodithiaz.), 7.54–7.73 (m, 2H, naphthyl), 7.89–8.20 (m, 4H, naphthyl), 8.95 (s, 1H, H-1, naphthyl) ppm. Anal. ($C_{19}H_{12}ClNO_3S_2$) C, H, N.

3.3.14. 6-Chloro-3-(4-chlorobenzoyl)-1,1-dioxo-1,4,2-benzodithiazine (34). Starting from benzenesulfonamide **17** (3.76 g); yield: 1.8 g (48%); mp 214–215 °C; IR (KBr) 1665 (C=O), 1580 (C=N), 1340, 1175 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.50–7.60 (m, 3H, arom.), 7.79 (dd, $J_{ortho} = 6.5$ Hz, $J_{meta} = 2.0$ Hz, 1H, H-6, benzodithiaz.), 8.16–8.23 (m, 3H, arom.). Anal. ($C_{14}H_7Cl_2NO_3S_2$) C, H, N.

3.3.15. 6-Chloro-3-(3-nitrobenzoyl)-1,1-dioxo-1,4,2-benzodithiazine (35). Starting from benzenesulfonamide **18** (3.87 g); yield: 1.9 g (50%); mp 176–177 °C; IR (KBr) 1695 (C=O), 1610 (C=N), 1350, 1175 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.86–7.94 (m, 2H, arom.), 8.21–8.30 (m, 2H, arom.), 8.42–8,59 (m, 2H, arom.), 8.85 (t, $J = 1.8$ Hz, 1H, H-2, 3-O_2NPh) ppm. Anal. ($C_{14}H_7ClN_2O_5S_2$) C, H, N.

3.3.16. 6-Chloro-3-(3,4-dichlorobenzoyl)-1,1-dioxo-1,4,2-benzodithiazine (36). Starting from benzenesulfonamide **19** (4.1 g); yield: 2.1 g (51%); mp 199–201 °C; IR (KBr) 1665 (C=O), 1630 (C=N), 1330, 1170 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.59–7.66 (m, 2H, arom.), 7.72 (dd, $J_{ortho} = 6.6$Hz, $J_{meta} = 1.9$ Hz, 1H, H-6, benzodithiaz.), 8.12–8.21 (m, 2H, arom.), 8.32 (d, $J = 2.0$ Hz, 1H, H-2, PhCO) ppm. Anal. ($C_{14}H_6Cl_3NO_3S_2$) C, H, N.

3.3.17. 6-Chloro-3-(2-naphthoyl)-1,1-dioxo-1,4,2-benzodithiazine (37). Starting from benzenosulfonamide **20** (3.92 g); yield: 2.0 g (51%); IR (KBr) 1660 (C=O), 1610 (C=N), 1340, 1175 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.57–7.74 (m, 4H, arom.), 7.90–8.05 (m, 3H, arom.), 8.14–8.23 (m, 2H, arom.), 8.89 (s, 1H, H-1, naphthyl) ppm. Anal. ($C_{18}H_{10}ClNO_3S_2$) C, H, N.

3.4. Materials, chemicals, and enzymes

All compounds were dissolved in DMSO and the stock solutions were stored at −20 °C. The γ [^{32}P]-ATP was purchased from either Amersham Biosciences or ICN. The expression systems for the wild-type IN, soluble mutant $IN^{F185KC280S}$, and the truncated mutant $IN^{50\text{-}212}$ (F185K) were generous gifts of Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD. The expression systems for the cysteine mutant was a generous gift of Dr. Anna Marie Skalka, Fox Chase Cancer Center, Philadelphia, PA and the enzymes were prepared as described.[17,18]

3.5. Preparation of oligonucleotide substrates

The oligonucleotides 21top, 5′-GTGTGGAAAATCTCTAGCAGT-3′ and 21bot, 5′-ACTGCTAGAGATTTTCCACAC-3′ were purchased from Norris Cancer Center Core Facility (University of Southern California) and purified by UV shadowing on polyacrylamide gel. To analyze the extent of 3′-processing and strand transfer using 5′-end labeled substrates, 21top was 5′-end labeled using T4 polynucleotide kinase (Epicentre, Madison, WI) and [^{32}P]-ATP (Amersham Biosciences or ICN). The kinase was heat-inactivated and 21bot was added in 1.5-M excess. The mixture was heated at 95 °C, allowed to cool slowly to room temperature, and run through a spin 25 mini-column (USA Scientific) to

separate annealed double-stranded oligonucleotide from unincorporated material.

3.6. Integrase assays

To determine the extent of 3′-processing and strand transfer, IN was preincubated at a final concentration of 200 nM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/mL bovine serum albumin, 10 mM 2-mercaptoethanol, 10% dimethyl sulfoxide, 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 the addition of an equal volume (16 μL) of 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 in a PhosphorImager cassette, and analyzed using a Typhoon 8610 Variable Mode Imager (Amersham Biosciences) and quantitated using ImageQuant 5.2. Percent inhibition (% I) was calculated using the following equation:

$$\%I = 100 \times [1 - (D - C)/(N - C)]$$

where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processing product) or strand transfer products for DNA alone, DNA plus IN, and IN plus drug, respectively. The IC_{50} values were determined by plotting the logarithm of drug concentration versus percent inhibition to obtain concentration that produced 50% inhibition.

3.7. Anti-HIV assays in cultured cell lines

The anti-HIV drug testing performed at NCI is based on a protocol described by Weislow et al.[19] In brief, all compounds were dissolved in DMSO and diluted in 1:100 in cell culture medium. Exponentially growing T4 lymphocytes (CEM cell line) were added at 5000 cells per well. Frozen virus stock solutions were thawed immediately before use, suspended in complete medium to yield the desired multiplicity of infection (≈0.1) and added to the microtiter wells, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. Cultures are incubated at 37 °C in a 5% CO_2 atmosphere for 6 days. The tetrazolium salt, XTT [2,3-bis (2-methoxy-4-nitro-5-sulfenyl)-2*H*-tetrazolium-5-carboxamide] was added to all wells, and cultures are incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazan production, and in addition are viewed microscopically for detection of viable cells and confirmation of protective activity.

3.8. X-ray structure analysis of 24

Crystal data for $C_{15}H_9C_{12}NO_3S_2$: monoclinic, space group $P21/_n$, $a = 7.2102(5)$, $b = 13.6632(7)$, $c = 15.6326(8)$ Å, $\beta = 91.406(5)°$, $V = 1539.57(15)$ Å^3, $Z = 4$, $d_x = 1.666\,g\,cm^{-3}$, $T = 155$ K. Data were collected for a crystal with dimensions $0.8 \times 0.15 \times 0.08$ mm with a KumaCCD diffractometer using graphite monochromated Mo K_α radiation. Final R indices for 2808 reflections with $I > 2\sigma(I)$ and 244 refined parameters are: $R_1 = 0.0388$, $wR_2 = 0.0921$ ($R_1 = 0.0455$, $wR_2 = 0.0964$ for all 3112 data). Atom labeling is shown in Figure 2.

Supporting information available: Detailed atomic coordinates, isotropic displacement parameters, bond length, bond angles, H-bond coordinates, and torsion angles (Tables 3–7) are available upon request.

Acknowledgements

We would like to thank the Drug Synthesis and Chemistry Branch, Developmental Therapeutics of the National Cancer Institute for the antiviral testing. The work in N.N.'s lab was supported by funds from the GlaxoSmithKline Drug Discovery Award.

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