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Original article

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

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Abstract

A series of novel 3-aroyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazines **15**–**28** as potential HIV-1 integrase (IN) inhibitors have been synthesized by the reduction of 3-aroyl-1,1-dioxo-1,4,2-benzodithiazines **1**–**14** with benzenesulfonyl hydrazide. All the compounds **15**–**28** inhibited IN mediated strand transfer reaction with IC_{50} values ranging from 3 to 30 μM. The 3-(4-bromobenzoyl)-6-chloro-7-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine **17** with the IC_{50} values of 4 ± 1 and 3 ± 1 μM for 3′-processing and strand transfer, respectively, was the most potent. Compound **17** as well its analogues were 5–20-fold less potent in Y99S and H114A mutants, implicating these residues as potential drug-binding site. This is a first report implicating Y99S and H114A of IN core domain in drug-binding interactions.

Keywords: 3-Aroyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazines; Synthesis; HIV-1 integrase inhibitors

1. Introduction

It is well known that compounds bearing arylsulfonamide moiety have been applied in various strategies aimed at discovery of novel antiviral agents [1–5]. HIV-1 integrase (IN) is an attractive target for selective blockade of viral infection since there are no known counterparts in the host cell [6]. Previously, we described the syntheses and anti-HIV activities of various 4-chloro-2-mercaptobenzenesulfonamide derivatives with the nitrogen atom of sulfonamide moiety attached to a variety heterocyclic ring systems (Fig. 1, structure **I**) [7–12]. Some of these compounds (**I**) [13,14], di(4-mercapto-3-nitrophenyl)sulfones (e.g., **II**) [15] and 1,2-di(2-mercaptobenzoyl)hydrazines (e.g., **III**) [16] were described as novel class of potent IN inhibitors (Fig. 1) [13–16]. In fact, among the recently reported dipyridine inhibitors, only compounds containing a free mercapto group showed antiviral activity and inhibited IN (e.g., **IV**, Fig. 1) [17]. A common theme in these leads is the requirement for a free mercaptoaryl group for antiviral activity as well as anti-IN potency (**I**–**IV**, Fig. 1) [10,12,13,15–17]. More recently, we found that cyclic analogues of 2-mercaptobenzenesulfonamides (Fig. 1, structure **V**) also showed anti-HIV and anti-IN properties [18]. This led us to an assumption that expansion of a series of candidate anti-IN agents of general formula **VI** (Fig. 1) may shed light on the structural features contributing to the IN inhibitors.

2. Results and discussion

2.1. Chemistry

The previously described methods were used to synthesize starting compounds, 3-aroyl-1,1-dioxo-1,4,2-benzodithiazines

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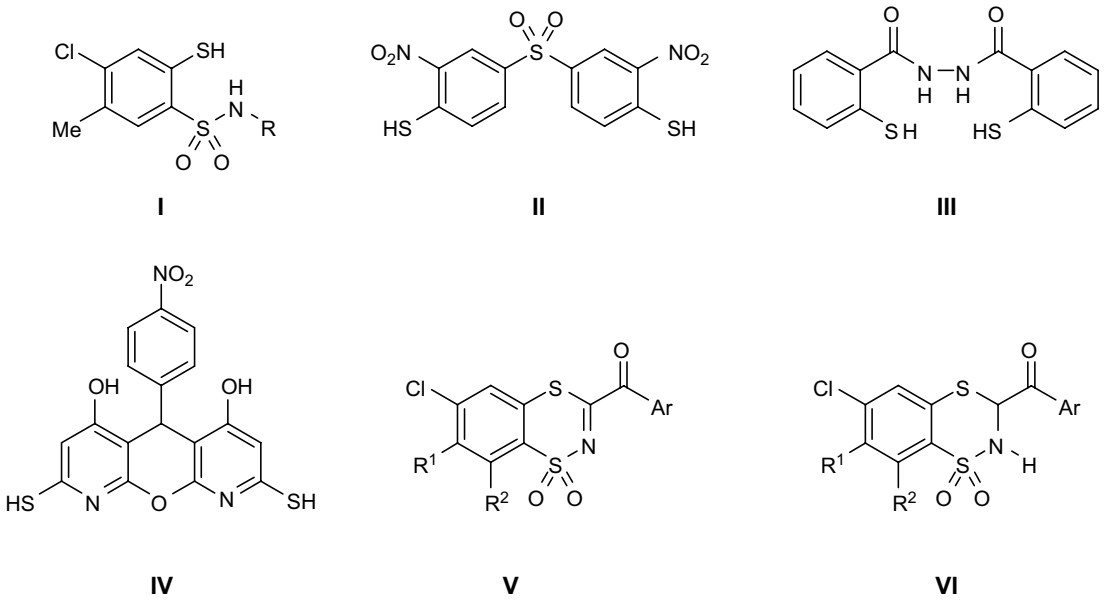


Fig. 1. Structures of HIV-1 integrase inhibitors.

1–**14** [18]. The synthesis of the target 3-aroyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazines **15**–**28** was achieved by reacting benzenesulfonyl hydrazide with the corresponding aroylbenzodithiazines **1**–**14** in methanol.

To our knowledge the above reaction is the first example of the reduction of C=N bond using arylsulfonylhydrazide. It has been well known that arylsulfonylhydrazides upon heating decompose to diimide which is convenient reducing agent for symmetrical double bonds such as C=C, N=N or C≡C. Under this conditions, however, polar C=N bond has not been reduced before [19]. Therefore, we propose two possible mechanisms A and B which can be operative for the reduction of compounds **1**–**14** incorporating formal α-imino-carbonyl moiety (Scheme 1). According to mechanism A, the initial step would consist in the formation of a spiro intermediate of type **A** by [2 + 2] cycloaddition of benzenesulfonyl hydrazide to the C3–C(=O)Ar bond of 3-aroyl-1,4,2-benzodithiazine 1,1-dioxides **1**–**14**, followed by the 1,2-diazetidine ring scission with subsequent loss of N_2 and benzenesulfinic acid. In the final stage of the reaction, the non-isolable enol **B** undergoes tautomerization to afford the corresponding ketones **15**–**28**. According to mechanism B, the addition of hydrazone NH_2 group to carbonyl C=O group of **1**–**14** may afford hemiaminal of type **C**, which undergoes a spontaneous retro-ene fragmentation with the formation of enol **B** (Scheme 1).

The ^{1}H NMR spectrum of the product **18** containing an aroyl group at position 3 of benzodithiazine ring showed two doublet signals attributed to the C3-H proton at δ 7.29 ppm and NH proton at δ 9.13 ppm with the coupling constant $J = 11.5$ Hz. Furthermore, the correlation between NH proton and both C-3 carbon atom at δ 64.04 and C=O carbon atom at δ 190.16 was found in the ^{1}H–^{13}C heterocorrelated spectrum (HMBC-heteronuclear multiple bond correlation), while the HSQC (heteronuclear single quantum coherence) spectrum showed the correlation between this C3-H proton and the C-3 carbon atom, and were fully consistent with the proposed structure.

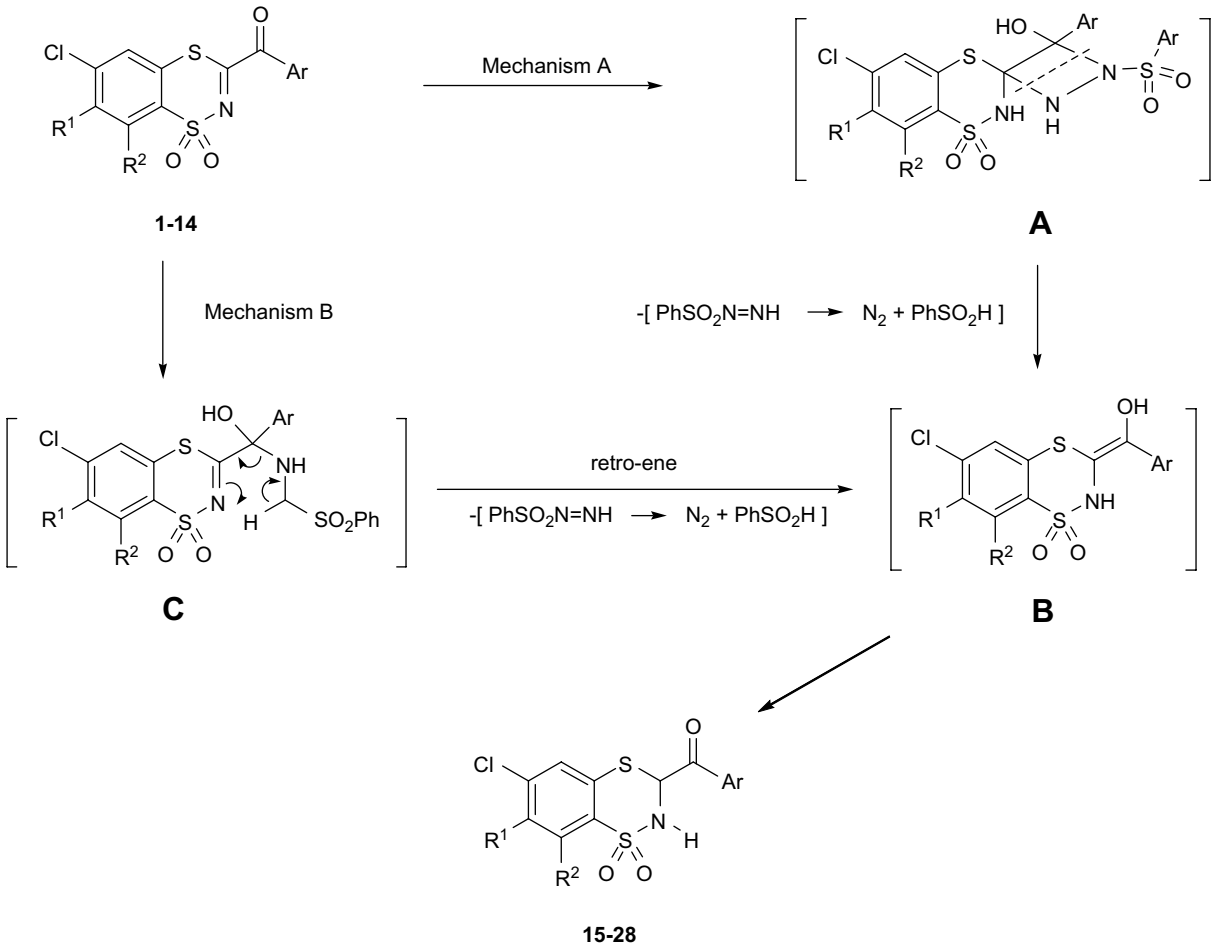
2.2. Biology

The IN inhibitory activities of the newly prepared compounds **15**–**28** compared to those of parent benzodithiazines **1**–**14** are presented in Table 1. All tested compounds are significantly active against wild-type IN in the presences of Mg^{2+}, albeit less effective as compared to Mn^{2+}, when added as a co-factor. This is in agreement with our previous studies [16].

For a series of compounds **1**–**7** with methyl group at position 7 of benzodithiazine ring, partial hydrogenation leads to compounds **15**–**21**, inhibitory activity of which is equal (compound **16**, Ar = 4-MeOPh), higher (**15**, Ar = Ph; **17**, Ar = 4-BrPh; **18**, Ar = 4-ClPh and **19**, Ar = 3-O_2NPh) or lower (**20**, Ar = 3,4-diClPh and **21**, Ar = 2-naphthyl). Thus, electronic and steric effects brought about by the Ar substituents seem to be crucial for biological activity of these compounds. The above pattern, however, is not observed for benzodithiazines **8**–**10** with 8-Me substituent, which upon hydrogenation gave **22**–**24** with similar inhibitory activities.

Interestingly, upon hydrogenation of compounds **11**–**13** lacking the methyl group at both 7 and 8 position of benzodithiazine ring, a very considerable improvement of inhibitory activity is noted for **25** (Ar = 4-ClPh) and **26** (Ar = 3,4-diClPh) thus obtained, while hydrogenated compound **27** (Ar = 2-naphthyl) retains a relatively high potency of **13**.

Summing up, all newly prepared compounds showed significant inhibition of purified IN with IC_{50} values ranging from 3 to 30 μM for strand transfer. The most active 3-(4-bromobenzoyl)-6-chloro-7-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**17**) with IC_{50} values of 4 ± 1 and 3 ± 1 μM for



Compds	X	R^1	R^2	Ar
1, 15	Cl	Me	H	Ph
2, 16	Cl	Me	H	4-MeOPh
3, 17	Cl	Me	H	4-BrPh
4, 18	Cl	Me	H	4-ClPh
5, 19	Cl	Me	H	3-O_2NPh
6, 20	Cl	Me	H	3,4-diClPh
7, 21	Cl	Me	H	2-naphthyl

Compds	X	R^1	R^2	Ar
8, 22	Cl	H	Me	4-ClPh
9, 23	Cl	H	Me	3,4-diClPh
10, 24	Cl	H	Me	2-naphthyl
11, 25	Cl	H	H	4-ClPh
12, 26	Cl	H	H	3,4-diClPh
13, 27	Cl	H	H	2-naphthyl
14, 28	H	H	H	3,4-diClPh

Scheme 1. Synthesis of 3-aroyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazines **15**–**28** and proposed mechanism of their formation. Reagents, conditions and yields: (a) benzenesulfonyl hydrazide (1.33 molar equiv), methanol, room temperature, 20 h, reflux, 12 h, 62–91%.

3′-processing and strand transfer, respectively, may serve as useful lead compound for further development of powerful anti-HIV agents.

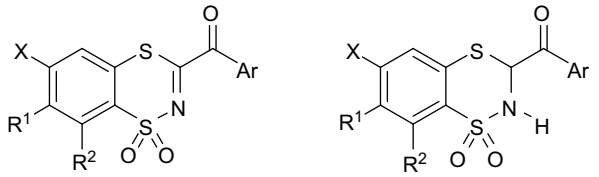
In our biological investigations we further used Y99S and H114A mutants of the enzyme. Importantly, the Y99S mutant in general was about fivefold more resistant than the H114A (Table 1). This implies that the tested compounds most likely bind to these novel sites. This is the first report implicating Y99 and H114 residues of IN catalytic site as drug-binding sites. Further studies are underway to provide a direct proof for these observations.

3. Experimental protocols

3.1. Synthesis

The following instruments and parameters were used: (melting points) Büchi 535 apparatus; (IR spectra) KBr

Table 1
Structure and anti-HIV integrase activities of previously described 3-aroyl-1,1-dioxo-1,4,2-benzodithiazines **1–14** [12], and their novel 2,3-dihydro analogues **15–28**



substrates **1-14** products **15-28**

Compd	X	R^1	R^2	Ar	Anti-HIV integrase activities					
					Compd **1–14**		Compd **15–28**		Y99S[c] Clv. ST	H114A[d] Clv. ST
					IC_{50} (μM)[a]		IC_{50} (μM)[a]			
					Cleavage (3′-processing)	Integration (strand transfer)	Cleavage (3′-processing)	Integration (strand transfer)		
1, **15**	Cl	Me	H	Ph	12	12	7 ± 1 (60)[b]	5 ± 1 (27)[b]	90	15
									18	9
2, **16**	Cl	Me	H	4-MeOPh	30	33	38 ± 11	17 ± 2		
3, **17**	Cl	Me	H	4-BrPh	30	30	4 ± 1	3 ± 1	75	20
									20	18
4, **18**	Cl	Me	H	4-ClPh	30	65	6 ± 2 (63)[b]	4 ± 1 (40)[b]	90	18
									20	16
5, **19**	Cl	Me	H	3-O_2NPh	57	50	32 ± 1	28 ± 6		
6, **20**	Cl	Me	H	3,4-diClPh	15	7	20 ± 13	10 ± 6		
7, **21**	Cl	Me	H	2-Naphthyl	28	3	57 ± 14	30 ± 13		
8, **22**	Cl	H	Me	4-ClPh	22	25	23 ± 5	14 ± 4		
9, **23**	Cl	H	Me	3,4-diClPh	17	25	20 ± 1	14 ± 4		
10, **24**	Cl	H	Me	2-Nahpthyl	22	7	9 ± 1 (83)[b]	6 ± 1 (51)[b]	70	33
									23	23
11, **25**	Cl	H	H	4-ClPh	100	90	8 ± 1 (51)[b]	4 ± 1 (25)[b]	100	20
									20	18
12, **26**	Cl	H	H	3,4-diClPh	61.2	124.0	9 ± 3 (58)[b]	5 ± 3 (25)[b]	90	18
									20	18
13, **27**	Cl	H	H	2-Naphthyl	8	8	8 ± 2 (55)[b]	3 ± 1 (28)[b]	100	9
									15	15
14, **28**	H	H	H	3,4-diClPh	NT	NT	22 ± 3	15 ± 4		

[a] Inhibitory concentration (μM) 50% required to reduce HIV-1 IN mediated 3′-processing (cleavage) or strand transfer (integration) reactions (carried out in the presences of Mn^{+2} as a co-factor).
[b] Values in the parenthesis were generated from experiments carried out in the presence of Mg^{+2} as a co-factor.
[c] IC_{50} values in μM refer to cleavage (top) and strand transfer (bottom) using Y99S mutant.
[d] IC_{50} values in μM refer to cleavage (top) and strand transfer (bottom) using H114A mutant.

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). HSQC and HMBC spectra were taken on Varian Unity 500 spectrometer. The results of elemental analyses for C, H and N were within ±0.4% of theoretical values.

*3.1.1. General procedure for the selective reduction of 3-aroyl-1,1-dioxo-1,4,2-benzodithiazines into 3-aroyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazines (**15–28**)*

A mixture of benzenesulfonyl hydrazide (0.69 g, 4 mmol) and the corresponding benzodithiazines **1–14** (3 mmol) in methanol (15 ml) was stirred at room temperature for 20 h, followed at reflux for 12 h. After cooling to room temperature and standing overnight the precipitate of the adequate 2,3-dihydro-1,4,2-benzodithiazine was collected by filtration, washed with methanol (4 × 2 ml) and dried.

In this manner, the following 2,3-dihydro-1,4,2-benzodithiazines were obtained.

*3.1.1.1. 3-Benzoyl-6-chloro-7-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**15**).* Starting from benzodithiazine **1** (1.06 g); yield: 0.79 g (74%); m.p. 165–166 °C; IR (KBr) 3228 (NH), 1690 (C=O), 1345, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 7.20 (d, $J = 11.5$ Hz, 1H, H-3), 7.57–7.78 (m, 4H, arom.), 7.92 (s, 1H, H-8), 7.98–8.07 (m, 2H, arom.), 9.11 (d, $J = 11.5$ Hz, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.18, 63.93, 127.64, 127.89, 129.17, 129.77, 132.22, 133.04, 133.38,

134.09, 134.75, 137.90, 190.90 ppm. Anal. ($C_{15}H_{12}ClNO_3S_2$) C, H, N.

*3.1.1.2. 6-Chloro-3-(4-methoxybenzoyl)-7-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**16**).* Starting from benzodithiazine **2** (1.15 g); yield: 0.98 g (85%); m.p. 172–174 °C; IR (KBr) 3230 (NH), 1665 (C=O), 1355, 1345, 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 3.88 (s, 3H, CH_3O), 7.11 (d, $J = 11.7$ Hz, 1H, H-3), 7.14 (d, $J = 8.8$ Hz, 2H, 4-MeOPh), 7.63 (s, 1H, H-5), 7.93 (s, 1H, H-9), 8.04 (d, $J = 8.8$ Hz, 2H, 4-MeOPh), 9.06 (d, $J = 11.7$ Hz, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.17, 56.05, 63.64, 114.55, 125.60, 127.64, 127.76, 127.85, 132.27, 133.54, 134.03, 137.85, 164.47, 188.87 ppm. Anal. ($C_{16}H_{14}ClNO_4S_2$) C, H, N.

*3.1.1.3. 3-(4-Bromobenzoyl)-6-chloro-7-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**17**).* Starting from benzodithiazine **3** (1.29 g); yield: 1.18 g (91%); m.p. 202–204 °C; IR (KBr) 3230 (NH), 1675 (C=O), 1345, 1335, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 7.19 (d, $J = 11.6$ Hz, 1H, H-3), 7.66 (s, 1H, H-5), 7.84 (d, $J = 8.4$ Hz, 2H, 4-BrPh), 7.86 (s, 1H, H-8), 7.96 (d, $J = 8.4$ Hz, 2H, 4-BrPh), 9.13 (d, $J = 11.6$ Hz, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.18, 64.00, 127.65, 127.76, 127.91, 128.96, 131.63, 132.17, 132.31, 133.20, 134.16, 137.95, 190.29 ppm. Anal. ($C_{15}H_{11}BrClNO_3S_2$) C, H, N.

*3.1.1.4. 6-Chloro-3-(4-chlorobenzoyl)-7-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**18**).* Starting from benzodithiazine **4** (1.16 g); yield: 0.96 g (82%); m.p. 185–186 °C; IR (KBr) 3235 (NH), 1680 (C=O), 1345, 1335, 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.38 (s, 3H, CH_3), 7.29 (d, $J = 11.5$ Hz, 1H, H-3), 7.66 (s, 1H, H-5), 7.77 (d, $J = 8.6$ Hz, 2H, 4-ClPh), 7.95 (s, 1H, H-8), 8.04 (d, $J = 8.6$ Hz, 2H, 4-ClPh), 9.13 (d, $J = 11.5$ Hz, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.18, 64.04, 127.65, 127.91, 129.35, 131.61, 131.82, 132.18, 133.22, 134.17, 137.95, 139.67, 190.16 ppm. Anal. ($C_{15}H_{11}Cl_2NO_3S_2$) C, H, N.

*3.1.1.5. 6-Chloro-3-(3-nitrobenzoyl)-7-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**19**).* Starting from benzodithiazine **5** (1.19 g); yield: 0.85 g (71%); m.p. 203–205 °C; IR (KBr) 3250 (NH), 1705 (C=O), 1345, 1330, 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 7.36 (d, $J = 11.3$ Hz, 1H, H-3), 7.72 (s, 1H, H-5), 7.79–7.91 (m, 2H, H-8, benzodithiazine and H-5, 3-O_2NPh), 8.41–8.46 (m, 1H, H-6, 3-O_2NPh), 8.55–8.60 (m, 1H, H-4, 3-O_2NPh), 8.77 (t, $J = 1.8$ Hz, 1H, H-2, 3-O_2NPh), 9.22 (d, $J = 11.3$ Hz, 1H, NH) ppm. Anal. ($C_{15}H_{11}ClN_2O_5S_2$) C, H, N.

*3.1.1.6. 6-Chloro-3-(3,4-dichlorobenzoyl)-7-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**20**).* Starting from benzodithiazine **6** (1.26 g); yield: 1.0 g (78%); m.p. 211–212 °C; IR (KBr) 3230 (NH), 1680 (C=O), 1340, 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 7.24 (d, $J = 11.3$ Hz, 1H, H-3), 7.68 (s, 1H, H-5), 7.90–8.00 (m, 3H, 3,4-diClPh), 8.22 (s, 1H, H-8), 9.16 (d, $J = 11.3$ Hz, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.18, 63.96, 127.67, 127.94, 129.67, 131.43, 131.62, 132.15, 132.20, 133.02, 133.35, 134.27, 137.52, 138.00, 189.45 ppm. Anal. ($C_{15}H_{10}Cl_3NO_3S_2$) C, H, N.

*3.1.1.7. 6-Chloro-7-methyl-3-(2-naphthoyl)-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**21**).* Starting from benzodithiazine **7** (1.21 g); yield: 1.1 g (90%); m.p. 191–192 °C; IR (KBr) 3300 (NH), 1685 (C=O), 1330, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.37 (s, 3H, CH_3), 7.32 (d, $J = 11.4$ Hz, 1H, H-3, benzodithiazine), 7.64–7.79 (m, 3H, arom.), 7.96–8.30 (m, 5H, arom.), 8.80 (d, $J = 5.5$ Hz, 1H, arom.), 9.13 (d, $J = 11.4$ Hz, 1H, NH) ppm. Anal. ($C_{19}H_{14}ClNO_3S_2$) C, H, N.

*3.1.1.8. 6-Chloro-3-(4-chlorobenzoyl)-8-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**22**).* Starting from benzodithiazine **8** (1.16 g); yield: 0.82 g (70%); m.p. 201–203 °C; IR (KBr) 3235 (NH), 1680 (C=O), 1330, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.63 (s, 3H, CH_3), 7.19 (d, $J = 12.0$ Hz, 1H, H-3), 7.33 (d, $J = 1.8$ Hz, 1H, H-7), 7.54 (d, $J = 1.8$ Hz, 1H, H-5), 7.70 (d, $J = 8.5$ Hz, 2H, 4-ClPh), 8.05 (d, $J = 8.5$ Hz, 2H, 4-ClPh), 9.30 (d, $J = 12.0$ Hz, 1H, NH) ppm. Anal. ($C_{15}H_{11}Cl_2NO_3S_2$) C, H, N.

*3.1.1.9. 6-Chloro-3-(3,4-dichlorobenzoyl)-8-methyl-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**23**).* Starting from benzodithiazine **9** (1.26 g); yield: 1.07 g (84%); m.p. 183–185 °C; IR (KBr) 3250 (NH), 1690 (C=O), 1335, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.63 (s, 3H, CH_3), 7.23 (d, $J = 11.7$ Hz, 1H, H-3), 7.34–7.36 (m, 1H, arom.), 7.56 (d, $J = 1.9$ Hz, 1H, arom.), 7.90–7.97 (m, 1H, arom.), 8.23 (d, $J = 1.6$ Hz, 1H, arom.), 9.33 (d, $J = 11.7$ Hz, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 20.71, 63.37, 125.51, 129.16, 129.69, 131.20, 131.61, 131.96, 132.12, 133.16, 136.23, 136.71, 137.62, 139.96, 189.52 ppm. Anal. ($C_{15}H_{10}Cl_3NO_3S_2$) C, H, N.

*3.1.1.10. 6-Chloro-8-methyl-3-(2-naphthoyl)-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**24**).* Starting from benzodithiazine **10** (1.21 g); yield: 1.08 g (89%); m.p. 190–191 °C; IR (KBr) 3205 (NH), 1685 (C=O), 1325, 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.65 (s, 3H, CH_3), 7.33 (d, $J = 11.6$ Hz, 1H, H-3, benzodithiazine), 7.46–7.84 (m, 4H, arom.), 8.03–8.14 (m, 4H, arom.), 8.82 (d, $J = 8.0$ Hz, 1H, arom.), 9.34 (d, $J = 11.6$ Hz, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 20.77, 63.03, 124.66, 125.39, 127.65, 128.17, 128.73, 129.03, 129.79, 130.04, 130.20, 132.11, 132.32, 134.40, 135.78, 136.15, 137.17, 139.92, 190.69 ppm. Anal. ($C_{19}H_{14}ClNO_3S_2$) C, H, N.

*3.1.1.11. 6-Chloro-3-(4-chlorobenzoyl)-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**25**).* Starting from benzodithiazine **11** (1.12 g); yield: 0.70 g (62%); m.p. 212–214 °C; IR (KBr) 3245 (NH), 1690 (C=O), 1365, 1340, 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.24 (d, $J = 11.5$ Hz, 1H, H-3), 7.42

(dd, $J_{ortho} = 8.5$ Hz, $J_{meta} = 2.1$ Hz, 1H, H-7), 7.70 (d, $J_{meta} = 2.1$ Hz, 1H, H-5), 7.72 (d, $J = 8.7$ Hz, 2H, 4-ClPh), 7.92 (d, $J_{ortho} = 8.5$ Hz, 1H, H-5), 8.03 (d, $J = 8.7$ Hz, 2H, 4-ClPh), 9.18 (d, $J = 11.5$ Hz, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 64.08, 126.34, 127.40, 127.63, 129.38, 131.60, 131.80, 132.27, 136.85, 137.44, 139.71, 190.07 ppm. Anal. ($C_{14}H_9Cl_2NO_3S_2$) C, H, N.

*3.1.1.12. 6-Chloro-3-(3,4-dichlorobenzoyl)-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**26**).* Starting from benzodithiazine **12** (1.22 g); yield: 0.80 g (65%); m.p. 185–186 °C; IR (KBr) 3225 (NH), 1675 (C=O), 1335, 1170 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.27 (d, $J = 11.2$ Hz, 1H, H-3), 7.42 (dd, $J_{ortho} = 8.4$ Hz, $J_{meta} = 1.9$ Hz, 1H, H-7), 7.72 (d, $J_{meta} = 1.9$ Hz, 1H, H-5), 7.84–7.95 (m, 3H, H-5,6 of 3,4-diClPh and H-8 of benzodithiazine), 8.23 (s, 1H, H-2, 3,4-diClPh), 9.22 (d, $J = 11.2$ Hz, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 63.99, 126.42, 127.42, 127.66, 129.67, 131.43, 131.65, 132.18, 132.29, 133.33, 136.66, 137.49, 137.57, 189.35 ppm. Anal. ($C_{14}H_8Cl_3NO_3S_2$) C, H, N.

*3.1.1.13. 6-Chloro-3-(2-naphthoyl)-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**27**).* Starting from benzodithiazine **13** (1.16 g); yield: 0.86 g (73%); m.p. 178–180 °C; IR (KBr) 3190 (NH), 1675 (C=O), 1350, 1325, 1170, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.36 (d, $J = 11.6$ Hz, 1H, H-3), 7.40 (dd, $J_{ortho} = 8.4$ Hz, $J_{meta} = 1.7$ Hz, 1H, H-7), 7.63–7.77 (m, 3H, arom.), 7.95 (d, $J_{ortho} = 8.4$ Hz, 1H, H-8), 8.01–8.29 (m, 4H, arom.), 8.82 (d, $J = 6.6$ Hz, 1H, arom.), 9.19 (d, $J = 11.6$ Hz, 1H, NH) ppm. Anal. ($C_{18}H_{12}ClNO_3S_2$) C, H, N.

*3.1.1.14. 3-(3,4-Dichlorobenzoyl)-2,3-dihydro-1,1-dioxo-1,4,2-benzodithiazine (**28**).* Starting from benzodithiazine **14** (1.12 g); yield: 0.8 g (71%); m.p. 206–207 °C; IR (KBr) 3205 (NH), 1685 (C=O), 1345, 1185 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.24 (d, $J = 11.3$ Hz, 1H, H-3), 7.37 (t, $J = 7.4$ Hz, 1H, H-6), 7.47–7.56 (m, 2H, arom.), 7.91 (t, $J = 7.4$ Hz, 1H, H-7), 7.93–8.00 (m, 2H, arom.), 8.23 (s, 1H, H-2, 3,4-diClPh), 9.20 (d, $J = 11.3$ Hz, 1H, NH) ppm. Anal. ($C_{14}H_9Cl_2NO_3S_2$) C, H, N.

3.2. HIV-1 IN inhibition assay

3.2.1. Materials

For biological testing 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.

3.2.2. Site directed mutagenesis

Site directed mutagenesis was conducted on the pET-15b-IN plasmid, a generous gift from Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD. The plasmid contains full length IN fused to a N-terminal histidine tag downstream from a T7 prompter. A Quickchange site directed mutagenesis kit (Stratagene) was employed to make the point mutations according to the manufacturer's instructions.

Nucleotide replacements were confirmed by sequencing at the USC/Norris Comprehensive Cancer Center Microchemical Core Facility (University of Southern California). The IN plasmid was expressed in *Escherichia coli* BL21(De3) PLYS S expression strain (Invitrogen) after induction by IPTG (1 mM) at an absorbance of 0.6–0.8 optical density at 595 nm. Protein purification was performed as described in Ref. [20].

3.2.3. Preparation of oligonucleotide substrates

The oligonucleotides 21top, 5′-GTGTGGAAAATCTCTAGCAGT-3′ and 21bot, 5′-ACTGCTAGAGATTTTCCACAC-3′ were purchased from the Norris Cancer Center Microsequencing 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 T_4 polynucleotide kinase (Epicentre, Madison, WI) and γ [^{32}P]-ATP (Amersham Biosciences or ICN). The kinase was heat-inactivated and 21bot was added in 1.5-molar 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.

3.2.4. IN assays

To determine the extent of 3′-processing and strand transfer, wild-type 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-lebeled linear oligonucleotide substrate was added, and incubation at 30 °C 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 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, 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:

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

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 the concentration that produced 50% inhibition.

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