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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel pyrazolobenzothiazine 5,5-dioxide derivatives as potent anti-HIV-1 agents

Matloob Ahmad · Sana Aslam · Mujahid Hussain Bukhari · Catherine Montero · Mervi Detorio · Masood Parvez · Raymond F. Schinazi

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Abstract A new series of pyrazolobenzothiazine-based carbohydrazides was prepared in a facile way, starting with commercially available sodium saccharine. The final products were sufficiently characterized by spectroscopic techniques. In addition, compound **5k** was confirmed with X-ray crystallography as well. All the compounds were screened for anti-HIV-1 and cytotoxicity activities. Overall, out of 15 compounds, seven exhibited good activity with EC_{50} values <20 μM. Compounds **5c**, **5d**, **5g**, **5j** and **5k** appeared as the potent anti-HIV-1 agents with EC_{50} values <5.0 μM. The structure–activity relationship would facilitate the discovery of new molecules with better profile of HIV inhibition activity.

Keywords Heterocyclic compounds · Biological activity · 1,2-Benzothiazine 1,1-dioxide · Pyrazolobenzothiazines · Anti-HIV-1 activity · Cytotoxic studies

This research article is dedicated to the late professor Dr. Hamid Latif Siddiqui, our honorable teacher (MA, SA and MHB) and friend (MP and RFS).

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Introduction

Human immunodeficiency virus (HIV) is a continuous threat to the human being. This pathogen is reported to have affected about 40 million people worldwide and is responsible for acquired immune deficiency syndrome (AIDS). However, this lethal infection has now been converted into a chronic condition by using a combination therapy of various antiviral drugs (Coffin *et al.*, 1986; De Clercq, 2006, 2007a, b). The first drug that showed inhibitory effects to HIV was Zidovudine (AZT). Now more than 20 drugs are approved for the treatment of HIV. Combinations of various antiviral drugs that inhibit different stages in the viral replication are widely used for the treatment of this infectious disease and are called highly active antiretroviral therapy (HAART). A handsome number of anti-HIV drugs contain heterocyclic ring systems as a basic skeleton such as Vicriviroc (I), Aplaviroc (II) (Fig. 1). These heterocyclic scaffolds include pyrimidines, pyrazoles, benzimidazoles heterocycles, etc. However, there is a continuous need for the development of new and effective anti-HIV drugs with less toxicity (Gallant *et al.*, 2006; Mitsuya *et al.*, 1985; Pozniak *et al.*, 2006; De Clercq, 2007a).

Benzothiazines are versatile biologically active heterocyclic precursors exhibiting a broad spectrum of biological activities. 1,2-Benzothiazine-based carboxamides are excellent in their anti-inflammatory and analgesic activities and are known as oxicams that include meloxicam, piroxicam, and sudoxicam (Lomabardino *et al.*, 1971; Marfat, 1985; Turck *et al.*, 1995). Various other derivatives of benzothiazines exhibit anticancer activity (Gupta *et al.*, 1993), endothelin receptor antagonistic activity (Berryman *et al.*, 1998), and calpain I inhibiting activity (Bihovsky *et al.*, 2004). While continuing our research in 1,2-benzothiazine derivatives, we have explored *N*′-arylmethyli dene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-2(4*H*)-yl)acetohydrazides (Ahmad *et al.*, 2010a, b), *N*′-(substituted-2-chloroquinolin-3-yl)methylidene-4-hydroxy-2*H*-1,2-benzothiazine-3-carbohydrazides 1,1-dioxides (Ahmad *et al.*, 2012), pyrazolobenzothiazine-based carboxamides as antioxidants (Ahmad *et al.*, 2013), and pyrazolobenzothiazine-based pyrimidines as antibacterial (Bukhari *et al.*, 2012). Recently, we have reported various pyrazolobenzothiazine derivatives as anti-HIV-1 agents (Aslam *et al.*, 2013).

The observation that heterocyclic ring systems act as basic skeleton in various anti-HIV drugs as depicted in Fig. 1 and biological significance of benzothiazine and pyrazolobenzothiazine heterocycles led us to assume the potent biological nature of our developed compounds. The hypothesis proved with the discovery of some pyrazolobenzothiazine derivatives as HIV inhibitors with EC_{50} values less than 5 μM (Scheme 1).

Results and discussion

Chemistry

N-Phenacylsaccharine (**1**), prepared by condensation of saccharine with phenacyl bromide, was subjected to ring expansion with sodium methoxide in MeOH to get 3-benzoyl-4-hydroxy-2*H*-1,2-benzothiazin-1,1-dioxide (**2**). Compound **3** was obtained from compound **2** by reaction with methyl chloroacetate, which was treated with hydrazine monohydrate that caused simultaneous ring closure to pyrazole ring as well as hydrazide formation. Thus, the strategy provides an efficient way to form pyrazole and benzothiazine hybrid compounds, i.e., pyrazolobenzothiazines (Scheme 1). This ring system is rarely reported in literature and could be further explored for a broad range of new derivatives possessing various functionalities and pharmacophores resulting in worthwhile molecules. We herein converted the hydrazide to respective imines by treating it with a range of substituted benzaldehydes.

Spectroscopic tools were effectively utilized for the characterization of products. Compound **1** was confirmed by the appearance of N–CH_2 moiety at δ value of 5.13 ppm in the ^{1}H NMR and in FTIR, the peak at 1,715 cm^{-1} confirmed the presence of carbonyl group. Compound **2** was confirmed by the appearance of singlet at δ value of 5.81 ppm (SO_2NH). Moreover, in FTIR spectrum, ketone peak is considerably shifted from 1,715 cm^{-1} (compound **1**) to 1,615 due to the formation of β-diketone moiety (enol form). Compound **3** bearing ester functionality was characterized by IR peaks of carbonyl at 1,754 cm^{-1} and the appearance of protons of –$COOCH_3$ group at δ 3.27 ppm and N–CH_2 protons at δ 3.98 ppm in ^{1}H NMR. Compound **4** (the precursor compound) was characterized sufficiently by the appearance of CONH protons at 14.23 ppm and NH_2 of carbohydrazide moiety at 4.42 ppm. The corresponding carbohydrazones obtained by treatment with substituted benzaldehydes were confirmed by the presence of CH=N group which appeared in aromatic range as singlet in ^{1}H NMR and in ^{13}C NMR, it was observed downfield in the range of 140–144 ppm. CHN analysis data confirmed the proposed structures (Table 1). The structure elucidation was finalized with the X-ray crystal structure of compound **5k** as represented compound of the series (Fig. 2).

Crystal structure of compound **5k**

The molecular structure of compound **5k** is shown in Fig. 2. The crystal data and structure refinement parameters are listed in Table 2. The heterocyclic thiazine ring adopts a twist chair conformation with atoms S1 and C1 displaced by 0.819(2) and 0.261(3) Å, respectively, from the mean plane formed by the remaining ring atoms (N1// C6–C8). The mean planes of the benzene rings C1–C6 and

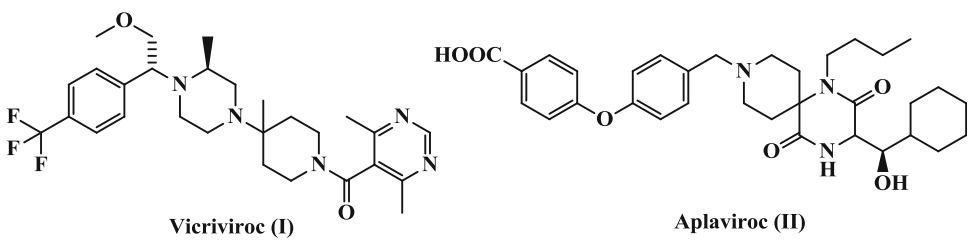
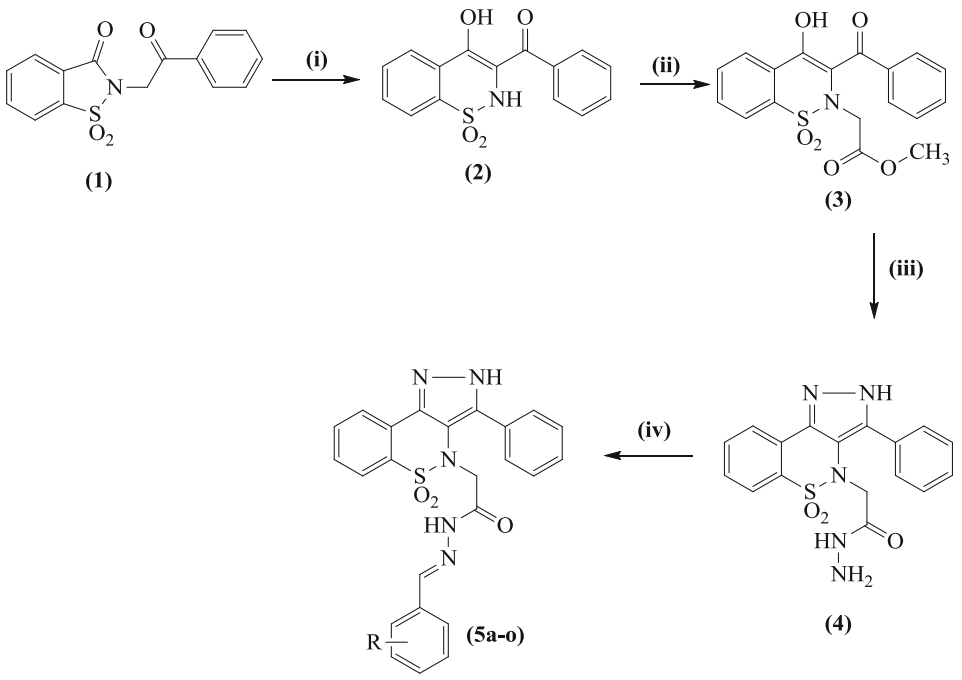


Fig. 1 Structures of HIV co-receptor antagonists Vicriviroc (**I**) and Aplaviroc (**II**)



Scheme 1 Layout for the synthesis of 2-(5,5-dioxido-3-phenylpyrazolo[4,3-*c*][1,2]benzothiazin-4(2*H*)-yl)-*N*′-[arylmethylidene] acetohydrazides (**5a–o**)

Table 1 CHN data for 2-(5,5-dioxido-3-phenylpyrazolo[4,3-*c*][1,2]benzothiazin-4(2*H*)-yl)-*N*′-[arylmethylidene]acetohydrazides (**5a–o**)

Sr. no.	Code no.	R	CHN analysis calculated (found)		
			C	H	N
1.	**5a**	2-F	60.62 (60.75)	3.82 (3.97)	14.73 (14.87)
2.	**5b**	3-Br	53.74 (53.89)	3.38 (3.51)	13.06 (13.21)
3.	**5c**	4-Br	53.74 (53.87)	3.38 (3.50)	13.06 (13.20)
4.	**5d**	3-Cl	58.59 (58.71)	3.69 (3.86)	14.24 (14.41)
5.	**5e**	4-Cl	58.59 (58.72)	3.69 (3.85)	14.24 (14.42)
6.	**5f**	2,4-$(Cl)_2$	54.76 (54.91)	3.26 (3.39)	13.30 (13.50)
7.	**5g**	2,6 $(Cl)_2$	54.76 (54.90)	3.26 (3.38)	13.30 (13.49)
8.	**5h**	4-NMe_2	62.38 (62.29)	4.83 (4.77)	16.79 (16.63)
9.	**5i**	4-NEt_2	63.62 (63.51)	5.34 (5.25)	15.90 (15.65)
10.	**5j**	2-NO_2	57.36 (57.47)	3.61 (3.75)	16.72 (16.87)
11.	**5k**	3-NO_2	57.36 (57.49)	3.61 (3.76)	16.72 (16.85)
12.	**5l**	4-NO_2	57.36 (57.48)	3.61 (3.78)	16.72 (16.86)
13.	**5m**	4-OMe	61.59 (61.73)	4.34 (4.47)	14.36 (14.43)
14.	**5n**	3,4-$(OMe)_2$	60.34 (60.75)	4.48 (4.63)	13.53 (13.70)
15.	**5o**	2,4-$(OMe)_2$	60.34 (60.74)	4.48 (4.65)	13.53 (13.72)

C10–C15 make dihedral angles 18.37(9) and 44.57(9)°, respectively, with the mean plane of the pyrazolyl ring (N2/N3/C7/C8/C9). The acetamide chain (O3/N4/N5/C17/C18) linking is essentially planar (rms deviation 0.0056 Å) and forms dihedral angles with the mean planes of the ring C19–C24 13.31(10)°. The nitro group (N6/O4/O5) is more

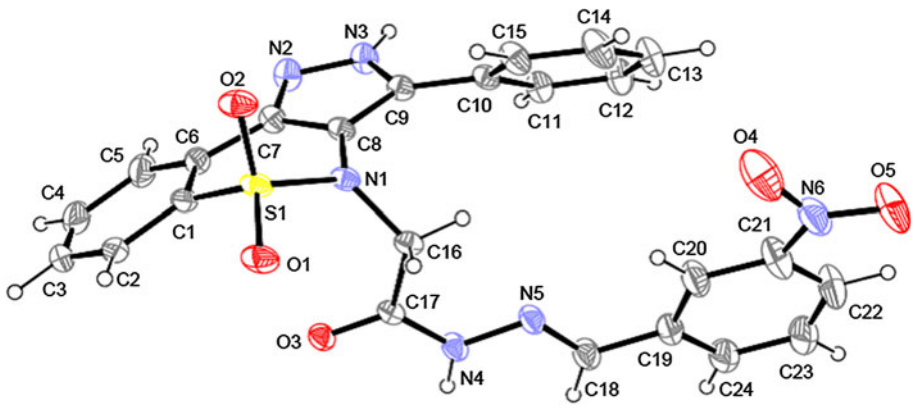


Fig. 2 The molecular structure of **5k** with the atom numbering scheme

Table 2 Crystal data and structure refinement parameters of compound **5k**

Parameters	Values	Parameters	Values
CCDC No.	881055	Crystal size (mm)	0.26 × 0.08 × 0.05
Moiety formula	C_{24} H_{18} N_6 O_5 S	T (K)	173(2)
Formula mass (g mol^{-1})	502.50	Index ranges	h: −35 to 35; k: −14 to 9; l: −19 to 19
Crystal system	Monoclinic	total data	7,424
Space group	C 2/c	Independent reflections	5,286 [R(int) = 0.013]
Unit cell dimensions			
a (Å)	27.722(7)	Completeness to θ = 27.5°	98.8 %
b (Å)	11.221(3)		
c (Å)	15.299(3)		
β (°)	101.549(16)	Absorption correction	Multi-scan method
V ($Å^3$)	4,662.7(19)	Max. and min. transmission	0.991 and 0.953
θ Ranges for data collection (°)	2.30–27.47	Data/restraints/parameters	5,286/0/335
Z	8	Final R indices[I >/2σ(I)]	R_1 = 0.0436; wR_2 = 0.0990
$\rho_{calc.}$ (g cm^{-3})	1.432	Goodness-of-fit on F^2	1.08
F(000)	2,080	Largest diff. peak and hole	0.25 and −0.46 e.$Å^{-3}$

or less co-planar with the benzene ring (C19–C24) with a dihedral angle between the mean planes being 9.6(11)°.

Pharmacology

Anti-HIV-1 activity

Among the series **5a–o**, seven compounds were found to be active against HIV-1 with EC_{50} values less than 20 μM (Table 3). Structure–activity relationship confirmed that the nature of substituent played an important role in the biological activity of the compounds.

Substituents having dominant electron donating effect are less active. Nitro- and halo-substituted compounds are more active than methoxy- and dialkyamino-substituted ones. All the methoxy- and *N*,*N*-dialkylamino-substituted compounds were either weakly active or inactive.

Among the nitro- and halo-substituted compounds, interestingly the substitution at 3-position results in enhanced activity. However, for bromo substitution, 4-bromo derivative was slightly more active than 3-bromo derivative. Among the nitro-substituted compounds, following order was observed: 3-NO_2 > 2-NO_2 > 4-NO_2 (Fig. 3). This order may be explained by the electron withdrawing nature of nitro group as it withdraws electrons from phenyl ring by inductive effect as well as by resonance effect, but overall, mesomeric effect dominates the inductive effect. Thus, NO_2 group at 3-position induces strong electron deficiency in the side chain containing hydrazone moiety. This proposed explanation is further supported by the X-ray data of **5k** (3-Nitro) where the nitro group (N6/O4/O5) is more or less co-planar with the benzene ring which is the mandatory condition for delocalization. Similarly, the order of biological activity of 2-nitro- and 4-nitro-substituted compounds is as expected. At ortho and para position, nitro group induces withdrawal of electrons by inductive effect which is 2-NO_2 > 4-NO_2.

Table 3 Anti-HIV-1 and cytotoxic activities of 2-(5,5-dioxido-3-phenylpyrazolo[4,3-*c*][1,2]benzothiazin-4(2*H*)-yl)-*N*′-[arylmethylidene] acetohydrazides (**5a–5o**)

Sr. no	R	Compound	Anti-HIV-1 activity in PBM cells (μM)[a]		Cytotoxicity (IC_{50}, μM)[a]		
			EC_{50}	EC_{90}	PBM	CEM	Vero
1		**4**	>100	>100	>100	15.1	>100
2	2-F	**5a**	>100	>100	20.7	15.4	15.5
3	3-Br	**5b**	6.0	35.9	11.7	6.8	12.2
4	4-Br	**5c**	4.1	13.4	13.0	12.6	48.5
5	3-Cl	**5d**	2.2	43.4	11.2	3.7	13.2
6	4-Cl	**5e**	6.5	29.7	31.6	11.9	14.2
7	2,4-$(Cl)_2$	**5f**	46.0	95.4	11.1	10.9	19.1
8	2,6 $(Cl)_2$	**5g**	2.7	52.5	12.9	<1.0	8.0
9	4-NMe_2	**5h**	>100	>100	>100	28.9	14.6
10	4-NEt_2	**5i**	27.2	61.4	52.2	12.5	62.6
11	2-NO_2	**5j**	4.8	47.3	47.8	2.0	59.3
12	3-NO_2	**5k**	3.3	30.2	22.0	15.2	53.1
13	4-NO_2	**5l**	25.1	>100	≥100	20.8	50.0
14	4-OMe	**5m**	42.0	81.0	76.3	31.6	66.0
15	3,4-$(OMe)_2$	**5n**	46.6	>100	75.8	48.6	46.1
16	2,4-$(OMe)_2$	**5o**	41.0	80.6	74.4	17.5	82.8
17	AZT		0.0033	0.031	>100	14.3	56.0

[a] All experiments were conducted in replicate

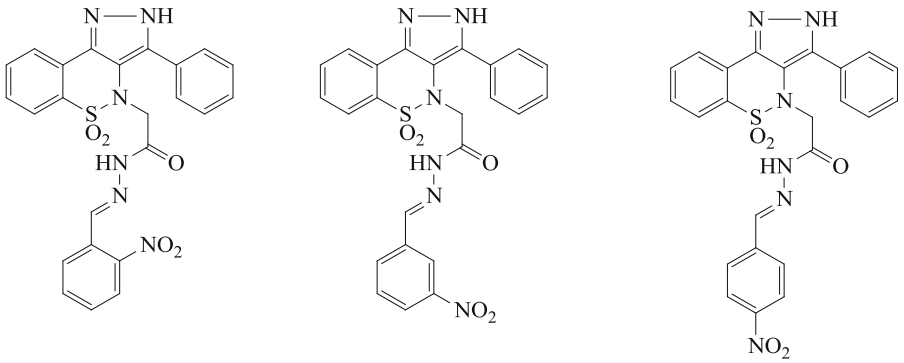
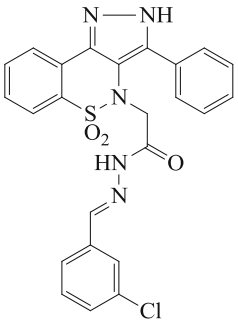


Fig. 3 Structure–activity relationship (anti-HIV-1) among the nitro-substituted compounds **5j**, **5k**, and **5l**

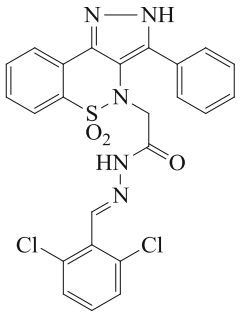
Compounds **5c**, **5d**, **5g**, **5j**, and **5k** bearing 4-Br, 3-Cl, 2,6 $(Cl)_2$, 2-NO_2, and 3-NO_2 substituents, respectively, were potentially active against HIV-1 with EC_{50} values 4.1, 2.2, 2.7, 4.8, and 3.3 μM, respectively (Fig. 4). However, substitutions by methoxy and *N*,*N*-dialkylamino groups resulted in reduced activity especially at position-4, e.g., 4-NEt_2 (**5i**) and 4-OMe (**5m**) displayed an EC_{50} values of 27.2 and 42.0 μM, respectively. Chlorosubstituted compounds (**5d** and **5g**) appeared as the most potent anti-HIV agents among the titled compound. These results support our recently published related work (Aslam *et al.*, 2013), where 3-chlorothiophenyl- and 2,5-dichlorothiophenyl-substituted products (**a**, Fig. 5) were the most potent and in the series of (2E)-3-(2-chloro-6-methyl/methoxyquinolin-3-yl)-1-(aryl)-prop-2-en-1-ones where 2,5-dichlorothiophenyl-substituted product (**b**, Fig. 5) was the most potent (Rizvi *et al.*, 2013).

Cytotoxicity studies

Compounds were screened for their cytotoxicity in human PBM, CEM, and Vero cells to determine their spectrum of toxicity. CEM cells are a line of lymphoblastic cells originally derived from a child with acute lymphoblastic leukemia whereas Vero cells are derived from African Green monkey kidney cells.



(**5d, EC_{50} = 2.2 μM**)



(**5g, EC_{50} = 2.7 μM**)

Fig. 4 Structures of the most potent anti-HIV-1 agents among compounds **5a–o**

In the series **5a–o**, all the halogen-substituted compounds were cytotoxic to human PBM, CEM, and Vero cells with IC_{50} values <50 μM. Among nitro-substituted compounds, **5l** (4-NO_2) showed no toxicity to human PBM and Vero cells. Methoxy-substituted compounds showed no toxicity in primary human PBM and Vero cells except compound **5n** which was toxic to Vero cells (Table 3) (Fig. 6).

Conclusion

In summary, we herein report the anti-HIV-1 activity of new acetohydrazides based on pyrazolobenzothiazine ring system. Seven compounds were active against HIV-1 with EC_{50} values less than 20.0 μM, which indicates the potential of these compounds as anti-HIV-1 agents. Among these seven active compounds, **5c**, **5d**, **5g**, **5j**, and **5k** were the potent anti-HIV-1 agents with EC_{50} values less than 5.0 μM. Compound **5d** appeared as the most potent of all having EC_{50} value of 2.2 μM. On these grounds, the structural modification of these compounds could lead us for new candidates of anti-HIV drugs with better inhibition profiles.

Experimental

Chemistry

All the chemicals were purchased from Alfa Aesar and were used without purification. ^{1}H NMR spectra were recorded on a Bruker DPX-400 instrument at 400 MHz. Chemical shifts are reported in ppm referenced to the residual solvent signal. FT-IR spectra were recorded on a Thermo Nicolet IR 200 spectrometer. Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected.

*Synthesis of 2-(2-oxo-2-phenylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (**1**)*

Compound (**1**) was prepared following our reported procedure (Ahmad *et al.*, 2010c). Phenacyl bromide (0.122 mol) was added to the suspension of sodium saccharine (25 g, 0.122 mol) in DMF (30 mL). The reaction mixture was stirred at 110 °C for 3 h and was poured on crushed ice. Precipitates formed were filtered, washed with excess water, and were dried after re-crystallization from ethanol.

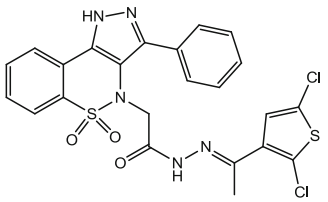
Yield: 84 %. FT-IR (KBr) ν_{max}:1715; 1335; 1175 cm^{-1}; ^{1}H NMR: (DMSO-d_6, 400 MHz) δ: 5.13 (s, 2H, *N*–CH_2), 7.49 (*t*, 2H, J = 7.6 Hz, Ar–*H*), 7.61 (*t*, 1H, J = 7.6 Hz, Ar–*H*), 7.83–7.91 (m, 2H, Ar–*H*), 7.94 (d, 1H, J = 6.8 Hz, Ar–*H*), 7.96 (d, 2H, J = 6.8 Hz, Ar–*H*), 8.09 (d, 1H, J = 8.0 Hz, Ar–H); ^{13}C NMR (DMSO-d_6): 51.3 (CH_2), 123.1 (Ar–C), 124 (Ar–C), 126.3 (Ar–C), 129.1 (2C, Ar), 129.4 (2C, Ar), 133.5 (Ar–C), 134.8 (Ar–C), 135.3 (Ar–C), 137 (Ar–C), 141.1 (Ar–C), 169.5 (C=O), 191.2 (Ar–CO); MS: ES−; 301.1 (M^+), +ES; 324.04 (M^++Na^+).

*Synthesis of (4-hydroxy-1,1-dioxido-2H-1,2-benzothiazin-3-yl) (phenyl)methanone (**2**)*

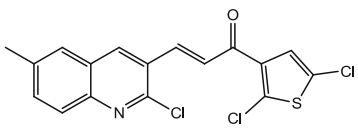
Compound (**2**) was prepared according to our reported methods (Ahmad *et al.*, 2010d). Sodium methoxide was freshly prepared by dissolving sodium metal (2.67 g, 0.116 mol) in 40 mL freshly dried methanol on reflux. The inert atmosphere was maintained by N_2 gas. Then, *N*-phenacylsaccharin (**1**) (5.0 g, 0.0166 mmol) was added to refluxing mixture of sodium methoxide and methanol. The solution turned to orange red color suspension immediately. The mixture was refluxed for half an hour and then poured on ice cold 10 % HCl. The white precipitates formed were collected and washed with excess water and dried.

Yield: 80 %. Appearance: white crystalline solid, m.p.156 °C; FT-IR (KBr) ν_{max}: 3157; 1615; 1358; 1151 cm^{-1}; ^{1}H NMR: (DMSO-d_6, 400 MHz) δ: 5.81 (s, 1H, SO_2NH), 7.64 (*t*, 1H, J = 8.0 Hz, Ar–*H*), 7.73 (d, 1H,

Fig. 5 Structures of the most potent compounds among our previously reported work (Aslam *et al.*, 2013; Rizvi *et al.*, 2013)



(**a,** EC_{50} = 3.2μM)



(**b,** EC_{50} = 1.1μM)

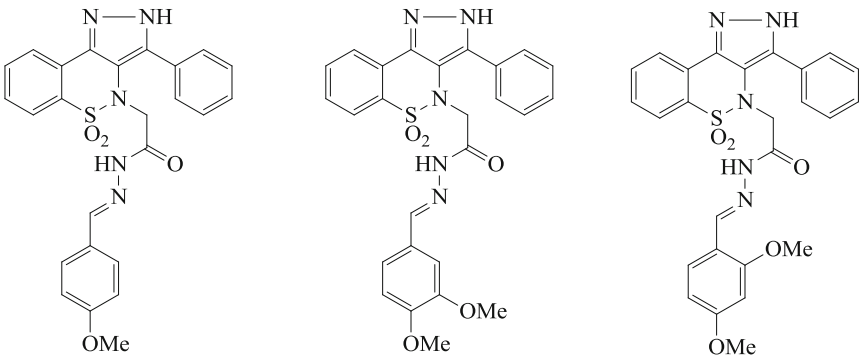


Fig. 6 Structure–activity relationship among methoxy-substituted compounds for cytotoxicity screening against human PBM cells

$J = 8.0$ Hz, Ar–*H*), 7.94–7.98 (m, 5H, Ar–*H*), 8.17 (*t*, 2H, $J = 3.6$ Hz, Ar–*H*), 14.63 (s, 1H, O–H); ^{13}C NMR (DMSO-d_6): 110.1 (C-3, thiazine), 122.9 (Ar–C), 127.1 (Ar–C), 128.1 (Ar–C), 128.3 (Ar–C), 129.1 (Ar–C), 129.5 (2C, Ar), 132.1 (Ar–C), 132.3 (Ar–C), 134 (Ar–C), 137.1 (2C, Ar), 151.6 (C-4, thiazine), 191.1 (C=O); LRMS (EI): ES−; 301.07 (M^+), +ES; 302.06 ($M^+ + H^+$).

*Synthesis of methyl [4-hydroxy-1,1-dioxido-3-(phenylcarbonyl)-2H-1,2-benzothiazin-2-yl]acetate (**3**)*

(4-Hydroxy-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl) (phenyl)methanone (2) (6.0 g, 0.020 mol) and anhydrous potassium carbonate (3.31 g, 0.024 mol) were dissolved in acetonitrile (30 mL). Then methyl chloroacetate (2.60 g, 0.024 mol) was added and allowed to reflux for 8 h. The completion of reaction was monitored by TLC. The solvent was removed under vacuum. Resulted residues were dissolved in cold water and maintain the neutral pH by dil. HCl to get the dirty yellow crystalline product.

Appearance: dirty yellow; m.p. 165 °C; FT-IR (KBr) ν_{max}: 1754; 1328; 1179 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.27 (s, 3H, –$COOCH_3$), 3.98 (s, 2H, N–CH_2), 7.63 (*t*, 1H, $J = 8.0$ Hz, Ar–*H*), 7.74 (d, 1H, $J = 8.0$ Hz, Ar–*H*), 7.90–7.95 (m, 5H, Ar–*H*), 8.16 (*t*, 2H, $J = 4.2$ Hz, Ar–*H*), 14.65 (s, 1H, O–H); ^{13}C NMR (DMSO-d_6): 43.2 (CH_2), 51.9 (O–CH_3), 114.1 (C-3, thiazine), 122.1 (Ar–C), 127.2 (Ar–C), 128.6 (2C, Ar), 128.9 (Ar–C), 129.3 (Ar–C), 129.5 (Ar–C), 132.1 (Ar–C), 133.2 (Ar–C), 134.6 (Ar–C), 134.8 (Ar–C), 138.1 (Ar–C), 156.7 (C-4, thiazine), 167.4 (C=O, ester), 190.3 (Ar–CO); MS *m*/*z*: ES−; 373.09 (M^+), +ES; 374.09 ($M^+ + H^+$).

Synthesis of 2-(5,5-dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)acetohydrazide (4)

A mixture of methyl [4-hydroxy-1,1-dioxido-3-(phenylcarbonyl)-2*H*-1,2-benzothiazin-2-yl]acetate (**3**) (5.0 g, 13.4 mmol) and hydrazine monohydrate (4.9 mL, 99 mmol) in 50 mL ethanol was refluxed for 4 h. Then the unreacted hydrazine monohydrate and ethanol was removed under vacuum. The crude product then dissolved in ice cold water. The resulted precipitates were filtered, recrystallized with ethanol, and finally dried.

Appearance: light dirty yellow; m.p. 190–192 °C; FT-IR (KBr) ν_{max}: 3378; 1685; 1333; 1162 cm^{-1}; ^{1}H NMR: (DMSO-d_6, 400 MHz) δ: 4.06 (s, 2H, *N*–CH_2), 4.42 (d, 2H, $J = 12.4$ Hz, NH_2), 7.50–7.66 (m, 3H, Ar–*H*), 7.73–7.85 (m, 4H, Ar–*H*), 7.97–8.03 (dd, 2H, $J_1 = 7.2$ Hz, $J_2 = 20.4$ Hz, Ar–*H*), 8.98 (s, 1H, NH), 14.23 (s, 1H, N–H); ^{13}C NMR (DMSO-d_6): 51.3 (CH_2), 126.9 (2C, Ar), 127.5 (Ar–C), 128.1 (Ar–C), 128.7 (Ar–C), 128.9 (2C, Ar), 129.2 (Ar–C), 131.1 (Ar–C), 131.3 (Ar–C), 131.6 (Ar–C), 133.1 (Ar–C), 134.2 (Ar–C), 134.4 (Ar–C), 136.2 (Ar–C), 166.8 (C=O); MS *m*/*z*: 369.1 (ES−), 392.10 (ES+, $M^+ + Na^+$).

*Synthesis of 2-(5,5-dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)-N′-[arylmethylidene] acetohydrazides (**5a–o**)*

Equimolar quantities of acetohydrazides **4** and corresponding benzaldehydes were refluxed in ethyl alcohol for 4–5 h. After completion of reaction, as indicated by TLC, the contents of the flask were concentrated on rotary evaporator. The precipitates formed were filtered, washed with excess ethanol, and dried.

*2-(5,5-Dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)-N′-[(2-fluorophenyl)methylidene]acetohydrazide (**5a**)* White powder; m.p. 239–240 °C; FT-IR (KBr) ν_{max}: 3727; 3229; 1684; 1587; 1324; 1157 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.70 (s, 2H, N–CH_2), 7.17–7.25 (m, 3H, Ar–*H*), 7.39 (*t*, 2H, $J = 6.8$ Hz, Ar–*H*), 7.55 (d, 2H, $J = 8.4$ Hz, Ar–*H*), 7.60–7.67 (m, 3H, Ar–*H*), 7.91 (d, 2H, $J = 8.4$ Hz, Ar–*H*),8.01 (s, 1H, C–H), 8.06 (d, 1H,

$J = 6.8$ Hz, Ar–*H*), 11.41 (s, 1H, N–H), 14.27 (s, 1H, N–H); ^{13}C NMR (DMSO-d_6): 51.2 (CH_2), 115.5 (Ar), 119.1 (Ar–C), 123.7 (Ar–C), 124.4 (Ar–C), 126.9 (Ar–C), 127.5 (Ar–C), 127.7 (Ar–C), 127.9 (Ar–C), 128.7 (Ar–C), 128.9 (Ar–C), 129.3 (Ar–C), 129.5 (Ar–C), 130.8 (Ar–C), 132.5 (Ar–C), 132.7 (Ar–C), 133.1 (Ar–C), 134.9 (Ar–C), 135.4 (Ar–C), 137.5 (Ar–C), 139.1 (Ar–C), 142.8 (N=CH), 148.9 (Ar–C), 167.7 (C=O); MS *m/z*: 475.15 (ES−, M^+), 476.14 (+ES, $M+H^+$).

N′-[(3-Bromophenyl)methylidene]-2-(5,5-dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)acetohydrazide ***(5b)*** Off white powder; m.p. 217–219 °C; FT-IR (KBr) ν_{max}: 3741; 3303; 1694; 1609; 1326; 1158 cm^{-1}; ^{1}H NMR: (DMSO-d_6, 400 MHz) δ: 4.69 (s, 2H, N–CH_2), 7.28 (*t*, 2H, $J = 7.6$ Hz, Ar–*H*), 7.34 (d, 1H, $J = 8.4$ Hz, Ar–*H*), 7.55 (d, 2H, $J = 7.6$ Hz, Ar–*H*), 7.62 (s, 1H, CH), 7.65 (d, 1H, $J = 8.4$ Hz, Ar–*H*), 7.74 (s, 1H, CH), 7.85 (d, 4H, $J = 10.4$ Hz, Ar–*H*), 8.00–8.07 (dd, 2H, $J_1 = 7.2$ Hz, $J_2 = 24.0$ Hz, Ar–*H*), 11.48 (s, 1H, NH), 14.26 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 51.7 (CH_2), 118.3 (Ar–C), 121.5 (Ar–C), 123.8 (Ar–C), 124.5 (Ar–C), 126.9 (Ar–C), 127.6 (Ar–C), 127.7 (Ar–C), 128.0 (Ar–C), 128.6 (Ar–C), 128.9 (Ar–C), 129.1 (Ar–C), 129.8 (Ar–C), 131.8 (Ar–C), 132.2 (Ar–C), 132.7 (Ar–C), 133.2 (Ar–C), 134.7 (Ar–C), 135.5 (Ar–C), 135.7 (Ar–C), 137.5 (Ar–C), 138.1 (Ar–C), 142.8 (N=CH), 168.9 (C=O); MS *m/z*:534.1, 536.06 (ES−), 558.04, 560.04 (+ES, $M+Na^+$) .

N′-[(4-Bromophenyl)methylidene]-2-(5,5-dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)acetohydrazide ***(5c)*** Off white powder; m.p. 254–255 °C; FT-IR (KBr) ν_{max}: 3325; 1685; 1609; 1328; 1154 cm^{-1}; ^{1}H NMR: (DMSO-d_6, 400 MHz) δ: 4.68 (s, 2H, N–CH_2), 7.29 (*t*, 2H, $J = 7.2$ Hz, Ar–*H*), 7.50–7.55 (m, 5H, Ar–*H*), 7.73 (s, 1H, CH), 7.83–7.88 (m, 4H, Ar–H), 8.03–8.07 (dd, 2H, $J_1 = 8.0$ Hz, $J_2 = 24.8$ Hz, Ar–*H*), 11.31 (s, 1H, NH), 13.90 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 51.4 (CH_2), 119.8 (Ar–C), 122.7 (Ar–C), 123.9 (Ar–C), 124.5 (Ar–C), 126.7 (Ar–C), 127.4 (Ar–C), 127.7 (Ar–C), 128.1 (Ar–C), 128.5 (Ar–C), 128.7 (Ar–C), 129.1 (Ar–C), 129.9 (Ar–C), 130.8 (Ar–C), 132.7 (Ar–C), 133.1 (Ar–C), 133.7 (Ar–C), 134.5 (Ar–C), 135.4 (Ar–C), 135.9 (Ar–C), 137.7 (Ar–C), 138.2 (Ar–C), 142.9 (N=CH), 168.9 (C=O); MS *m/z*: 534.05, 536.05 (ES−), 558.03, 560.06 (ES+, $M+Na^+$).

N′-[(3-Chlorophenyl)methylidene]-2-(5,5-dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)acetohydrazide ***(5d)*** White powder; m.p. 218–219 °C; FT-IR (KBr) ν_{max}: 3305; 1693; 1609; 1326; 1158 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.74 (s, 2H, N–CH_2), 7.33 (*t*, 1H, $J = 5.2$ Hz, Ar–*H*), 7.37–7.44 (dd, 2H, $J_1 = 7.6$ Hz, $J_2 = 18.4$ Hz, Ar–*H*), 7.47 (s, 1H, CH), 7.49 (d, 1H, $J = 6.8$ Hz, Ar–*H*), 7.63–7.70 (dd, 2H, $J_1 = 7.2$ Hz, $J_2 = 18.4$ Hz, Ar–*H*), 7.75 (s, 1H, CH), 7.83–7.88 (m, 4H, Ar–*H*), 7.99–8.07 (dd, 2H, $J_1 = 7.6$ Hz, $J_2 = 24.4$ Hz, Ar–*H*), 11.47 (s, 1H, NH), 14.25 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 51.3 (CH_2), 120.9 (Ar–C), 122.7 (Ar–C), 123.8 (Ar–C), 124.4 (Ar–C), 126.4 (Ar–C), 127.2 (Ar–C), 127.7 (Ar–C), 128.1 (Ar–C), 128.4 (Ar–C), 128.7 (Ar–C), 129.0 (Ar–C), 129.7 (Ar–C), 130.9 (Ar–C), 132.5 (Ar–C), 132.9 (Ar–C), 133.4 (Ar–C), 133.8 (Ar–C), 134.7 (Ar–C), 135.9 (Ar–C), 137.7 (Ar–C), 138.7 (Ar–C), 142.9 (N=CH), 168.1 (C=O); MS *m/z*: 492.11 (ES−), 514.09 (ES+, $M+Na^+$).

N′-[(4-Chlorophenyl)methylidene]-2-(5,5-dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)acetohydrazide ***(5e)*** Light yellow powder; m.p. 252–253 °C; FT-IR (KBr) ν_{max}: 3315; 1685; 1609; 1328; 1155 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.68 (s, 2H, N–CH_2), 7.16 (*t*, 2H, $J = 8.4$ Hz, Ar–*H*), 7.42 (s, 1H, CH), 7.52 (*t*, 2H, $J = 6.8$ Hz, Ar–*H*), 7.58 (*t*, 2H, $J = 6.8$ Hz, Ar–*H*), 7.76–7.90 (m, 5H, Ar–*H*), 7.99–8.07 (dd, 2H, $J_1 = 7.2$ Hz, $J_2 = 24.0$ Hz, Ar–*H*), 11.26 (s, 1H, NH), 14.25 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 51.4 (CH_2), 121.1 (Ar–C), 122.7 (Ar–C), 123.8 (Ar–C), 124.5 (Ar–C), 126.7 (Ar–C), 127.1 (Ar–C), 127.7 (Ar–C), 128.3 (Ar–C), 128.7 (Ar–C), 129.1 (Ar–C), 129.3 (Ar–C), 129.8 (Ar–C), 130.8 (Ar–C), 132.5 (Ar–C), 132.8 (Ar–C), 133.7 (Ar–C), 134.2 (Ar–C), 134.8 (Ar–C), 135.7 (Ar–C), 137.9 (Ar–C), 139.1 (Ar–C), 142.8 (N=CH), 168.3 (C=O); MS *m/z*: 492.1 (ES−), 514.09 (ES+, $M+Na^+$).

N′-[(2,4-Dichlorophenyl)methylidene]-2-(5,5-dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)acetohydrazide ***(5f)*** Off white powder; m.p. 237–238 °C; FT-IR (KBr) ν_{max}: 3648; 3313; 1686; 1601; 1324; 1157 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.69 (s, 2H, N–CH_2), 7.36–7.61 (m, 5H, Ar–*H*), 7.65 (s, 1H, CH), 7.76–7.88 (m, 4H, Ar–*H*), 7.99 (d, 2H, $J = 7.2$ Hz, Ar–*H*), 8.05 (d, 1H, $J = 8.0$ Hz, Ar–*H*), 11.48 (s, 1H, NH), 14.25 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 51.3 (CH_2), 122.8 (Ar–C), 123.9 (Ar–C), 124.7 (Ar–C), 126.9 (Ar–C), 127.1 (Ar–C), 127.8 (Ar–C), 128.1 (Ar–C), 128.7 (Ar–C), 129.0 (Ar–C), 129.5 (Ar–C), 129.8 (Ar–C), 131.8 (Ar–C), 132.5 (Ar–C), 132.7 (Ar–C), 134.1 (Ar–C), 134.9 (Ar–C), 135.3 (Ar–C), 135.5 (2C, Ar), 137.7 (Ar–C), 139.8 (Ar–C), 140.8 (N=CH), 168.7 (C=O); MS *m/z*: 526.06 (ES−), 550.04 (ES+, $M+Na^+$).

N′-[(2,6-Dichlorophenyl)methylidene]-2-(5,5-dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)acetohydrazide ***(5g)*** Off white powder; m.p. 254–255 °C; FT-IR (KBr) ν_{max}: 3222; 1692; 1606; 1324; 1158 cm^{-1}; ^{1}H NMR: (DMSO-d_6, 400 MHz) δ: 4.63 (s, 2H, N–CH_2), 7.35 (*t*, 2H, $J = 8.0$ Hz, Ar–H), 7.47 (d, 4H, $J = 8.0$ Hz, Ar–

H), 7.61–7.70 (m, 3H, Ar–*H*), 7.79–7.86 (m, 3H, Ar–*H*), 8.07 (s, 1H, CH), 11.46 (s, 1H, N–H), 14.25 (s, 1H, N–H); ^{13}C NMR (DMSO-d_6): 51.3, 122.9 (Ar–C), 124.0 (Ar–C), 124.8 (Ar–C), 126.8 (Ar–C), 127.1 (Ar–C), 127.7 (Ar–C), 128.0 (2C, Ar), 129.1 (Ar–C), 129.5 (Ar–C), 129.8 (Ar–C), 131.7 (Ar–C), 132.4 (2C, Ar), 132.7 (Ar–C), 134.2 (Ar–C), 135.1 (Ar–C), 136.5 (2C, Ar), 137.5 (Ar–C), 139.8 (Ar–C), 142.7 (N=CH), 168.9 (C=O); MS *m/z*: 524.07 (ES−), 548.05 (ES+, M+Na$^+$).

*N′-[4-(Dimethylamino)phenyl]methylidene-2-(5,5-dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)acetohydrazide (**5h**)* Dirty yellow; m.p. 255–257 °C; FT-IR (KBr) ν_{max}: 3747; 3322; 1685; 1598; 1322; 1154 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.91 (s, 3H, N–CH$_3$), 2.94 (s, 3H, N–CH$_3$), 4.64 (s, 2H, N–CH$_2$), 6.62 (*t*, 2H, $J = 8.4$ Hz, Ar–*H*), 7.16 (*t*, 1H, $J = 5.6$ Hz, Ar–*H*), 7.32–7.35 (dd, 1H, $J_1 = 4.4$ Hz, $J_2 = 7.6$ Hz, Ar–H), 7.51–7.56 (dd, 2H, $J_1 = 4.8$ Hz, $J_2 = 15.2$ Hz, Ar–H), 7.61 (s, 1H, CH), 7.64 (v 2H, $J = 8.4$ Hz, Ar–H), 7.79–7.90 (m, 4H, Ar–*H*), 8.02–8.05 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 25.2$ Hz, Ar–*H*), 11.01 (s, 1H, NH), 14.23 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 40.7 (2C, N(CH$_3$)$_2$), 51.4 (CH$_2$), 115.1 (2C, Ar), 122.7 (Ar–C), 124.1 (Ar–C), 124.7 (Ar–C), 126.8 (Ar–C), 127.3 (Ar–C), 127.5 (Ar–C), 128.1 (Ar–C), 128.5 (Ar–C), 129.1 (Ar–C), 129.5 (Ar–C), 129.8 (Ar–C), 131.7 (Ar–C), 132.4 (Ar–C), 132.7 (Ar–C), 134.7 (Ar–C), 135.1 (Ar–C), 137.5 (Ar–C), 139.8 (Ar–C), 142.7 (N=CH), 151.9 (Ar–C), 168.7 (C=O); MS *m/z*: 500.2 (ES−), 523.17 (ES+, M+Na$^+$).

*N′-[4-(Diethylamino)phenyl]methylidene-2-(5,5-dioxido-3-phenylpyrazolo[4,3 c][1,2]benzothiazin-4(2H)-yl)acetohydrazide (**5i**)* Dirty yellow; m.p. 255–257 °C; FT-IR (KBr) ν_{max}: 3747; 3322; 1685; 1598; 1322; 1154 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.10 (s, 3H, CH$_3$), 1.13 (s, 3H, CH$_3$), 2.87 (s, 2H, N–CH$_2$), 2.90 (s, 2H, N–CH$_2$), 4.60 (s, 2H, N–CH$_2$), 6.60 (*t*, 2H, $J = 8.4$ Hz, Ar–*H*), 7.15 (*t*, 1H, $J = 5.6$ Hz, Ar–*H*), 7.31–7.35 (dd, 1H, $J_1 = 4.4$ Hz, $J_2 = 7.6$ Hz, Ar–H), 7.50–7.54 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 15.2$ Hz, Ar–H), 7.59 (s, 1H, CH), 7.62 (*t*, 2H, $J = 8.4$ Hz, Ar–H), 7.81–7.90 (m, 4H, Ar–*H*), 8.01–8.03 (dd, 2H, $J_1 = 8.0$ Hz, $J_2 = 25.2$ Hz, Ar–*H*), 11.02 (s, 1H, NH), 14.20 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 12.9 (2C, CH$_3$(ethyl)), 44.5 (2C, CH$_2$(ethyl)), 51.3 (*N*–CH$_2$), 115.1 (2C, Ar), 122.7 (Ar–C), 124.0 (Ar–C), 124.8 (Ar–C), 126.8 (Ar–C), 127.1 (Ar–C), 127.7 (Ar–C), 128.0 (Ar–C), 128.5 (Ar–C), 129.1 (Ar–C), 129.4 (Ar–C), 129.7 (Ar–C), 131.7 (Ar–C), 132.5 (Ar–C), 132.7 (Ar–C), 134.5 (Ar–C), 135.0 (Ar–C), 137.5 (Ar–C), 139.7 (Ar–C), 142.5 (N=CH), 151.4 (Ar–C), 168.7 (C=O); MS *m/z*:528.2 (ES−), 551.17 (ES+, M+Na$^+$).

*2-(5,5-Dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)-N′-[(2-nitrophenyl)methylidene]acetohydrazide (**5j**)* Light yellow powder; m.p. 274 °C; FT-IR (KBr) ν_{max}: 3226; 1688; 1588; 1322; 1156 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.69 (s, 2H, N–CH$_2$), 7.52–7.69 (m, 5H, Ar–*H*), 7.78–7.89 (m, 4H, Ar–*H*), 7.98 (d, 2H, $J = 8.0$ Hz, Ar–*H*), 8.06 (d, 2H, $J = 7.6$ Hz, Ar–*H*), 8.16 (s, 1H, CH=N), 11.55 (s, 1H, NH), 14.27 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 51.3 (CH$_2$), 122.7 (Ar–C), 124.0 (Ar–C), 124.7 (Ar–C), 126.7 (Ar–C), 127.1 (Ar–C), 127.7 (Ar–C), 128.0 (Ar–C), 128.3 (Ar–C), 128.7 (Ar–C), 129.5 (Ar–C), 129.8 (Ar–C), 131.7 (Ar–C), 132.5 (Ar–C), 132.7 (Ar–C), 134.6 (Ar–C), 135.1 (Ar–C), 135.5 (Ar–C), 135.9 (Ar–C), 137.5 (Ar–C), 139.7 (Ar–C), 142.5 (N=CH), 147.7 (Ar–C), 170.1 (C=O); MS *m/z*: 502.14 (ES−), 525.11 (ES+, M+Na$^+$).

*2-(5,5-Dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)-N′-[(3-nitrophenyl)methylidene]acetohydrazide (**5k**)* Dirty yellow powder; m.p. 248–249 °C; FT-IR (KBr) ν_{max}: 3319; 1689; 1599; 1328; 1158 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.74 (s, 2H, N–CH$_2$), 7.42 (d, 2H, $J = 7.6$ Hz, A–r–*H*), 7.64 (*t*, 2H, $J = 7.2$ Hz, Ar–*H*), 7.81–7.89 (m, 5H, Ar–*H*), 8.04 (s, 1H), 8.11 (*t*, 1H, $J = 10.0$ Hz, Ar–*H*), 8.20 (*t*, 2H, $J = 8.4$ Hz, Ar–*H*), 8.37 (d, 1H, $J = 10.0$ Hz, Ar–*H*), 11.75 (s, 1H, NH), 14.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 51.3 (CH$_2$), 122.5 (Ar–C), 124.1 (Ar–C), 124.7 (Ar–C), 126.8 (Ar–C), 127.0 (Ar–C), 127.7 (Ar–C), 128.1 (Ar–C), 128.4 (Ar–C), 128.7 (Ar–C), 129.4 (Ar–C), 129.8 (Ar–C), 131.9 (Ar–C), 132.5 (Ar–C), 132.8 (Ar–C), 134.5 (Ar–C), 135.0 (Ar–C), 135.4 (Ar–C), 135.8 (Ar–C), 137.5 (Ar–C), 139.8 (Ar–C), 142.7 (N=CH), 148.9 (Ar–C), 169.9 (C=O); MS *m/z*: 502.14 (ES−), 525.11 (ES+, M+Na$^+$).

*2-(5,5-Dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)-N′-[(4-nitrophenyl)methylidene]acetohydrazide (**5l**)* Light yellow powder; m.p. 238–240 °C; FT-IR (KBr) ν_{max}: 3748; 3321; 1688; 1587; 1327; 1155 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.74 (s, 2H, N–CH$_2$), 7.42 (d, 2H, $J = 7.2$ Hz, Ar–*H*), 7.51–7.66 (m, 5H, Ar–*H*), 7.80–7.87 (dd, 4H, $J_1 = 8.4$ Hz, $J_2 = 18.8$ Hz, Ar–*H*), 8.01 (s, 1H, CH), 8.16 (*t*, 2H, $J = 9.4$ Hz, Ar–*H*), 11.58 (s, 1H, NH), 14.27 (s, 1H, N-H); ^{13}C NMR (DMSO-d_6): 51.3 (CH$_2$), 122.7 (Ar–C), 124.0 (Ar–C), 124.7 (Ar–C), 126.7 (Ar–C), 127.1 (Ar–C), 127.7 (Ar–C), 128.1 (Ar–C), 128.5 (Ar–C), 128.7 (Ar–C), 129.5 (Ar–C), 129.7 (Ar–C), 132.0 (Ar–C), 132.4 (Ar–C), 132.7 (Ar–C), 134.7 (Ar–C), 135.0 (Ar–C), 135.7 (Ar–C), 135.9 (Ar–C), 137.5 (Ar–C), 139.8 (Ar–C), 142.5 (N=CH), 150.4 (Ar–C), 170.1 (C=O); MS *m/z*: 502.16 (ES−), 541.16 (ES+, M + K$^+$).

*2-(5,5-Dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)-N′-[(4-methoxyphenyl)methylidene]acetohydrazide (**5m**)* Off white powder; m.p. 255–257 °C; IR (KBr) ν_{max}: 3717; 3229; 1674; 1587; 1324; 1157 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.75 (s, 3H, O–CH_3), 4.68 (s, 2H, N–CH_2), 6.93 (d, 2H, $J = 5.6$ Hz, Ar–*H*), 7.24 (*t*, 1H, $J = 7.6$ Hz, Ar–*H*), 7.47–7.69 (m, 3H, Ar–*H*), 7.74 (s, 1H, CH), 7.78–7.89 (m, 5H, Ar–*H*), 8.00–8.08 (dd, 2H, $J_1 = 7.2$ Hz, $J_2 = 24.0$ Hz, Ar–*H*), 11.34 (s, 1H, NH), 14.25 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 48.5 (O–CH_3), 51.6 (CH_2), 111.1 (Ar–C), 116.5 (Ar–C), 122.5 (Ar–C), 124.3 (Ar–C), 126.5 (Ar–C), 127.1 (Ar–C), 127.7 (Ar–C), 128.1 (Ar–C), 128.7 (Ar–C), 129.1 (Ar–C), 129.4 (Ar–C), 129.7 (Ar–C), 130.7 (Ar–C), 132.5 (Ar–C), 132.7 (Ar–C), 134.7 (Ar–C), 135.0 (Ar–C), 135.3 (Ar–C), 137.7 (Ar–C), 139.5 (Ar–C), 142.7 (N=CH), 161.4 (Ar–C), 168.7 (C=O); MS *m/z*: 487.17 (ES−, M^+), 488.16 (ES+, $M + H^+$).

*2-(5,5-Dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)-N′-[(3,4,5-trimethoxyphenyl)methylidene]acetohydrazide (**5n**)* Off white powder; m.p. 279–281 °C; FT-IR (KBr) ν_{max}: 3610; 3230; 1692; 1604; 1323; 1121 cm^{-1}; ^1H NMR: (DMSO-d_6, 400 MHz) δ: 3.69 (s, 3H, O–CH_3), 3.79 (s, 3H, O–CH_3), 3.83 (s, 3H, O–CH_3), 4.66 (s, 2H, N–CH_2), 6.76 (*t*, 2H, $J = 8.8$ Hz, Ar–*H*), 7.03 (*t*, 1H, $J = 8.8$ Hz, Ar–*H*), 7.34 (d, 1H, $J = 8.0$ Hz, Ar–*H*), 7.54–7.67 (m, 4H, Ar–*H*), 7.77–7.88 (m, 3H, Ar–*H*), 7.94 (s, 1H, CH), 11.09 (s, 1H, NH), 14.24 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 49.8 (2C, OCH_3), 51.5 (CH_2), 54.1 (OCH_3), 113.5 (Ar–C), 115.7 (Ar–C), 122.7 (Ar–C), 124.1 (Ar–C),126.4 (Ar–C), 127.1 (Ar–C), 127.7 (Ar–C), 128.0 (Ar–C), 128.5 (Ar–C), 129.1 (Ar–C), 129.5 (Ar–C), 129.8 (Ar–C), 131.3 (Ar–C), 132.4 (Ar–C), 132.7 (Ar–C), 135.0 (Ar–C), 135.5 (Ar–C), 135.9 (Ar–C), 137.5 (Ar–C), 142.5 (N=CH), 149.7 (2C, Ar), 168.3 (C=O); MS *m/z*: 547.2 (M^+).

*2-(5,5-Dioxido-3-phenylpyrazolo[4,3-c][1,2]benzothiazin-4(2H)-yl)-N′-[(2,3,4-trimethoxyphenyl)methylidene]acetohydrazide (**5o**)* Off white powder; m.p. 275–277 °C; FT-IR (KBr) ν_{max}: 3619; 3250; 1682; 1588; 1324; 1156 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.67 (s, 3H, O–CH_3), 3.74 (s, 3H, O–CH_3), 3.77 (s, 3H, O–CH_3), 4.67 (s, 2H, N–CH_2), 6.80 (d, 2H, $J = 18.4$ Hz, Ar–H), 7.46–7.67 (m, 5H, Ar–*H*), 7.70 (s, 1H, CH), 7.82 (*t*, 2H, $J = 7.2$ Hz, Ar–*H*), 8.01–8.07 (dd, 2H, $J_1 = 6.8$ Hz, $J_2 = 22.0$ Hz, Ar–H), 11.25 (s, 1H, N–H), 14.25 (s, 1H, N–H); ^{13}C NMR (DMSO-d_6): 51.6 (CH_2), 51.8 (OCH_3), 52.3 (OCH_3), 55.1 (OCH_3), 105.5 (2C, Ar), 111.9 (Ar–C), 122.7 (Ar–C), 124.0 (Ar–C), 125.9 (Ar–C), 127.1 (Ar–C), 127.8 (Ar–C), 128.1 (Ar–C), 128.7 (Ar–C), 129.0 (Ar–C), 129.4 (Ar–C), 129.8 (Ar–C), 132.5 (Ar–C), 132.7 (Ar–C), 135.1 (Ar–C), 135.5 (Ar–C), 137.7 (Ar–C), 142.5 (N=CH), 149.9 (Ar–C), 160.7 (Ar–C), 161.1 (Ar–C), 168.5 (C=O); MS m/z: 547.58 (M^+).

X-ray crystallographic studies

A pale yellow needle-shaped crystals of **5k** was used for data collection. The crystals were coated with Paratone 8277 oil (Exxon) and mounted on a glass fiber. All measurements were made on a Nonius Kappa CCD diffractometer with graphite-monochromated Mo-Kα radiation. Details of crystal data and structure refinement have been provided in Table 2. The data were collected at a temperature of 173(2) K using ω and φ scans, corrected for Lorentz and polarization effects and for absorption using multi-scan method (Hooft *et al.*, 1998; Otwinowski and Minor, 1997).

The structure was solved by the direct methods (Altomare *et al.*, 1993) and expanded using Fourier techniques (Beurskens *et al.*, 1994). The non-hydrogen atoms were refined anisotropically. Nitro group in **5k** was disordered. Hydrogen atoms were included at geometrically idealized positions and were not refined. The final cycle of full-matrix least-squares refinement using SHELXL97 (Sheldrick, 2008) converged with unweighted and weighted agreement factors, R and $wR = 0.0436$ and 0.0990 (all data) for **5k**, and goodness of fit, $S = 1.079$. The weighting schemes were based on counting statistics and the final difference Fourier maps were essentially featureless. The figures were plotted with the aid of ORTEP-3 (Farrugia, 1997).

Anti-HIV-1 assay

The synthesized compounds were screened for their in vitro antiviral effects in human peripheral blood mononuclear (PBM) cells according to the standardized assay (Schinazi *et al.*, 1990). Cells obtained from Life South Community Blood Centers (Atlanta, GA) were isolated by Histopaque (Sigma-Aldrich, St. Louis, MO) and discontinuous gradient centrifugation from healthy seronegative donors. The median effective concentration (EC_{50}) was determined using a reported method (Belen'kii and Schinazi, 1994). Assays were conducted using at least two different donor cells in duplicate or triplicate. The results are expressed in Table 3.

Cytotoxicity assay

Primary human PBM, CEM, and Vero cells were cultured in 96-well plates (5×10^4 cells per well) along with increasing concentrations of the test compound. Cell viability was measured after 5-day incubation period using the cell titer 96 aqueous one solution cell proliferation assay (Promega, Madison, WI) by incubating in an incubator at

37 °C with 5 % CO_2 for human PBM cells (Stuyver *et al*., 2002). The results are summarized in Table 3.

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