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# Synthesis, molecular docking and antiviral screening of novel *N'*-substitutedbenzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazides

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**Abstract** *N'*-Substitutedbenzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazides (**7a–u**) were synthesized through a multistep reaction. The final compounds were screened for anti-HIV-1 and cytotoxic activities. Among these compounds, **7l** and **7m** exhibited the most significant anti-HIV-1 activity with EC<sub>50</sub> values of 5.4 and 3.3 μM, respectively, while **7j** showed moderate anti-HIV activity with an EC<sub>50</sub> value 17.1 μM. Molecular docking into HIV-1 Reverse Transcriptase also showed the best fit for **7l** and **7m** followed by **7j**. In addition, compounds **7b**, **7f**, **7h**, **7k**, **7o**, and **7s** exhibited no toxicity in all the three cell lines used i.e., primary human PBM, CEM, and Vero cells, while **7e**, **7g**, **7i**, **7n**,

**7p**, **7q**, and **7t** exhibited no cytotoxic activity in primary human PBM cells. Moreover, it was found through molecular docking that compounds **7l**, **7m**, and **7j** bound efficiently in the NTP-binding pocket of HIV-1 Reverse Transcriptase. The structure–activity relationship established on these results would facilitate the further development of new HIV inhibitors based on this skeleton.

**Keywords** Anti-HIV-1 activity · 1,2-Benzothiazine · Cytotoxic activity · Hydrazides · Pyrazolobenzothiazine dioxides

**Electronic supplementary material** The online version of this article (doi:10.1007/s00044-013-0879-7) contains supplementary material, which is available to authorized users.

This research article is dedicated to Dr. Hamid Latif Siddiqui (Institute of Chemistry, University of the Punjab, Lahore-Pakistan), our honorable teacher (SA and MA), our friend and colleague (MMA, JMG, MP, and RFS) who passed away on July 26, 2012.

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## Introduction

The 1,2-benzothiazines ring system is a heterocyclic scaffold of paramount importance in drug chemistry. The potent anti-inflammatory drugs based on this pharmacophore, e.g., piroxicam (Lomabardino *et al.*, 1971),

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tenoxicam, meloxicam (Turck *et al.*, 1995; Engelhardt *et al.*, 1995), ampiroxicam (Lombardino *et al.*, 1973; Myung and Soon, 2000; Marfat, 1985), lornoxicam (Caruso *et al.*, 1994), and droxicam (Soler, 1986), are classified as oxicams within the broad class of NSAIDs. Various derivatives of this class have been established as antimicrobial (Giuseppe *et al.*, 1987), antiallergic (Ikeda *et al.*, 1992), anti-inflammatory (Suh *et al.*, 1987), antianxiety, antidepressant (Krapcho and Turk, 1975; Krapcho, 1973), and antithrombotic agents (Constantine, 1967). Recently, pyrazolobenzothiazine derivatives are reported as potent HCV replication inhibitors (Barreca *et al.*, 2013).

Human Immunodeficiency Virus (HIV) is responsible for Acquired Immune Deficiency Syndrome (AIDS). It is a disease characterized by extensive immunosuppression that predisposes patients to life-threatening opportunistic infections and unusual forms of neoplasms (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Siegal *et al.*, 1981; Francis *et al.*, 1983; Broder and Gallo, 1984; Shaw *et al.*, 1985; Ferionio *et al.*, 1985). AIDS is thought to result from infection of T cells by a pathogenic human retrovirus, human T-lymphotropic virus type III (HTLV-III) or lymphadenopathy-associated virus (LAV) (Barrd-Sinoussi *et al.*, 1983; Klatzman *et al.*, 1984). This virus preferentially infects and destroys OKT4<sup>+</sup> (helper/inducer) T-cells. Among the most potent anti-HIV compounds, ribavirin (Fig. 1) (McCormick *et al.*, 1984), antimoniotungstate (HPA-23) (Rosenbaum *et al.*, 1985), ansamycin and phosphonoformic acid (Sandstrom *et al.*, 1985), and Crixivan (Fig. 1) are of significant importance.

To date, many drugs are known to inhibit HIV at various stages of its life cycle. Yet, there remains an urgent need for the development of new anti-HIV drugs and therapeutic strategies. Our endeavors on the versatile synthesis of a range of 1,2-benzothiazines led us to report the synthesis of a number of libraries of biologically active benzothiazine derivatives (Ahmad *et al.*, 2010a, b, 2012, 2013a, b; Bukhari *et al.*, 2011). Recently, we have reported pyrazolobenzothiazine derivatives as the first example of HIV inhibitors from this family of heterocyclic compounds (Aslam *et al.*, 2013). Noting the involvement of heterocyclic templates in a number of antiviral agents (Fig. 1), we herein describe the potential of various new derivatives of pyrazolobenzothiazine ring system as anti-HIV-1 agents.

## Results and discussion

### Chemistry

*N'*-substitutedbenzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazides (**7a–u**) were synthesized via the reaction of 2-(4-methyl-3-phenyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-1(4*H*)-yl)acetohydrazide (**6**) with a range of substituted benzaldehydes (Scheme 2).

The starting material (*N*-phenacilsaccharine, **1**) was synthesized according to our previously reported thermal method (Ahmad *et al.*, 2010c), as well as using ultrasound and microwave irradiation techniques (Table 1). The corresponding 1,2-benzothiazine derivative was synthesized by the ring expansion reaction of 2-(2-oxo-2-phenylethyl)-1,2-benzisothiazol-3(2*H*)-one-1,1-dioxide (**1**) using NaOMe in MeOH (Ahmad *et al.*, 2010d). *N*-Methylation of 3-benzoyl-4-hydroxy-2*H*-1,2-benzothiazine-1,1-dioxide (**2**) was also attempted using ultrasound (Table 2), which reduced reaction times to 10–15 min with 85 % yield. The formation of alkylated product was confirmed by the appearance of N-CH<sub>3</sub> peak at 2.89 ppm in the <sup>1</sup>H NMR spectrum and by HR-MS spectrum. Treatment of compound **3** with hydrazine monohydrate in refluxing ethanol resulted in the cyclization of pyrazole ring to give 4-methyl-3-phenyl-2,4-dihydrobenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazine 5,5-dioxide (**4**) (Scheme 1). In FTIR, NH and C=N functionalities were

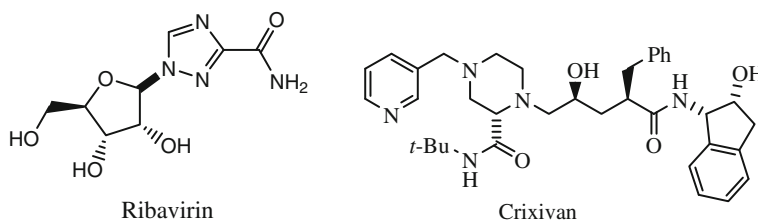
**Table 1** Comparison of thermal and ultrasonic reaction conditions for *N*-alkylation of 3-benzoyl-4-hydroxy-2*H*-1,2-benzothiazine-1,1-dioxide (**3**)

Entry	Technique used	Reaction time (min)	Yield (%)
1	Thermal method	30–40	80
2	Ultrasonic irradiation	10–15	85

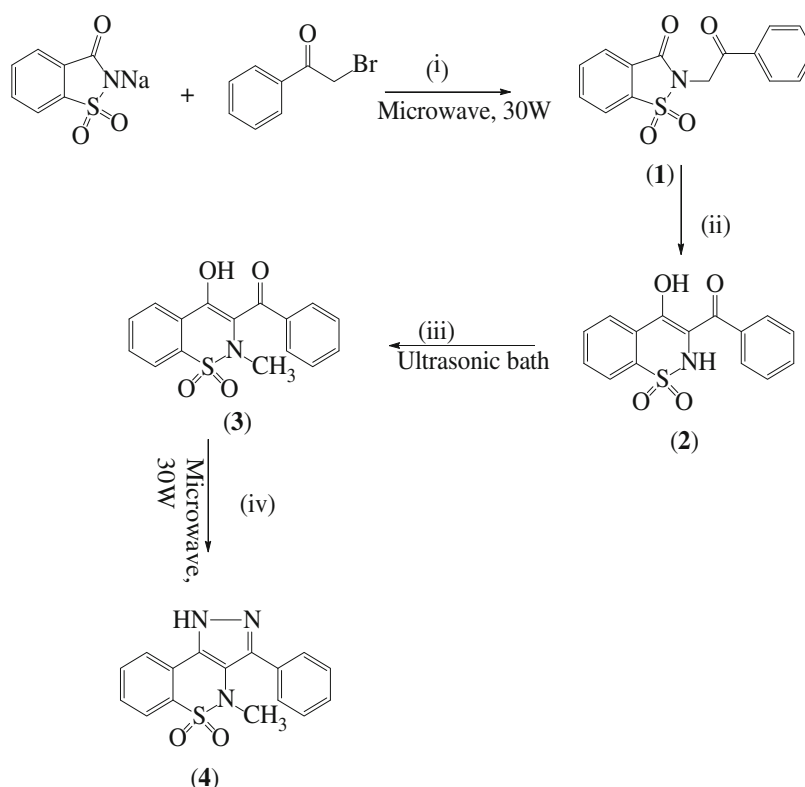
**Table 2** Synthesis of 2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazide (**6**) under different reaction conditions

Entry	Technique used	Reaction time (min)	Yield (%)
1	Thermal method	240	80
2	Ultrasonic irradiation	30–40	71
3	Microwave irradiation	10–20	75

**Fig. 1** The structures of anti-HIV drugs, Ribavirin and Crixivan (Indinavir)



**Scheme 1** Microwave-assisted synthesis of 4-methyl-3-phenyl-2,4-dihydrobenzo[*e*]pyrazole [4,3-*c*][1,2]thiazine 5,5-dioxide (**4**)



**Chemicals and reagents:** (i) dimethylformamide (ii) NaOMe/MeOH, reflux  
(iii)  $(\text{CH}_3)_2\text{SO}_4/\text{NaOH}$ , acetone (iv) hydrazine monohydrate

observed at  $3,368$  and  $1,655\text{ cm}^{-1}$ , respectively. *N*-Alkylation of compound (**4**) with various alkyl chloroacetates was affected using  $\text{K}_2\text{CO}_3$  as a base. The structure was confirmed from the  $\text{O}-\text{CH}_3$  peak at  $3.27$  ppm and  $\text{N}-\text{CH}_2$  at  $5.77$  ppm in  $^1\text{H}$  NMR spectrum and a carbonyl stretch at  $1,685\text{ cm}^{-1}$  in the FTIR spectrum. For **5b**, observation of a quartet at  $3.96$  ppm due to the  $\text{O}-\text{CH}_2$  and a triplet at  $1.09$  ppm for the  $\text{C}-\text{CH}_3$  group supported structural assignment. Molecular ion peaks were observed at  $383.06$  and  $397.96$  in the MS for compounds **5a** and **5b**, respectively.

Compound (**5**) was refluxed with hydrazine monohydrate in absolute ethanol to provide 2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazide (**6**). Microwave irradiation resulted in 75 % yield after a much reduced reaction time of only 10 min, compared to the 4 h required for the thermal reaction. Absorption peaks for  $\text{C}=\text{N}$  and  $\text{NH}$  groups appeared at  $1,675$  and  $3,468\text{ cm}^{-1}$  in the FTIR spectrum, respectively. In the  $^1\text{H}$  NMR spectrum,  $\text{NH}_2$  and  $\text{NH}$  gave rise to resonances at  $4.39$  and  $11.94$  ppm, respectively. *N'*-Substitutedbenzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazides (**7a–u**) (Scheme 2) were obtained by the reaction of compound **6** with a range of substituted benzaldehydes in refluxing ethanol using the conventional thermal method, the microwave irradiation, and also using an ultrasonic bath. The reaction parameters are discussed in Table 3,

and it was observed that although the obtained yields were almost same for all three methods, there was a significant reduction in reaction times using microwave irradiation compared to the thermal reaction times.

#### X-ray crystallographic studies

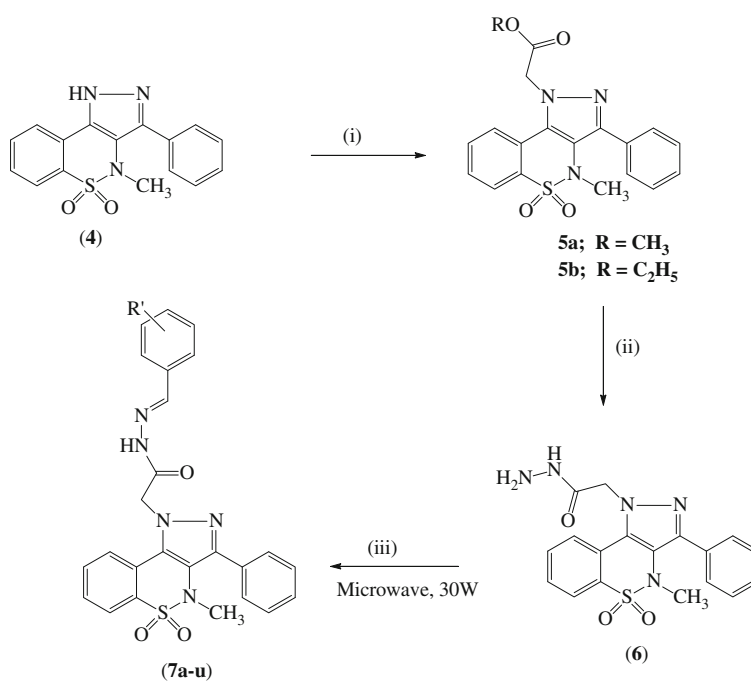
The molecular structure of **7b** is shown in the ORTEP (Fig. 2). The heterocyclic thiazine ring adopts a twist chair conformation with atoms S1 and C6 displaced by  $0.732(2)$  and  $-0.245(3)\text{ \AA}$ , respectively, from the mean plane formed by the remaining ring atoms (N1//C1/C7/C8). The mean planes of benzene rings C1—C6 and C20—C25 make dihedral angles  $22.94(8)^\circ$  and  $9.90(10)^\circ$ , respectively, with the mean plane of the pyrazolyl ring (N2/N3/C7/C8/C10). The acetamide chain (O3/C12/N4/N5/C13) is fully extended. The crystal data and structure refinement parameters are described in Table 4.

#### Virology

##### Anti-HIV-1 activity

The ability of these *N'*-substitutedbenzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-

**Scheme 2** Layout for microwave-assisted synthesis of the titled compounds (**7a–u**)

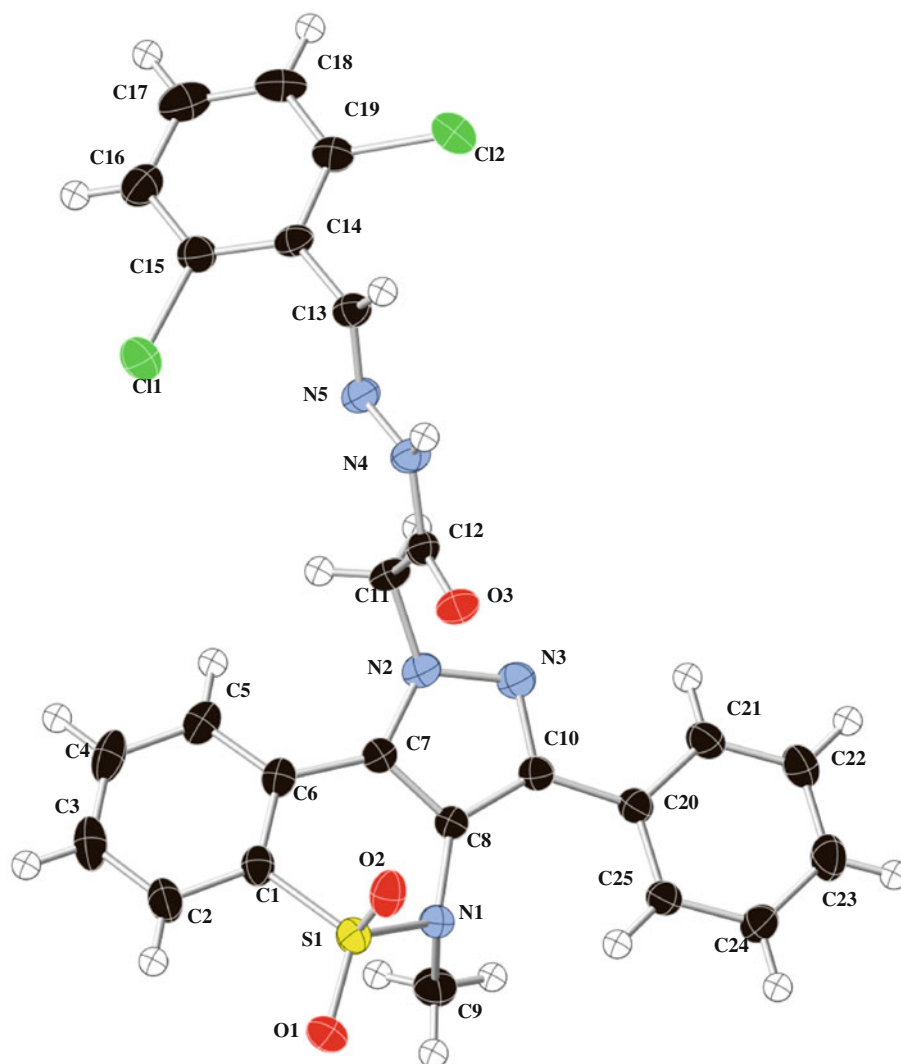


**Chemical and Reagents:** (i) alkyl chloroacetates, K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>CN (ii) hydrazine monohydrate, EtOH (iii) RCHO, EtOH, 0.2 mL acetic acid

**Table 3** Synthesis of *N'*-substitutedbenzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4*H*)-yl)acetohydrazide (**7a–u**) under microwave irradiation, ultrasonic irradiation, and thermal reaction

Entry	Products	<i>R'</i>	Conventional thermal method		Microwaves irradiation		Ultrasonic bath	
			Reaction time (min)	Yield (%)	Reaction time (min)	Yield (%)	Reaction time (min)	Yield (%)
1	<b>7a</b>	4-Bromophenyl	26	81	3	83	4	79
2	<b>7b</b>	2,6-Dichlorophenyl	25	82	2	84	4	79
3	<b>7c</b>	4-Chlorophenyl	20	81	2.5	87	4	84
4	<b>7d</b>	4-Dimethyl aminophenyl	22	80	2	82	5	82
5	<b>7e</b>	2-Nitrophenyl	17	79	2.5	85	4	81
6	<b>7f</b>	3-Nitrophenyl	20	80	2	86	6	83
7	<b>7g</b>	4-Diethyl aminophenyl	21	80	2.5	85	5	85
8	<b>7h</b>	2,4-Dichlorophenyl	24	81	3	85	4	83
9	<b>7i</b>	2,3,4-Trimethoxyphenyl	21	80	2	90	4	89
10	<b>7j</b>	4-Methoxyphenyl	22	82	3.5	90	3	87
11	<b>7k</b>	2,4-Dimethoxyphenyl	23	85	4.5	87	3	81
12	<b>7l</b>	3-Ethoxy-4-hydroxyphenyl	30	80	3.5	81	7	79
13	<b>7m</b>	2,4-Dihydroxyphenyl	28	77	2.5	80	7	78
14	<b>7n</b>	2-Fluorophenyl	20	79	3.5	82	8	80
15	<b>7o</b>	3,4,5-Trimethoxyphenyl	18	82	3.5	84	8	80
16	<b>7p</b>	2-Bromophenyl	17	82	2.5	85	6	83
17	<b>7q</b>	4-Acetamidophenyl	24	80	3	86	5	81
18	<b>7r</b>	3,4-Dichlorophenyl	20	80	3.5	84	6	80
19	<b>7s</b>	4-Nitrophenyl	21	79	3	81	5	80
20	<b>7t</b>	3-Chlorophenyl	19	80	2.5	84	6	81
21	<b>7u</b>	3-Bromophenyl	20	79	2.5	83	4	82

**Fig. 2** ORTEP diagram for *N'*-(2,6-dichlorobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazide (**7b**)



1(4*H*)-yl)acetohydrazides (**7a–u**) to inhibit viral growth in activated primary human peripheral blood mononuclear (PBM) cells was determined by screening against HIV-1 according to previously reported methodology (Schinazi *et al.* 1990). The results obtained for these compounds are summarized in Table 5. The data were compared with the antiviral activity of 3'-azido-3'-deoxythymidine (AZT), a potent clinical anti-HIV nucleoside reverse transcriptase inhibitor (NRTI). From the results of the anti-HIV assay, it is difficult to establish an obvious structure activity relationship (SAR) for the final compounds. Two compounds, **7l** and **7m**, exhibited the most significant anti-HIV-1 activity with  $EC_{50}$  values 5.4 and 3.3  $\mu$ M, respectively, while **7j** showed moderate anti-HIV-1 activity with an  $EC_{50}$  value 17.1  $\mu$ M. The SAR was established with the help of molecular docking.

#### Cytotoxic activity

The final compounds were also tested for their cytotoxicity in primary human PBM, CEM, and Vero cells as described by Stuyver *et al.*, 2002. The results of cell-based assays are expressed as  $IC_{50}$  (median inhibitory concentration) (Table 5). Out of the 21 *N'*-substitutedbenzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazides evaluated (**7a–u**), six (**7b**, **7f**, **7h**, **7k**, **7o**, and **7s**) showed significant nontoxic behavior in all three cell lines used i.e., primary human PBM, CEM, and Vero cells, while **7e**, **7g**, and **7i** were nontoxic to primary human PBM and CEM cells. Moreover, compounds **7n**, **7p**, **7q**, and **7t** were nontoxic in primary human PBM and Vero cells. Compound **7j**, which is moderately active for anti-HIV-1 activity, is nontoxic to human PBM cells. The nontoxic behavior in

**Table 4** Crystal data and structure refinement for *N'*-(2,6-dichlorobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazide (**7b**)

Parameters	Values	Parameters	Values
CCDC number	915138	Theta range for data collection	1.96°–27.67°
Empirical formula	C <sub>25</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>5</sub> O <sub>3</sub> S	Index ranges	−10 ≤ <i>h</i> ≤ 10, −14 ≤ <i>k</i> ≤ 14, −19 ≤ <i>l</i> ≤ 19
Formula weight	540.41	Reflections collected	9869
Temperature	173(2) K	Independent reflections	5385 [ <i>R</i> (int) = 0.029]
Wavelength	0.71073 Å	Completeness to theta = 27.67°	98.2 %
Crystal system	Triclinic	Absorption correction	multiscan
Space group	<i>P</i> −1	Max. and min. transmission	0.980 and 0.916
Unit cell dimensions	<i>a</i> = 7.8666(3) Å, <i>α</i> = 89.500(2)° <i>b</i> = 10.8263(5) Å, <i>β</i> = 75.029(2)° <i>c</i> = 14.7797(6) Å, <i>γ</i> = 74.588(2)°	Refinement method	Full-matrix least-squares on <i>F</i> <sup>2</sup>
Volume	1169.85(8) Å <sup>3</sup>	Data/restraints/parameters	5385/0/329
Z	2	Goodness-of-fit on <i>F</i> <sup>2</sup>	1.10
Density (calculated)	1.534 Mg/m <sup>3</sup>	Final <i>R</i> indices [ <i>I</i> > 2σ( <i>I</i> )]	<i>R</i> <sup>1</sup> = 0.050, <i>wR</i> <sup>2</sup> = 0.103
Absorption coefficient	0.407 mm <sup>−1</sup>	<i>R</i> indices (all data)	<i>R</i> <sup>1</sup> = 0.060, <i>wR</i> <sup>2</sup> = 0.110
<i>F</i> (000)	556	Largest diff. peak and hole	0.35 and −0.37 e·Å <sup>−3</sup>
Crystal size	0.22 × 0.22 × 0.05 mm <sup>3</sup>		

**Table 5** Anti-HIV-1 and cytotoxicity screening of *N'*-substituted benzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazides (**7a–u**)

Anti- HIV-1 activity in primary human PBM cells (μM)				Cytotoxicity (IC <sub>50</sub> , μM)		
Compound Codes	<i>R'</i>	EC <sub>50</sub>	EC <sub>90</sub>	PBM	CEM	Vero
<b>7a</b>	4-Bromophenyl	>100	>100	97.0	46.9	36.4
<b>7b</b>	2,6-Dichlorophenyl	>100	>100	>100	78.6	>100
<b>7c</b>	4-Chlorophenyl	>100	>100	>100	44.9	20.5
<b>7d</b>	4-Dimethylaminophenyl	>100	>100	14.4	>100	>100
<b>7e</b>	2-Nitrophenyl	>100	>100	>100	88.9	45.8
<b>7f</b>	3-Nitrophenyl	>100	>100	>100	99.6	>100
<b>7g</b>	4-Diethylaminophenyl	>100	>100	>100	>100	23.5
<b>7h</b>	2,4-Dichlorophenyl	>100	>100	>100	>100	88.9
<b>7i</b>	2,3,4-Trimethoxyphenyl	>100	>100	>100	>100	19.6
<b>7j</b>	4-Methoxyphenyl	17.1	53.9	>100	20.7	15.4
<b>7k</b>	2,4-Dimethoxyphenyl	99.8	>100	>100	>100	>100
<b>7l</b>	3-Ethoxy-4-hydroxyphenyl	5.4	15.5	8.7	<1	9.2
<b>7m</b>	2,4-Dihydroxyphenyl	3.3	5.9	7.8	<1	4.3
<b>7n</b>	2-Fluorophenyl	>100	>100	>100	23.4	>100
<b>7o</b>	3,4,5-Trimethoxyphenyl	>100	>100	>100	75.4	>100
<b>7p</b>	2-Bromophenyl	>100	>100	>100	36.6	>100
<b>7q</b>	4-Acetamido phenyl	>100	>100	>100	44.6	≥100
<b>7r</b>	3,4-Dichlorophenyl	>100	>100	38.0	19.3	>100
<b>7s</b>	4-Nitrophenyl	>100	>100	>100	>100	>100
<b>7t</b>	3-Chlorophenyl	>100	>100	51.6	12.1	78.5
<b>7u</b>	3-Bromophenyl	>100	>100	31.8	17.6	>100
<b>AZT</b>		0.00037	0.014	>100	14.3	56.0

Where EC<sub>50</sub> and IC<sub>50</sub> are the median effective (antiviral) and inhibitory (cytotoxic) concentrations, respectively. All experiments were conducted in replicate



**Table 6** The interactions of compounds with HIV-1 reverse transcriptase

Compound	EC <sub>50</sub> (μM)	S score	Interacting residues
<b>7l</b>	5.4	−10.41616	Ser 105
<b>7m</b>	3.3	−10.16434	Lys 102
<b>7j</b>	17.1 μM	−9.33224	Thr 107
<b>AZT</b>		−16.21676	Glu28, Lys32, Pro140, Lys103

human cell lines supports the potential of these compounds for their medicinal use.

### Molecular Docking

The mechanism of anti-HIV activity of these compounds was attempted by in silico investigation of active compounds. The compounds (**7m**, **7l**, and **7j**) which showed the most significant anti-HIV activity were docked into the HIV-1 reverse transcriptase crystal structure. The HIV-1 reverse transcriptase structure was downloaded from the PDB database using ID: 1C1B and was docked with active compounds. After docking, the top conformations were selected on the basis of the minimum S-score and were analyzed to find potential interactions among compounds and HIV-1 to understand the mechanism of inhibition. Compound **7l** was ranked as the top conformation, followed sequentially by **7m** and **7j**. Compounds **7l** and **7m** exhibited the most significant anti-HIV-1 activity, while **7j** exhibited lower anti-HIV-1 activity with EC<sub>50</sub> value 17.1 μM. Compound **7l**, which had significant anti-HIV-1 activity (EC<sub>50</sub> = 5.4 μM), was also ranked as the top conformation in docking results, followed in order by **7m** and **7j**. The residue interactions of these compounds with HIV-1 Reverse Transcriptase are listed in Table 6. AZT, which is a potent anti-HIV drug, was also docked with this binding pocket of HIV-1 reverse transcriptase to validate the docking procedure. These three compounds and AZT bound efficiently with the NTP-binding pocket of HIV-1 reverse transcriptase.

### Interaction analysis

After postdocking analysis, it was found that the compound **7l** had strong interactions with Ser 105 and also had close contact with other pocket residues Lys102, Lys 103, Lys104, Val 106, Asp 192, Ile195, Pro236, Tyr318, Tyr319, Asp 320, Pro321, Lys 323, and Glu344 of the HIV reverse transcriptase. Interactions are shown in Fig. 3a. Compound **7m** had strong interaction with Lys 102 and also had contact with residues Ly103, Lys104, Tyr319, Val317, Tyr318, Asp 320, Pro321, Lys323, and Glu344 (Fig. 3b). Compound **7j** had the strong interactions with Thr107 and also had contact with Ly102, Lys103, Lys104,

Ser105, Val106, Asp192, Ile195, Pro236, Asp237, and Asp320 (Fig. 3c). 3D images of **7m**, **7l**, and **7j** docked in the NTRI-binding site are shown in Fig. 4. Docking of AZT with HIV-1 reverse transcriptase showed that it had the interactions with Glu28, Lys32, Pro140, and Lys103 while also having contact with Ile31, Lys101, Lys102, Ile132, Pro133, Ser134, Thr139, Gly141, Asp177, Val179, Asp192, and Pro321 (Fig. 5).

### Conclusion

In short, we report an efficient synthesis of new *N'*-substituted benzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazides (**7a–u**). Furthermore, 4-methyl-3-phenyl-2,4-dihydrobenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazine 5,5-dioxide (**4**) was efficiently synthesized in high yield from low cost starting materials using microwave irradiation. Two compounds **7l** and **7m** emerged as potent anti-HIV-1 agents with EC<sub>50</sub> values of 5.4 and 3.3 μM, respectively, while **7j** showed moderate activity with an EC<sub>50</sub> value of 17.1 μM. Notably, six compounds (**7b**, **7f**, **7h**, **7k**, **7o**, and **7s**) showed no toxicity in the three cell lines used. Compound **7j** showed no toxicity in human PBM cells and thus could have medicinal use. The mechanism of HIV inhibition was studied through molecular docking, and thus, a rationale for the efficacy of three lead compounds can be provided. This provides a valuable basis for further SAR-driven optimization.

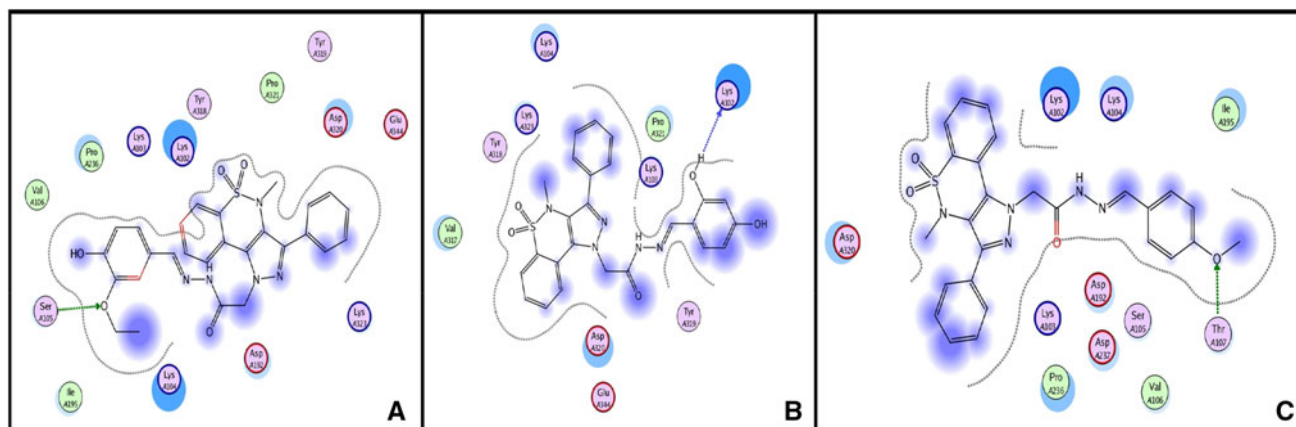
### Experimental

All the chemicals were purchased from E.Merck, Sigma/Aldrich or Fluka and used without purification. General melting points were obtained on Gallenkamp melting point apparatus and were uncorrected. IR spectra were recorded in KBr pellets on Perkin Elmer infrared spectrophotometer. <sup>1</sup>H NMR spectra were recorded in DMSO-*d*<sub>6</sub> on Bruker NMR (400 MHz), and TMS was used as internal standard (chemical shifts, δ in ppm). Mass spectra were recorded on a Jeol MS Route instrument. Ultrasonic-mediated reactions were carried out in Clifton Ultrasonic Bath (2 9 T2A, 300W, DU-4) made by Nickel Electro Ltd.

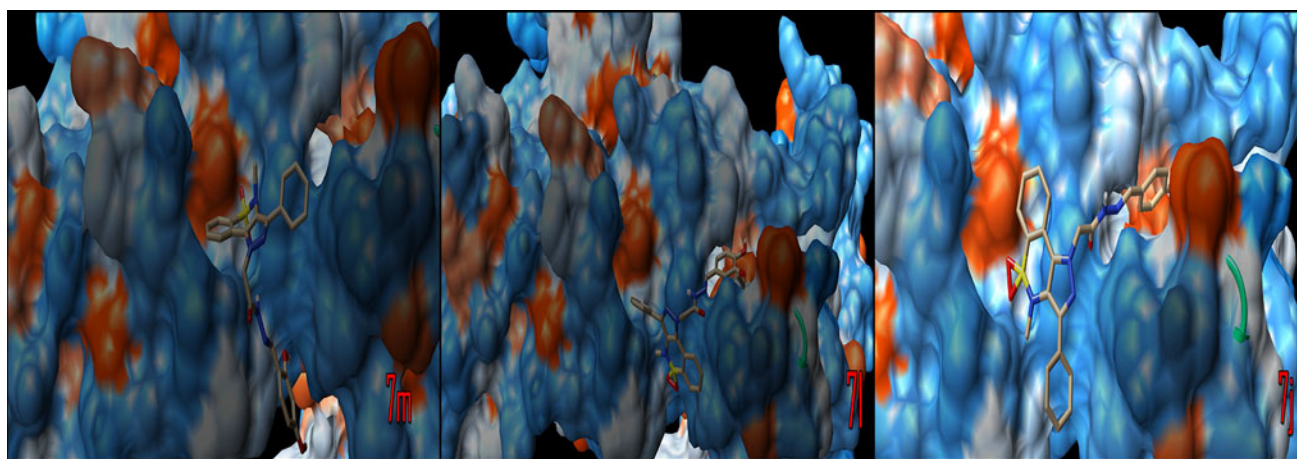
General procedure for the synthesis of 2-(2-oxo-2-phenylethyl)-1,2-benzisothiazol-3(2*H*)-one-1,1-dioxide (**1**)

Phenacyl bromide (0.222 mol) was added to the oven-dried sodium saccharine (45.55 g, 0.222 mol) using DMF (20 mL) as reaction medium. The reaction was completed in 25 min under microwave irradiation of 30 W. Then, the reaction mixture was cooled to ambient temperature and poured onto crushed ice. The resulting white precipitates

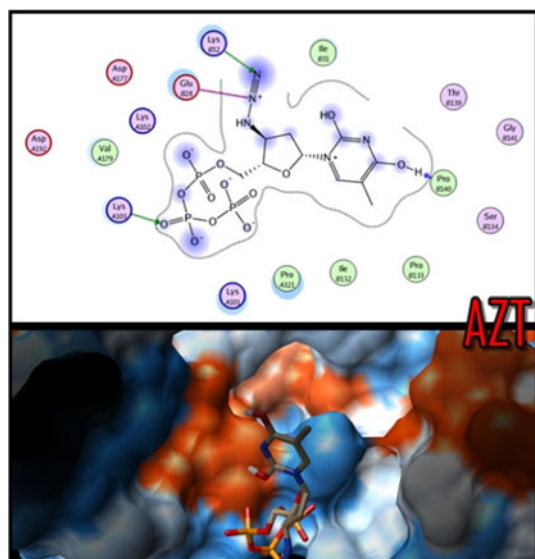




**Fig. 3** The binding interactions of compounds **7l**, **7m**, and **7j**, respectively, with HIV-1 reverse transcriptase



**Fig. 4** 3D binding interaction of **7l**, **7m**, and **7j** with HIV-1 reverse transcriptase



**Fig. 5** Interactions of AZT bound to HIV-1 reverse transcriptase

were filtered, washed with water, and recrystallized from ethanol affording pure **1** (87 %). FT-IR (KBr)  $\nu_{\text{max}}$ : 1715; 1345; 1165  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: (DMSO- $d_6$ , 400 MHz)  $\delta$ : 5.15 (2H, s, N-CH<sub>2</sub>), 7.47 (2H, t,  $J$  = 7.4 Hz, Ar-H), 7.59 (1H, t,  $J$  = 7.4 Hz, Ar-H), 7.81–7.90 (2H, m, Ar-H), 7.92 (1H, d,  $J$  = 6.6 Hz, Ar-H), 7.94 (2H, d,  $J$  = 6.8 Hz, Ar-H), 8.07 (1H, d,  $J$  = 7.8 Hz, Ar-H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 51.3 (CH<sub>2</sub>), 123.2 (Ar-C), 124.1 (Ar-C), 126.3 (Ar-C), 129.2 (2C, Ar), 129.4 (2C, Ar), 133.5 (Ar-C), 134.8 (Ar-C), 135.3 (Ar-C), 137.0 (Ar-C), 141.1 (Ar-C), 169.5 (C=O), 191.3 (Ar-CO); MS  $m/z$ : ES<sup>−</sup>; 301.1 (M<sup>+</sup>), +ES; 324.04 (M<sup>+</sup>+Na<sup>+</sup>).

General procedure for the synthesis of 3-benzoyl-4-hydroxy-2*H*-1,2-benzothiazine-1,1-dioxide (**2**)

Fresh sodium methoxide was first prepared by refluxing sodium metal (5.34 g, 0.232 mol) in 60 mL of freshly dried methanol under inert atmosphere maintained by N<sub>2</sub>

gas. Subsequently, *N*-phenacylsaccharin (**1**) (10.0 g, 0.232 mol) was added to the sodium methoxide in refluxing methanol. The reaction mixture immediately turned an orange–red color. The mixture was further refluxed for 20 min and then cooled to room temperature and poured into ice-cold 10 % HCl solution. The resulting white precipitates were filtered and washed with excess water and dried to afford **2** as a white crystalline solid (82 %). m.p. 156 °C FT-IR (KBr)  $\text{cm}^{-1}$  3353; 3133; 1625; 1358; 1157;  $^1\text{H}$  NMR: (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 5.79 (1H, s,  $\text{SO}_2\text{NH}$ ), 7.61 (1H, t,  $J = 7.8$  Hz, Ar-H), 7.71 (1H, d,  $J = 7.8$  Hz, Ar-H), 7.95–7.99 (5H, m, Ar-H), 8.15 (2H, t,  $J = 4.6$  Hz, Ar-H), 14.67 (1H, s, O-H);  $^{13}\text{C}$  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), 129.4 (2C, Ar), 132.1 (Ar-C), 132.3 (Ar-C), 134.0 (Ar-C), 137.1 (2C, Ar), 151.6 (C-4, thiazine), 191.1 (C=O); MS  $m/z$ : 301.07 [ $\text{M}^+$ ].

General procedure for the synthesis of 3-benzoyl-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-1,1-dioxide (**3**)

3-Benzoyl-4-hydroxy-2*H*-1,2-benzothiazine-1,1-dioxide (**2**) (10.0 g, 33.2 mmol) and aqueous sodium hydroxide (2.66 g in 5 mL water) were mixed in 50 mL of acetone. The mixture was allowed to be stirred at ambient temperature for 10 min. Dimethyl sulfate (8.06 mL, 64 mol) was added into the reaction mixture drop wise. The mixture was placed in ultrasonic media for 15 min at ambient temperature. Light yellow precipitates were formed on addition of cold dilute HCl (20 mL; 5 %), were filtered, washed with excess water, and then dried to afford **3** as a light yellow crystalline solid (85 %). m.p. 147–148 °C. IR (KBr)  $\text{cm}^{-1}$ : 3470; 2970; 1610; 1586; 1357; 1184;  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 2.89 (3H, s, N- $\text{CH}_3$ ), 7.59 (2H, t,  $J = 7.6$  Hz, Ar-H), 7.69 (1H, d,  $J = 7.8$  Hz, Ar-H), 7.73–7.77 (2H, dd,  $J_1 = 2.8$  Hz,  $J_2 = 8.4$  Hz, Ar-H), 7.94–7.97 (3H, m, Ar-H), 8.17 (1H, t,  $J = 4.6$  Hz, Ar-H), 14.69 (1H, s, O-H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 39.8 (N- $\text{CH}_3$ ), 121.9 (C-3, thiazine), 123.2 (Ar-C), 124.2 (Ar-C), 124.7 (Ar-C), 127.2 (Ar-C), 128.6 (Ar-C), 129.2 (Ar-C), 130.1 (Ar-C), 131.0 (Ar-C), 133.0 (Ar-C), 134.2 (Ar-C), 136.5 (Ar-C), 138.2 (Ar-C), 167.2 (C-4, thiazine), 190.0 (C=O); MS  $m/z$ : 315.34 [ $\text{M}^+$ ]. HR-MS (+ES): 315.0571 ( $\text{M}+\text{H}^+$ ) ( $\text{C}_{16}\text{H}_{13}\text{NO}_4\text{S}$ ).

General procedure for the synthesis of 4-methyl-3-phenyl-2,4-dihydrobenzo[*e*]pyrazolo [4,3-*c*][1,2]thiazine 5,5-dioxide (**4**)

A mixture of 3-benzoyl-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-1,1-dioxide (**3**) (5.0 g, 13.08 mmoles) and hydrazine monohydrate (4.9 mL, 99 mmol) in 50 mL ethanol was

refluxed in microwave oven at 30 W for 20 min. The unreacted hydrazine monohydrate and ethanol were removed under vacuum. The crude product was then dissolved in ice-cold water. The resulting precipitates were filtered and recrystallized from ethanol to afford pure **4** as an off-white crystalline solid (87 %). FT-IR (KBr)  $\text{cm}^{-1}$ : 3368; 2965; 1655; 1337; 1165;  $^1\text{H}$  NMR: (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.91 (3H, s, N- $\text{CH}_3$ ), 7.49 (1H, t,  $J = 7.8$  Hz, Ar-H), 7.61 (2H, d,  $J = 8.4$  Hz, Ar-H), 7.64–7.71 (1H, dd,  $J_1 = 1.2$  Hz,  $J_2 = 7.6$  Hz,  $J_3 = 11.2$  Hz,  $J_4 = 16.8$  Hz, Ar-H), 7.73–7.79 (1H, dd,  $J_1 = 1.5$  Hz,  $J_2 = 8.4$  Hz, Ar-H), 7.94–8.01 (3H, m, Ar-H), 8.17 (1H, d,  $J = 7.6$  Hz, Ar-H), 13.78 (1H, s, N-H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 39.9 (N- $\text{CH}_3$ ), 122.2 (Ar-C), 124.2 (Ar-C), 124.7 (Ar-C), 124.9 (Ar-C), 125.8 (Ar-C), 125.9 (Ar-C), 127.3 (Ar-C), 128.6 (Ar-C), 130.1 (Ar-C), 130.3 (Ar-C), 130.4 (Ar-C), 134.2 (Ar-C), 134.4 (Ar-C), 138.2 (Ar-C), 140.4 (N=C, Ar-C); MS  $m/z$ : 312.36 (ES+), HR-MS (+ES): 312.0801 ( $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$ ).

General procedure for the synthesis of alkyl 2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetate (**5**)

4-Methyl-3-phenyl-2,4-dihydrobenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazine 5,5-dioxide (**4**) (6.2 g, 0.020 mol) and anhydrous potassium carbonate (3.31 g, 0.024 mol) were mixed in acetonitrile (30 mL). Then, methyl chloroacetate (2.60 g, 0.024 mol)/ethyl chloroacetate (0.024 mol) was added and allowed to reflux for 8 h. The completion of reaction was checked by TLC. The solvent was removed under reduced pressure. The resulting residues were dissolved in cold water, and neutral pH was maintained by addition of dilute HCl to provide the dirty yellow crystalline product **5**.

Methyl 2-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetate (**5a**)

Yield: 89 %. FT-IR (KBr)  $\text{cm}^{-1}$ : 3078; 2965; 1685; 1339; 1275; 1168;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.91 (3H, s, N- $\text{CH}_3$ ), 3.27 (3H, s, O- $\text{CH}_3$ ), 5.77 (2H, s, N- $\text{CH}_2$ ), 7.44 (3H, t,  $J = 7.6$  Hz, Ar-H), 7.63–7.67 (1H, dd,  $J_1 = 2.4$  Hz,  $J_2 = 7.8$  Hz, Ar-H), 7.75–7.79 (1H, dd,  $J_1 = 3.2$  Hz,  $J_2 = 8.0$  Hz, Ar-H), 7.94–8.01 (2H, dt,  $J_1 = 2.8$  Hz,  $J_2 = 7.8$  Hz,  $J_3 = 14.8$  Hz, Ar-H), 8.2 (2H, d,  $J = 7.6$  Hz, Ar-H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 39.8 (N- $\text{CH}_3$ ), 51.4 (O- $\text{CH}_3$ ), 53.9 (N- $\text{CH}_2$ ), 122.3 (Ar-C), 124.2 (Ar-C), 124.7 (Ar-C), 124.9 (Ar-C), 125.9 (Ar-C), 126.0 (Ar-C), 127.2 (Ar-C), 128.6 (Ar-C), 130.2 (Ar-C), 130.4 (Ar-C), 130.6 (Ar-C), 132.4 (Ar-C), 134.29 (Ar-C), 134.41 (Ar-C), 139.0 (N=C, Ar-C), 167.8 (C=O); MS  $m/z$ : 383.06 ( $\text{M}^+$ ).

*Ethyl 2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetate (5b)*

Dirty yellow crystalline; FT-IR (KBr)  $\text{cm}^{-1}$ : 3082; 2959; 2901; 1681; 1344; 1272; 1161;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 1.09 (3H, t,  $J = 8.4$  Hz, C-CH<sub>3</sub>), 2.91 (3H, s, N-CH<sub>3</sub>), 3.96 (2H, q,  $J = 8.4$  Hz, O-CH<sub>2</sub>), 5.75 (2H, s, N-CH<sub>2</sub>), 7.41 (3H, t,  $J = 7.6$  Hz, Ar-H), 7.61–7.65 (1H, dd,  $J_1 = 2.8$  Hz,  $J_2 = 8.0$  Hz, Ar-H), 7.74–7.78 (1H, dd,  $J_1 = 3.2$  Hz,  $J_2 = 8.4$  Hz, Ar-H), 7.95–8.02 (2H, dt,  $J_1 = 2.8$  Hz,  $J_2 = 7.8$  Hz,  $J_3 = 14.8$  Hz, Ar-H), 8.25 (2H, d,  $J = 7.6$  Hz, Ar-H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 13.4 (CH<sub>3</sub>, ester), 39.8 (N-CH<sub>3</sub>), 51.1 (O-CH<sub>2</sub>), 53.9 (N-CH<sub>2</sub>), 122.2 (Ar-C), 124.3 (Ar-C), 124.8 (Ar-C), 124.9 (Ar-C), 125.8 (Ar-C), 126.0 (Ar-C), 127.2 (Ar-C), 128.62 (Ar-C), 130.2 (Ar-C), 130.4 (Ar-C), 130.8 (Ar-C), 132.6 (Ar-C), 134.2 (Ar-C), 134.4 (Ar-C), 139.0 (N=C, Ar-C), 167.8 (C=O); MS  $m/z$ : 397.96 ( $\text{M}^+$ ).

General procedure for the synthesis of 2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (6)

A mixture of methyl 2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetate (5a) (5.0 g, 13.04 mmol) and hydrazine monohydrate (4.9 mL, 99 mmol) in 50 mL absolute ethanol was refluxed for 4 h. The unreacted hydrazine monohydrate and ethanol were then removed under vacuum. The crude product was dissolved in ice-cold water, and the pH was maintained at about 5.0–5.5 by addition of dil. HCl. The resulting precipitates were filtered and finally dried to provide pure 6 (80 %). Reaction in an ultrasonic bath was complete in 30 min with 71 % yield, and reaction in a microwave oven was complete in 15 min with 75 % yield. FT-IR (KBr)  $\text{cm}^{-1}$ : 3468; 3010; 1675; 1337, 1165;  $^1\text{H}$  NMR: (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.90 (3H, s, N-CH<sub>3</sub>), 4.39 (2H, s, NH<sub>2</sub>), 5.78 (2H, s, N-CH<sub>2</sub>), 7.49 (3H, t,  $J = 7.6$  Hz, Ar-H), 7.59–7.61 (1H, dd,  $J_1 = 2.8$  Hz,  $J_2 = 8.4$  Hz, Ar-H), 7.71–7.73 (1H, dd,  $J_1 = 2.8$  Hz,  $J_2 = 8.4$  Hz, Ar-H), 7.83 (2H, t,  $J = 8.4$  Hz, Ar-H), 8.11 (2H, d,  $J = 7.8$  Hz, Ar-H) 11.94 (1H, br-s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 39.8 (N-CH<sub>3</sub>), 53.9 (N-CH<sub>2</sub>), 122.2 (Ar-C), 124.2 (Ar-C), 124.7 (Ar-C), 124.9 (Ar-C), 125.9 (Ar-C), 126.0 (Ar-C), 127.2 (Ar-C), 128.6 (Ar-C), 130.2 (Ar-C), 130.4 (Ar-C), 130.6 (Ar-C), 132.4 (Ar-C), 134.2 (Ar-C), 134.4 (Ar-C), 138.9 (N=C, Ar-C), 165.6 (C=O); MS  $m/z$ : 383.07 ( $\text{M}^+$ ).

General procedure for the synthesis of *N'*-substitutedbenzylidene-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (7a–u)

2-(4-Methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (6) and substituted

benzaldehyde were dissolved in equimolar quantity in ethanol followed by the addition of 0.2–0.5 mL of glacial acetic acid. The mixture was allowed to reflux for half to 1 h depending on the reactivity of aromatic aldehydes. The reaction was also carried out under microwave irradiation of 30 W or alternatively using an ultrasonic bath. The reaction parameters are described in Table 3. The resulting precipitates were filtered and washed with hot methanol and dried.

*N'-(4-Bromobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (7a)*

Off-white crystals; Yield: 83 %; m.p. 260 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3469; 3060; 2948; 1694; 1604; 1486; 1348; 1279; 1189; 1021; 754;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 5.87 (2H, s, N-CH<sub>2</sub>), 7.43 (1H, t,  $J = 7.6$  Hz, Ar-H), 7.53 (1H, t,  $J = 7.6$  Hz, Ar-H), 7.62 (1H, d,  $J = 8.8$  Hz, Ar-H), 7.67 (1H, d,  $J = 4$  Hz, Ar-H), 7.71–7.73 (3H, dd,  $J_1 = 2.8$  Hz,  $J_2 = 8.4$  Hz, Ar-H), 7.83 (2H, t,  $J = 8.4$  Hz, Ar-H), 7.96 (2H, t,  $J = 7.2$  Hz, Ar-H), 8.03 (2H, d,  $J = 8.0$  Hz, Ar-H), 8.69 (1H, s, CH), 11.94 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 53.65 (N-CH<sub>2</sub>), 122.3 (Ar-C), 123.80 (Ar-C), 124.6 (Ar-C), 125.0 (Ar-C), 125.4 (Ar-C), 125.77 (Ar-C), 128.6 (Ar-C), 129.0 (Ar-C), 129.2 (Ar-C), 129.8 (Ar-C), 130.0 (Ar-C), 130.2 (Ar-C), 130.7 (Ar-C), 130.8 (Ar-C), 131.4 (Ar-C), 131.5 (Ar-C), 131.8 (Ar-C), 132.0 (Ar-C), 133.5 (Ar-C), 135.1 (Ar-C), 142.5 (N=C, Ar-C), 146.9 (N=CH), 167.9 (C=O); MS  $m/z$ : 549.0 ( $\text{M}^+$ ), 551.0 ( $\text{M}^+ + 2$ ); Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>BrN<sub>5</sub>O<sub>3</sub>S: C, 54.55; H, 3.66; N, 12.72. Found: C, 54.63; H, 3.60; N, 12.68.

*N'-(2,6-Dichlorobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (7b)*

Off-white crystals; Yield: 84 %; m.p. 240 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3457; 3069; 2964; 1685; 1598; 1466; 1345; 1291; 1168; 1065; 747;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 5.79 (2H, s, N-CH<sub>2</sub>), 7.44 (2H, q,  $J = 7.2$  Hz, Ar-H), 7.53 (1H, t,  $J = 7.6$  Hz, Ar-H), 7.58 (2H, d,  $J = 8.0$  Hz, Ar-H), 7.75 (1H, t,  $J = 7.2$  Hz, Ar-H), 7.80 (1H, d,  $J = 7.6$  Hz, Ar-H), 7.85 (2H, d,  $J = 7.2$  Hz, Ar-H), 7.96–8.02 (3H, dd,  $J_1 = 7.2$  Hz,  $J_2 = 8.0$  Hz, Ar-H), 8.38 (1H, s, CH), 12.17 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 53.5 (N-CH<sub>2</sub>), 122.3 (Ar-C), 123.8 (Ar-C), 124.5 (Ar-C), 125.1 (Ar-C), 125.4 (Ar-C), 125.8 (Ar-C), 128.6 (Ar-C), 129.0 (Ar-C), 129.2 (Ar-C), 129.5 (Ar-C), 129.8 (Ar-C), 130.1 (Ar-C), 130.2 (Ar-C), 130.8 (Ar-C), 130.8 (Ar-C), 131.3 (Ar-C), 131.5 (Ar-C), 131.7 (Ar-C), 132.0 (Ar-C), 133.5 (Ar-C), 142.2 (N=C, Ar-C), 145.9 (N=CH), 168.8 (C=O);

MS  $m/z$ : 539.0 ( $M^+$ ); Anal. Calcd. for  $C_{25}H_{19}Cl_2N_5O_3S$  C, 55.56; H, 3.54; N, 12.96. Found: C, 55.50; H, 3.58; N, 12.90.

*N'*-(4-Chlorobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (**7c**)

Off-white powder; Yield: 87 %; m.p. 256 °C; FT-IR (KBr)  $cm^{-1}$ : 3467; 3061; 2949; 1684; 1614; 1486; 1345; 1274; 1182; 1021;  $^1H$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 5.88 (2H, s, N-CH<sub>2</sub>), 7.44 (1H, d,  $J = 7.2$  Hz, Ar-H), 7.51 (1H, q,  $J = 7.8$  Hz, Ar-H), 7.57 (3H, d,  $J = 8.8$  Hz, Ar-H), 7.74–7.80 (2H, dd,  $J_1 = 8.4$  Hz,  $J_2 = 16.4$  Hz, Ar-H), 7.89 (3H, d,  $J = 8.4$  Hz, Ar-H), 7.97 (2H, d,  $J = 7.2$  Hz, Ar-H), 8.00 (1H, d,  $J = 7.4$  Hz, Ar-H), 8.70 (1H, s, CH), 11.88 (1H, s, NH);  $^{13}C$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 53.6 (CH<sub>2</sub>), 122.4 (Ar-C), 123.9 (Ar-C), 124.8 (Ar-C), 125.3 (Ar-C), 126.0 (Ar-C), 126.7 (Ar-C), 128.6 (Ar-C), 128.8 (Ar-C), 129.2 (Ar-C), 129.8 (Ar-C), 130.1 (Ar-C), 130.7 (Ar-C), 130.8 (Ar-C), 131.3 (Ar-C), 131.5 (Ar-C), 131.8 (Ar-C), 132.5 (Ar-C), 135.9 (Ar-C), 137.4 (Ar-C), 137.8 (Ar-C), 142.5 (N=C, Ar-C), 146.9 (N=CH), 161.1 (C=O); MS  $m/z$ : 505.0 ( $M^+$ ); Anal. Calcd. for  $C_{25}H_{20}ClN_5O_3S$  C, 59.34; H, 3.98; N, 13.84. Found: C, 59.30; H, 3.86; N, 13.77.

*N'*-(4-(Dimethylamino)benzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo [4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (**7d**)

Dirty off-white powder; Yield: 82 %; m.p. 234 °C; FT-IR (KBr)  $cm^{-1}$ : 3422; 3082; 2968; 1685; 1531; 1467; 1344; 1287; 1184; 1021; 749;  $^1H$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 2.95 (6H, s, N-CH<sub>3</sub>), 5.81 (2H, s, N-CH<sub>2</sub>), 6.68–6.74 (2H, dd,  $J_1 = 11.6$  Hz,  $J_2 = 18.4$  Hz, Ar-H), 7.44 (1H, q,  $J = 9.2$  Hz, Ar-H), 7.53 (2H, t,  $J = 8.0$  Hz, Ar-H), 7.58 (2H, t,  $J = 6.8$  Hz, Ar-H), 7.84 (3H, t,  $J = 7.8$  Hz, Ar-H), 7.96–7.99 (2H, dd,  $J_1 = 2.4$  Hz,  $J_2 = 10.0$  Hz, Ar-H), 8.02 (1H, d,  $J = 8.0$  Hz, Ar-H), 8.14 (1H, s, CH), 11.60 (1H, s, NH);  $^{13}C$  NMR (DMSO- $d_6$ ): 38.7 (N-CH<sub>3</sub>), 40.2 (2C, N-CH<sub>3</sub>), 53.8 (CH<sub>2</sub>), 111.6 (Ar-C), 111.8 (Ar-C), 121.1 (Ar-C), 122.2 (Ar-C), 122.9 (Ar-C), 123.9 (Ar-C), 124.3 (Ar-C), 124.6 (Ar-C), 125.9 (Ar-C), 126.7 (Ar-C), 128.6 (Ar-C), 128.8 (Ar-C), 128.9 (Ar-C), 129.1 (Ar-C), 129.2 (Ar-C), 129.4 (Ar-C), 131.2 (Ar-C), 132.9 (Ar-C), 133.5 (Ar-C), 142.0 (N=C, Ar-C), 146.7 (N=CH), 151.5 (Ar-C), 168.1 (C=O); MS  $m/z$ : 514.0 ( $M^+$ ); Anal. Calcd. for  $C_{27}H_{26}N_6O_3S$  C, 63.02; H, 5.09; N, 16.33. Found: C, 63.08; H, 5.17; N, 16.30.

2-(4-Methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)-*N'*-(2-nitrobenzylidene)acetohydrazide (**7e**)

Dirty yellow powder; Yield: 85 %; m.p. 227 °C; FT-IR (KBr)  $cm^{-1}$ : 3427; 3082; 2970; 1655; 1569; 1519; 1443; 1343; 1206; 1184; 953; 747;  $^1H$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 5.89 (2H, s, N-CH<sub>2</sub>), 7.08–7.14 (2H, dd,  $J_1 = 8.4$  Hz,  $J_2 = 18.4$  Hz, Ar-H), 7.32–7.45 (3H, m, Ar-H), 7.50 (2H, t,  $J = 7.8$  Hz, Ar-H), 7.58 (1H, d,  $J = 7.2$  Hz, Ar-H), 7.68 (2H, t,  $J = 7.8$  Hz, Ar-H), 7.96 (2H, d,  $J = 9.2$  Hz, Ar-H), 8.03 (1H, d,  $J = 7.6$  Hz, Ar-H), 8.13 (1H, s, CH), 11.69 (1H, s, NH);  $^{13}C$  NMR (DMSO- $d_6$ ): 39.9 (N-CH<sub>3</sub>), 54.8 (CH<sub>2</sub>), 121.4 (Ar-C), 122.3 (Ar-C), 122.7 (Ar-C), 123.3 (Ar-C), 123.8 (Ar-C), 124.0 (Ar-C), 124.7 (Ar-C), 125.1 (Ar-C), 125.6 (Ar-C), 125.8 (Ar-C), 127.8 (Ar-C), 128.6 (Ar-C), 129.0 (Ar-C), 129.6 (Ar-C), 130.8 (Ar-C), 131.6 (Ar-C), 132.1 (Ar-C), 133.9 (Ar-C), 134.4 (Ar-C), 142.6 (N=C, Ar-C), 146.8 (N=CH), 148.8 (Ar-C), 169.7 (C=O); MS  $m/z$ : 516.0 ( $M^+$ ); Anal. Calcd. for  $C_{25}H_{20}N_6O_5S$  C, 58.13; H, 3.90; N, 16.27. Found: C, 58.17; H, 3.97; N, 16.19.

2-(4-Methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)-*N'*-(3-nitrobenzylidene)acetohydrazide (**7f**)

Off-white crystalline; Yield: 86 %; m.p. 204 °C; FT-IR (KBr)  $cm^{-1}$ : 3457; 3085; 2956; 1687; 1598; 1528; 1466; 1348; 1267; 1183; 1022; 734;  $^1H$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 5.94 (2H, s, N-CH<sub>2</sub>), 7.43 (1H, d,  $J = 9.2$  Hz, Ar-H), 7.53 (2H, t,  $J = 7.6$  Hz, Ar-H), 7.72 (2H, t,  $J = 7.8$  Hz, Ar-H), 7.82 (2H, t,  $J = 8.0$  Hz, Ar-H), 7.97 (2H, d,  $J = 7.2$  Hz, Ar-H), 8.03 (1H, t,  $J = 7.6$  Hz, Ar-H), 8.24 (1H, t,  $J = 8.4$  Hz, Ar-H), 8.30 (1H, d,  $J = 7.6$  Hz, Ar-H), 8.36–8.38 (1H, dd,  $J_1 = 2.4$  Hz,  $J_2 = 7.2$  Hz, Ar-H), 8.92 (1H, s, CH), 12.19 (1H, s, NH);  $^{13}C$  NMR (DMSO- $d_6$ ): 39.7 (N-CH<sub>3</sub>), 54.8 (CH<sub>2</sub>), 121.4 (Ar-C), 122.3 (Ar-C), 122.6 (Ar-C), 123.3 (Ar-C), 123.8 (Ar-C), 124.3 (Ar-C), 124.5 (Ar-C), 125.0 (Ar-C), 125.4 (Ar-C), 125.8 (Ar-C), 128.6 (Ar-C), 129.0 (Ar-C), 129.8 (Ar-C), 130.0 (Ar-C), 130.6 (Ar-C), 133.2 (Ar-C), 133.5 (Ar-C), 134.2 (Ar-C), 135.5 (Ar-C), 142.5 (N=C, Ar-C), 145.7 (N=CH), 148.2 (Ar-C), 168.6 (C=O); MS  $m/z$ : 516.0 ( $M^+$ ); Anal. Calcd. for  $C_{25}H_{20}N_6O_5S$  C, 58.13; H, 3.90; N, 16.27. Found: C, 58.11; H, 3.99; N, 16.19.

*N'*-(4-(Diethylamino)benzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (**7g**)

Bright yellow powder; Yield: 85 %; m.p. 245 °C; FT-IR (KBr)  $cm^{-1}$ : 3445; 3069; 2976; 1679; 1595; 1468; 1347;

1266; 1182; 1021; 766;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 1.14 (6H, t,  $J = 6.4$  Hz, C-CH<sub>3</sub>), 2.88 (3H, s, N-CH<sub>3</sub>), 3.44 (4H, q,  $J = 6.8$  Hz, C-CH<sub>2</sub>), 5.80 (2H, s, N-CH<sub>2</sub>), 6.69–6.74 (2H, dd,  $J_1 = 2.4$  Hz,  $J_2 = 7.2$  Hz, Ar-H), 7.44 (2H, d,  $J = 7.6$  Hz, Ar-H), 7.42–7.51 (2H, dd,  $J_1 = 2.0$  Hz,  $J_2 = 9.8$  Hz, Ar-H), 7.59 (2H, d,  $J = 8.8$  Hz, Ar-H), 7.84 (1H, t,  $J = 8.4$  Hz, Ar-H), 7.93–7.99 (4H, m, Ar-H), 8.44 (1H, s, CH), 11.49 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 12.4 (2C, C-CH<sub>3</sub>), 38.8 (N-CH<sub>3</sub>), 43.7 (2C, N-CH<sub>2</sub>), 53.8 (CH<sub>2</sub>), 110.9 (Ar-C), 120.2 (Ar-C), 120.7 (Ar-C), 122.1 (Ar-C), 122.8 (Ar-C), 123.9 (Ar-C), 124.3 (Ar-C), 124.6 (Ar-C), 125.8 (Ar-C), 126.6 (Ar-C), 128.6 (Ar-C), 128.9 (Ar-C), 129.0 (Ar-C), 129.2 (Ar-C), 129.4 (Ar-C), 129.8 (Ar-C), 130.3 (Ar-C), 130.8 (Ar-C), 133.5 (Ar-C), 142.8 (N=C, Ar-C), 146.3 (N=CH), 151.1 (Ar-C), 169.5 (C=O); MS  $m/z$ : 542.1 ( $\text{M}^+$ ); Anal. Calcd. for C<sub>29</sub>H<sub>30</sub>N<sub>6</sub>O<sub>3</sub>S C, 64.19; H, 5.57; N, 15.49. Found: C, 64.27; H, 5.44; N, 15.51.

*N'*-(2,4-Dichlorobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazide (**7h**)

Light yellow powder; Yield: 85 %; m.p. 212 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3447; 3078; 2960; 1682; 1599; 1467; 1343; 1274; 1155; 1050; 747;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.76 (3H, s, N-CH<sub>3</sub>), 5.45 (2H, s, N-CH<sub>2</sub>), 7.04 (1H, s, Ar-H), 7.45–7.47 (1H, dd,  $J_1 = 2.8$  Hz,  $J_2 = 8.4$  Hz, Ar-H), 7.58 (3H, d,  $J = 4.4$  Hz, Ar-H), 7.69 (2H, t,  $J = 3.2$  Hz, Ar-H), 7.83 (2H, t,  $J = 7.6$  Hz, Ar-H), 7.92 (1H, d,  $J = 7.6$  Hz, Ar-H), 7.97 (1H, d,  $J = 8.8$  Hz, Ar-H), 8.03 (1H, d,  $J = 7.6$  Hz, Ar-H), 8.31 (1H, s, CH), 11.94 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 53.5 (N-CH<sub>2</sub>), 121.5 (Ar-C), 122.3 (Ar-C), 122.7 (Ar-C), 124.5 (Ar-C), 125.1 (Ar-C), 125.4 (Ar-C), 125.9 (Ar-C), 128.6 (Ar-C), 129.0 (Ar-C), 129.2 (Ar-C), 129.5 (Ar-C), 129.8 (Ar-C), 130.1 (Ar-C), 130.2 (Ar-C), 130.8 (Ar-C), 131.3 (Ar-C), 131.5 (Ar-C), 131.7 (Ar-C), 132.0 (Ar-C), 133.5 (Ar-C), 142.2 (N=C, Ar-C), 145.9 (N=CH), 168.8 (C=O); MS  $m/z$ : 538.9 ( $\text{M}^+$ ); Anal. Calcd. for C<sub>25</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>3</sub>S C, 55.56; H, 3.54; N, 12.96. Found: C, 55.51; H, 3.65; N, 12.99.

2-(4-Methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)-*N'*-(2,3,4-trimethoxybenzylidene)acetohydrazide (**7i**)

Off-white powder; Yield: 90 %; m.p. 241 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3449; 3067; 2940; 1673; 1593; 1462; 1343; 1266; 1168; 1066; 741;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 3.88 (9H, s, O-CH<sub>3</sub>), 5.83 (2H, s, N-CH<sub>2</sub>), 6.86–6.93 (1H, dd,  $J_1 = 8.8$  Hz,  $J_2 = 18.4$  Hz, Ar-H), 7.53 (3H, t,  $J = \text{Hz}$ , Ar-H), 7.58 (1H, d,  $J = 4.8$  Hz, Ar-H), 7.65 (1H, d,  $J = 9.2$  Hz, Ar-H), 7.83–7.87 (2H, dd,

$J_1 = 5.2$  Hz,  $J_2 = 6.4$  Hz, Ar-H), 7.98 (2H, t,  $J = 7.2$  Hz, Ar-H), 8.01 (1H, d,  $J = 8.0$  Hz, Ar-H), 8.28 (1H, s, CH), 11.76 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 55.4 (CH<sub>2</sub>), 58.9 (O-CH<sub>3</sub>), 59.9 (O-CH<sub>3</sub>), 60.3 (O-CH<sub>3</sub>), 106.5 (Ar-C), 120.1 (Ar-C), 120.7 (Ar-C), 121.2 (Ar-C), 122.1 (Ar-C), 122.8 (Ar-C), 123.9 (Ar-C), 124.2 (Ar-C), 124.9 (Ar-C), 125.6 (Ar-C), 125.9 (Ar-C), 127.2 (Ar-C), 128.7 (Ar-C), 129.4 (Ar-C), 129.9 (Ar-C), 130.9 (Ar-C), 132.5 (Ar-C), 139.5 (Ar-C), 141.9 (N=C, Ar-C), 146.6 (N=CH), 149.9 (Ar-C), 150.2 (Ar-C), 167.9 (C=O); MS  $m/z$ : 561.4 ( $\text{M}^+$ ); Anal. Calcd. for C<sub>28</sub>H<sub>27</sub>N<sub>5</sub>O<sub>6</sub>S C, 59.88; H, 4.85; N, 12.47. Found: 59.80; H, 4.81; N, 12.59.

*N'*-(4-Methoxybenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazide (**7j**)

Off-white powder; 90 % Yield; m.p. 208 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3440; 3056; 2940; 1677; 1595; 1470; 1344; 1269; 1167; 1069; 746;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 3.79 (3H, s, O-CH<sub>3</sub>), 5.85 (2H, s, N-CH<sub>2</sub>), 6.99 (2H, d,  $J = 9.2$  Hz, Ar-H), 7.44 (1H, d,  $J = 7.6$  Hz, Ar-H), 7.53 (3H, t,  $J = 7.6$  Hz, Ar-H), 7.69 (2H, q,  $J = 8.8$  Hz, Ar-H), 7.84 (1H, t,  $J = 10.2$  Hz, Ar-H), 7.98 (3H, t,  $J = 7.2$  Hz, Ar-H), 8.03 (1H, d,  $J = 8.0$  Hz, Ar-H), 8.23 (1H, s, CH), 11.69 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 55.6 (N-CH<sub>2</sub>), 58.9 (O-CH<sub>3</sub>), 110.4 (Ar-C), 120.4 (Ar-C), 120.8 (Ar-C), 121.4 (Ar-C), 122.1 (Ar-C), 122.8 (Ar-C), 123.3 (Ar-C), 124.3 (Ar-C), 125.4 (Ar-C), 125.9 (Ar-C), 127.2 (Ar-C), 128.5 (Ar-C), 128.9 (Ar-C), 129.6 (Ar-C), 130.1 (Ar-C), 130.6 (Ar-C), 133.2 (Ar-C), 133.5 (Ar-C), 134.2 (Ar-C), 141.8 (N=C, Ar-C), 145.8 (N=CH), 159.8 (Ar-C), 168.1 (C=O); MS  $m/z$ : 501.4 ( $\text{M}^+$ ); Anal. Calcd. for C<sub>26</sub>H<sub>23</sub>N<sub>5</sub>O<sub>4</sub>S C, 62.26; H, 4.62; N, 13.96. Found: C, 62.30; H, 4.67; N, 13.89.

*N'*-(2,4-Dimethoxybenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazide (**7k**)

Light yellow powder; Yield: 87 %; m.p. 178 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3454; 3065; 2965; 1685; 1610; 1460; 1341; 1274; 1155; 1067; 746;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 3.79 (3H, s, O-CH<sub>3</sub>), 3.86 (3H, s, O-CH<sub>3</sub>), 5.82 (2H, s, N-CH<sub>2</sub>), 6.64 (1H, s, Ar-H), 7.34 (1H, d,  $J = 7.4$  Hz, Ar-H), 7.53 (3H, t,  $J = 8$  Hz, Ar-H), 7.72 (2H, t,  $J = 8.0$  Hz, Ar-H), 7.83 (2H, t,  $J = 8.8$  Hz, Ar-H), 7.96–8.01 (3H, dd,  $J_1 = 7.2$  Hz,  $J_2 = 12.4$  Hz, Ar-H), 8.34 (1H, s, CH), 11.69 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 55.4 (CH<sub>2</sub>), 59.4 (O-CH<sub>3</sub>), 59.9 (O-CH<sub>3</sub>), 106.9 (Ar-C), 120.1 (Ar-C), 120.7 (Ar-C), 121.2 (Ar-C), 122.1 (Ar-C), 122.8 (Ar-C), 123.8 (Ar-C), 124.2 (Ar-C), 124.9 (Ar-C), 125.7 (Ar-C), 127.2 (Ar-C),



128.6 (Ar–C), 129.0 (Ar–C), 129.4 (Ar–C), 130.2 (Ar–C), 130.9 (Ar–C), 132.5 (Ar–C), 133.5 (Ar–C), 142.0 (N=C, Ar–C), 146.4 (N=CH), 159.9 (Ar–C), 160.7 (Ar–C), 168.3 (C=O); MS  $m/z$ : 531.4 ( $M^+$ ); Anal. Calcd. for  $C_{27}H_{25}N_5O_5S$  C, 61.00; H, 4.74; N, 13.17. Found: C, 61.09; H, 4.82; N, 13.24.

*N'-(3-Ethoxy-4-hydroxybenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (7l)*

Off-white powder; Yield: 81 %; m.p. 196 °C; FT-IR (KBr)  $cm^{-1}$ : 3449; 3367; 3077; 2941; 1693; 1599; 1482; 1349; 1267; 1178; 1056; 743;  $^1H$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 1.48 (3H, t, C–CH<sub>3</sub>), 2.89 (3H, s, N–CH<sub>3</sub>), 4.89 (2H, q, O–CH<sub>2</sub>), 5.85 (2H, s, N–CH<sub>2</sub>), 7.38 (2H, t,  $J = 7.2$  Hz, Ar–H), 7.49 (3H, d,  $J = 7.6$  Hz, Ar–H), 7.53 (2H, t,  $J = 8.4$  Hz, Ar–H), 7.60 (1H, s, Ar–H), 7.67–7.85 (2H, dd,  $J_1 = 5.2$  Hz,  $J_2 = 6.4$  Hz, Ar–H), 7.96 (2H, d,  $J = 10.8$  Hz, Ar–H), 8.21 (1H, s, CH), 9.87 (1H, s, OH), 11.91 (1H, s, NH);  $^{13}C$  NMR (DMSO- $d_6$ ): 12.6 (CH<sub>3</sub>), 38.9 (N–CH<sub>3</sub>), 55.5 (N–CH<sub>2</sub>), 59.9 (O–CH<sub>2</sub>), 111.4 (Ar–C), 120.5 (Ar–C), 120.9 (Ar–C), 121.4 (Ar–C), 122.2 (Ar–C), 122.9 (Ar–C), 123.8 (Ar–C), 124.3 (Ar–C), 125.8 (Ar–C), 127.4 (Ar–C), 128.5 (Ar–C), 128.8 (Ar–C), 129.6 (Ar–C), 130.1 (Ar–C), 130.6 (Ar–C), 133.7 (Ar–C), 134.4 (Ar–C), 135.2 (Ar–C), 142.8 (N=C, Ar–C), 145.6 (N=CH), 147.8 (Ar–C), 150.3 (Ar–C), 168.8 (C=O); MS  $m/z$ : 531.1( $M^+$ ); Anal. Calcd. for  $C_{27}H_{25}N_5O_5S$  C, 61.00; H, 4.74; N, 13.17. Found: C, 61.09; H, 4.72; N, 13.22.

*N'-(2,4-Dihydroxybenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (7m)*

Off-white powder; Yield: 80 %; m.p. 201 °C; FT-IR (KBr)  $cm^{-1}$ : 3529; 3396; 3071; 2961; 1695; 1595; 1489; 1345; 1267; 1173; 1057; 740;  $^1H$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N–CH<sub>3</sub>), 5.84 (2H, s, N–CH<sub>2</sub>), 7.34 (2H, d,  $J = 7.6$  Hz, Ar–H), 7.51 (1H, s, Ar–H), 7.53 (2H, t,  $J = 7.8$  Hz, Ar–H), 7.75 (2H, t,  $J = 8.4$  Hz, Ar–H), 7.82–7.87 (2H, dd,  $J_1 = 7.6$  Hz,  $J_2 = 14.6$  Hz, Ar–H), 7.90 (1H, t,  $J = 8.8$  Hz, Ar–H), 7.96–8.03 (2H, dd,  $J_1 = 7.6$  Hz,  $J_2 = 12.6$  Hz, Ar–H), 8.43 (1H, s, CH), 9.89 (2H, s, OH), 11.89 (1H, s, NH);  $^{13}C$  NMR (DMSO- $d_6$ ): 38.9 (N–CH<sub>3</sub>), 53.5 (N–CH<sub>2</sub>), 120.2 (Ar–C), 121.2 (Ar–C), 122.3 (Ar–C), 123.7 (Ar–C), 124.5 (Ar–C), 125.4 (Ar–C), 125.8 (Ar–C), 127.2 (Ar–C), 128.6 (Ar–C), 128.9 (Ar–C), 129.5 (Ar–C), 129.8 (Ar–C), 130.0 (Ar–C), 130.7 (Ar–C), 131.3 (Ar–C), 132.5 (Ar–C), 133.5 (Ar–C), 134.1 (Ar–C), 142.2 (N=C, Ar–C), 146.6 (N=CH), 149.7 (Ar–C), 150.2 (Ar–C), 168.1 (C=O); MS  $m/z$ : 503.4 ( $M^+$ ); Anal. Calcd. for  $C_{25}H_{20}N_6O_5S$  C, 59.63; H, 4.20; N, 13.91. Found: C, 59.54; H, 4.12; N, 13.97.

*N'-(2-Fluorobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (7n)*

Off-white powder; Yield: 82 %; m.p. 213 °C; FT-IR (KBr)  $cm^{-1}$ : 3447; 3078; 2960; 1682; 1599; 1467; 1343; 1274; 1155; 1050; 747;  $^1H$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.89 (3H, s, N–CH<sub>3</sub>), 5.88 (2H, s, N–CH<sub>2</sub>), 7.26–7.30 (2H, m, Ar–H), 7.53 (2H, t,  $J = 7.6$  Hz, Ar–H), 7.58 (1H, d,  $J = 4.4$  Hz, Ar–H), 7.74 (3H, t,  $J = 7.8$  Hz, Ar–H), 7.85 (2H, d,  $J = 9.2$  Hz, Ar–H), 7.92 (1H, d,  $J = 7.4$  Hz, Ar–H), 7.97–8.02 (2H, dd,  $J_1 = 7.2$  Hz,  $J_2 = 13.2$  Hz, Ar–H), 8.31 (1H, s, CH), 11.99 (1H, s, NH);  $^{13}C$  NMR (DMSO- $d_6$ ): 38.9 (N–CH<sub>3</sub>), 53.5 (N–CH<sub>2</sub>), 122.4 (Ar–C), 123.9 (Ar–C), 124.8 (Ar–C), 125.5 (Ar–C), 125.9 (Ar–C), 126.8 (Ar–C), 128.7 (Ar–C), 128.9 (Ar–C), 129.3 (Ar–C), 129.8 (Ar–C), 130.2 (Ar–C), 130.8 (Ar–C), 130.9 (Ar–C), 131.3 (Ar–C), 131.5 (Ar–C), 131.8 (Ar–C), 132.2 (Ar–C), 133.0 (Ar–C), 137.4 (Ar–C), 142.3 (N=C, Ar–C), 143.5 (Ar–C), 146.9 (N=CH), 167.9 (C=O); MS  $m/z$ : 489.4( $M^+$ ); Anal. Calcd. for  $C_{25}H_{20}FN_5O_3S$  C, 61.34; H, 4.12; N, 14.31. Found: C, 61.39; H, 4.17; N, 14.27.

*2-(4-Methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)-N'-(3,4,5-trimethoxybenzylidene)acetohydrazide (7o)*

Off-white powder; Yield: 84 %; m.p. 238 °C; FT-IR (KBr)  $cm^{-1}$ : 3445; 3055; 2954; 1687; 1590; 1461; 1344; 1264; 1155; 1027; 746;  $^1H$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.89 (3H, s, N–CH<sub>3</sub>), 3.69 (3H, s, O–CH<sub>3</sub>), 3.81 (6H, s, O–CH<sub>3</sub>), 5.88 (2H, s, N–CH<sub>2</sub>), 7.04 (1H, s, Ar–H), 7.09 (1H, s, Ar–H), 7.54 (2H, t,  $J = 8.0$  Hz, Ar–H), 7.75 (2H, t,  $J = 8.0$  Hz, Ar–H), 7.86 (1H, d,  $J = 7.6$  Hz, Ar–H), 7.97 (2H, d,  $J = 7.2$  Hz, Ar–H), 8.02 (2H, t,  $J = 6.4$  Hz, Ar–H), 8.21 (1H, s, CH), 11.94 (1H, s, NH);  $^{13}C$  NMR (DMSO- $d_6$ ): 38.9 (N–CH<sub>3</sub>), 55.4 (CH<sub>2</sub>), 59.2 (O–CH<sub>3</sub>), 59.8 (O–CH<sub>3</sub>), 60.1 (O–CH<sub>3</sub>), 105.7 (Ar–C), 106.5 (Ar–C), 120.7 (Ar–C), 121.2 (Ar–C), 122.8 (Ar–C), 124.2 (Ar–C), 125.6 (Ar–C), 125.9 (Ar–C), 127.2 (Ar–C), 128.7 (Ar–C), 129.0 (Ar–C), 129.4 (Ar–C), 130.0 (Ar–C), 130.2 (Ar–C), 130.9 (Ar–C), 132.6 (Ar–C), 133.5 (Ar–C), 142.1 (N=C, Ar–C), 146.5 (N=CH), 158.0 (Ar–C), 159.7 (Ar–C), 160.1 (Ar–C), 168.0 (C=O); MS  $m/z$ : 561.5 ( $M^+$ ); Anal. Calcd. for  $C_{28}H_{27}N_5O_6S$  C, 59.88; H, 4.85; N, 12.47. Found: C, 59.80; H, 4.80; N, 12.40.

*N'-(2-Bromobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (7p)*

Off-white powder; Yield: 85 %; m.p. 256 °C; FT-IR (KBr)  $cm^{-1}$ : 3465; 3069; 2946; 1695; 1599; 1486; 1348; 1275;

1188; 1024; 754;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 5.89 (2H, s, N-CH<sub>2</sub>), 7.36–7.43 (2H, m, Ar-H), 7.54 (2H, t,  $J$  = 7.4 Hz, Ar-H), 7.59 (3H, d,  $J$  = 10.8 Hz, Ar-H), 7.69 (1H, d,  $J$  = 7.6 Hz, Ar-H), 7.83–7.85 (2H, m, Ar-H), 7.97 (1H, d,  $J$  = 7.6 Hz, Ar-H), 8.01 (1H, t,  $J$  = 8.0 Hz, Ar-H), 8.06–8.08 (1H, dd,  $J_1$  = 1.6 Hz,  $J_2$  = 7.6 Hz, Ar-H), 8.32 (1H, s, CH), 11.92 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 53.6 (N-CH<sub>2</sub>), 122.3 (Ar-C), 123.8 (Ar-C), 124.6 (Ar-C), 125.1 (Ar-C), 125.4 (Ar-C), 125.8 (Ar-C), 128.6 (Ar-C), 129.1 (Ar-C), 129.2 (Ar-C), 129.8 (Ar-C), 130.0 (Ar-C), 130.2 (Ar-C), 130.5 (Ar-C), 130.8 (Ar-C), 131.3 (Ar-C), 131.5 (Ar-C), 131.8 (Ar-C), 132.0 (Ar-C), 133.6 (Ar-C), 142.2 (N=C, Ar-C), 143.5 (Ar-C), 146.9 (N=CH), 167.9 (C=O); MS  $m/z$ : 549.4 ( $\text{M}^+$ ), 551.4 ( $\text{M}^+ + 2$ ); Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>BrN<sub>5</sub>O<sub>3</sub>S C, 54.55; H, 3.66; N, 12.72. Found: C, 54.52; H, 3.68; N, 12.85.

*4-((2-(2-(4-Methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetyl)hydrazono)methyl)benzamide (7q)*

Off-white powder; Yield: 86 %; m.p. 145 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3449; 3066; 2968; 1679; 1654; 1483; 1345; 1268; 1184; 1022; 747;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 5.84 (2H, s, N-CH<sub>2</sub>), 7.43 (2H, t,  $J$  = 7.6 Hz, Ar-H), 7.53 (3H, t,  $J$  = 7.6 Hz, Ar-H), 7.66 (4H, d,  $J$  = 9.8 Hz, Ar-H), 7.84 (2H, s, NH<sub>2</sub>), 7.98 (3H, t,  $J$  = 7.2 Hz, Ar-H), 8.04 (1H, d,  $J$  = 9.6 Hz, Ar-H), 8.22 (1H, s, CH), 11.80 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 39.7 (N-CH<sub>3</sub>), 55.6 (N-CH<sub>2</sub>), 118.8 (Ar-C), 120.4 (Ar-C), 120.8 (Ar-C), 121.4 (Ar-C), 122.1 (Ar-C), 122.8 (Ar-C), 123.4 (Ar-C), 123.8 (Ar-C), 124.3 (Ar-C), 125.7 (Ar-C), 125.9 (Ar-C), 127.8 (Ar-C), 128.5 (Ar-C), 128.9 (Ar-C), 129.0 (Ar-C), 129.8 (Ar-C), 130.6 (Ar-C), 133.2 (Ar-C), 133.5 (Ar-C), 134.2 (Ar-C), 141.8 (N=C, Ar-C), 145.7 (N=CH), 168.1 (C=O, hydrazide), 171.8 (C=O); MS  $m/z$ : 528.5 ( $\text{M}^+$ ); Anal. Calcd. for C<sub>26</sub>H<sub>22</sub>N<sub>6</sub>O<sub>4</sub>S C, 60.69; H, 4.31; N, 16.33. Found: C, 60.61; H, 4.38; N, 16.25.

*N'-(3,4-Dichlorobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (7r)*

Off-white powder; Yield: 84 %; m.p. 245 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3454; 3068; 2966; 1682; 1597; 1462; 1344; 1290; 1168; 1066; 746;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 5.91 (2H, s, N-CH<sub>2</sub>), 7.43 (1H, t,  $J$  = 7.6 Hz, Ar-H), 7.53 (2H, t,  $J$  = 7.6 Hz, Ar-H), 7.57 (1H, d,  $J$  = 4.4 Hz, Ar-H), 7.67 (1H, d,  $J$  = 3.2 Hz, Ar-H), 7.72–7.76 (2H, dd,  $J_1$  = 7.8 Hz,  $J_2$  = 9.2 Hz, Ar-H), 7.83 (2H, d,  $J$  = 8.4 Hz, Ar-H), 7.95–8.02 (1H, m, Ar-H),

8.06 (1H, s, Ar-H), 8.08 (1H, d,  $J$  = 5.8 Hz, Ar-H), 8.28 (1H, s, CH), 12.04 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 53.6 (CH<sub>2</sub>), 122.4 (Ar-C), 123.7 (Ar-C), 124.5 (Ar-C), 125.1 (Ar-C), 125.5 (Ar-C), 125.7 (Ar-C), 127.3 (Ar-C), 128.6 (Ar-C), 128.9 (Ar-C), 129.0 (Ar-C), 129.2 (Ar-C), 129.8 (Ar-C), 130.0 (Ar-C), 130.2 (Ar-C), 130.9 (Ar-C), 131.4 (Ar-C), 131.6 (Ar-C), 131.8 (Ar-C), 132.0 (Ar-C), 133.6 (Ar-C), 142.1 (N=C, Ar-C), 145.9 (N=CH), 168.8 (C=O); MS  $m/z$ : 539.5 ( $\text{M}^+$ ); Anal. Calcd. for C<sub>25</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>3</sub>S C, 55.56; H, 3.54; N, 12.96. Found: C, 55.63; H, 3.58; N, 12.88.

*2-(4-Methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)-N'-(4-nitrobenzylidene)acetohydrazide (7s)*

Off-white powder; Yield: 81 %; m.p. 243 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3452; 3085; 2952; 1689; 1597; 1525; 1460; 1349; 1267; 1186; 1026; 738;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.89 (3H, s, N-CH<sub>3</sub>), 5.93 (2H, s, N-CH<sub>2</sub>), 7.43 (1H, t,  $J$  = 7.2 Hz, Ar-H), 7.54 (2H, t,  $J$  = 7.6 Hz, Ar-H), 7.58 (1H, d,  $J$  = 4.8 Hz, Ar-H), 7.85 (2H, t,  $J$  = 7.8 Hz, Ar-H), 7.97 (2H, d,  $J$  = 7.2 Hz, Ar-H), 8.03 (2H, t,  $J$  = 7.6 Hz, Ar-H), 8.19 (1H, s, CH), 8.24 (3H, t,  $J$  = 7.8 Hz, Ar-H), 12.17 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 39.9 (N-CH<sub>3</sub>), 54.9 (CH<sub>2</sub>), 121.4 (Ar-C), 122.7 (Ar-C), 123.3 (Ar-C), 123.8 (Ar-C), 124.0 (Ar-C), 124.8 (Ar-C), 125.1 (Ar-C), 125.6 (Ar-C), 125.8 (Ar-C), 127.8 (Ar-C), 128.6 (Ar-C), 129.0 (Ar-C), 129.4 (Ar-C), 129.6 (Ar-C), 130.8 (Ar-C), 131.6 (Ar-C), 132.2 (Ar-C), 133.9 (Ar-C), 134.4 (Ar-C), 142.2 (N=C, Ar-C), 144.6 (Ar-C), 148.8 (N=CH), 169.8 (C=O); MS  $m/z$ : 516.4 ( $\text{M}^+$ ); Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>N<sub>6</sub>O<sub>5</sub>S C, 58.13; H, 3.90; N, 16.27. Found: C, 58.17; H, 3.97; N, 16.35.

*N'-(3-Chlorobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-1(4H)-yl)acetohydrazide (7t)*

Off-white powder; Yield: 84 %; m.p. 233 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3469; 3067; 2949; 1684; 1609; 1486; 1345; 1272; 1180; 1021; 738;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N-CH<sub>3</sub>), 5.91 (2H, s, N-CH<sub>2</sub>), 7.44 (2H, q,  $J$  = 8.4 Hz, Ar-H), 7.53 (2H, t,  $J$  = 8.0 Hz, Ar-H), 7.58 (2H, d,  $J$  = 4.8 Hz, Ar-H), 7.68–7.71 (2H, dd,  $J_1$  = 3.6 Hz,  $J_2$  = 7.6 Hz, Ar-H), 7.84 (1H, d,  $J$  = 4.8 Hz, Ar-H), 7.91 (1H, t,  $J$  = 8.0 Hz, Ar-H), 7.98 (2H, t,  $J$  = 8.4 Hz, Ar-H), 8.02 (1H, s, Ar-H), 8.08 (1H, s, CH), 11.99 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ): 38.9 (N-CH<sub>3</sub>), 53.5 (CH<sub>2</sub>), 122.3 (Ar-C), 123.9 (Ar-C), 124.8 (Ar-C), 125.3 (Ar-C), 125.9 (Ar-C), 126.6 (Ar-C), 128.6 (Ar-C), 128.8 (Ar-C), 129.2 (Ar-C), 129.8 (Ar-C), 130.1 (Ar-C), 130.7 (Ar-C), 130.8 (Ar-C), 131.4 (Ar-C), 131.5 (Ar-C), 131.8 (Ar-C), 132.5 (Ar-C), 132.9 (Ar-C), 137.5 (Ar-C), 142.3 (N=C, Ar-C),



143.5 (Ar–C), 146.9 (N=CH), 167.1 (C=O); MS  $m/z$ : 505.4( $M^+$ ); Anal. Calcd. for  $C_{25}H_{20}ClN_5O_3S$  C, 59.34; H, 3.98; N, 13.84. Found: C, 59.38; H, 3.87; N, 13.88.

*N'-(3-Bromobenzylidene)-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-*c*][1,2]thiazin-1(4*H*)-yl)acetohydrazide (7u)*

Off-white powder; Yield: 83 %; m.p. 251 °C; FT-IR (KBr)  $cm^{-1}$ : 3465; 3069; 2946; 1695; 1599; 1486; 1348; 1275; 1188; 1024; 754;  $^1H$ NMR (DMSO- $d_6$ ) (400 MHz)  $\delta$ : 2.88 (3H, s, N–CH<sub>3</sub>), 5.91 (2H, s, N–CH<sub>2</sub>), 7.39 (1H, t,  $J$  = 8.0 Hz, Ar–H), 7.45 (1H, t,  $J$  = 7.6 Hz, Ar–H), 7.53 (3H, t,  $J$  = 7.6 Hz, Ar–H), 7.58 (2H, d,  $J$  = 5.8 Hz, Ar–H), 7.75 (1H, dd,  $J_1$  = 4.8 Hz,  $J_2$  = 7.6 Hz, Ar–H), 7.90 (2H, d,  $J$  = 5.2 Hz, Ar–H), 7.95 (2H, t,  $J$  = 8.4 Hz, Ar–H), 8.02 (1H, s, Ar–H), 8.07 (1H, s, CH), 11.99 (1H, s, NH);  $^{13}C$  NMR (DMSO- $d_6$ ): 38.9 (N–CH<sub>3</sub>), 53.6 (N–CH<sub>2</sub>), 122.3 (Ar–C), 123.7 (Ar–C), 124.6 (Ar–C), 125.0 (Ar–C), 125.5 (Ar–C), 125.7 (Ar–C), 128.6 (Ar–C), 129.0 (Ar–C), 129.3 (Ar–C), 129.8 (Ar–C), 130.0 (Ar–C), 130.2 (Ar–C), 130.7 (Ar–C), 130.8 (Ar–C), 131.3 (Ar–C), 131.5 (Ar–C), 131.7 (Ar–C), 131.9 (Ar–C), 133.5 (Ar–C), 142.2 (Ar–C), 143.5 (N=C, Ar–C), 146.9 (N=CH), 167.8 (C=O); MS  $m/z$ : 549.3 ( $M^+$ ), 551.3 ( $M^+ + 2$ ); Anal. Calcd. for  $C_{25}H_{20}BrN_5O_3S$  C, 54.55; H, 3.66; N, 12.72. Found: C, 54.58; H, 3.60; N, 12.79.

### X-ray crystallographic studies

A colorless plate crystal of (7b) was coated with Paratone 8277 oil (Exxon) and mounted on a glass fiber. All measurements were made on a Nonius KappaCCD diffractometer with graphite monochromated Mo-K $\alpha$  radiation. Details of crystal data and structure refinement have been provided in Table 4. The data were collected at a temperature of 173(2) K using  $\omega$  and  $\phi$  scans, corrected for Lorentz and polarization effects and for absorption using multiscan method (Hooft, 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 nonhydrogen atoms were refined anisotropically. Hydrogen atoms were included at geometrically idealized positions and were not refined except for the amino H atom which was located from a difference map and was allowed to refine freely. 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 (all data) and 0.0919 (all data) and the goodness of fit,  $S$  = 1.079. The weighting scheme was based on counting statistics, and the final

difference Fourier map was essentially featureless. The Figure was 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 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<sub>50</sub>) 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 5.

### Cytotoxicity assay

Primary human PBM, CEM, and Vero cells were cultured in 96-well plates ( $5 \times 10^4$  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<sub>2</sub> for human PBM cells (Stuyver *et al.*, 2002). The results are summarized in Table 5.

### Molecular docking

The high resolution structure of HIV-1 Reverse Transcriptase was retrieved from the Protein data bank using accession no. 1C1B. The docking procedure was carried out using Molecular operating environment (MOE). The 3D structure was optimized by removing water molecules and then protonating the structure. Energy of the structure was minimized using parameters such as gradient: 0.05, Force Field: MMFF94X + Solvation, and Chiral Constraint: Current Geometry. The structures of ligands were drawn using ChemDraw software and were converted to 3D structures. Energy was minimized to optimize the structures using parameters such as gradient: 0.05, Force Field: MMFF94X, Chiral Constraint, and Current Geometry. The docking algorithm of MOE software was utilized to dock the compounds with binding pocket of HIV-1 reverse transcriptase. After docking, top-ranking conformations were selected on the basis of S-score for interaction analysis. Interaction analysis was done using Chimera structure visualization software.

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