Synthesis and Anticancer Activities of New Benzothiadiazinyl Hydrazinecarboxamides and Anilino[1,2,4]triazolo[1,5-*b*][1,2,4]thiadiazine 5,5-diones

Ahmed Kamal,[a,*] Y. V. V. Srikanth,[a] Md. Ashraf,[a] M. Naseer A. Khan,[a] Thokhir Basha Shaik,[a] Shasi V. Kalivendi,[a] Nitasha Suri[b] and A. K. Saxena[b]

[a] *Division of Organic Chemistry, Indian Institute of Chemical Technology, Hyderabad 500 607, India*

[b] *Pharmacology Division, Indian Institute of Integrative Medicine, Canal Road, Jammu, 180001, India*

Abstract: Two series of compounds (**5-14** and **15-23**) based on the scaffolds of 2-(1,1-dioxido-4-phenyl-4*H*-benzo[*e*][1,2,4]thiadiazin-3-yl)-*N*-(4-methoxyphenyl)hydrazinecarboxamide (**5**) and 2-((4-methoxyphenyl)amino)-10-phenyl-10*H*-benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4]thiadiazine 5,5-dioxide (**15**) respectively, were designed and synthesized. These compounds were tested for anticancer activity against various cancer cell lines including lung, ovary, prostate, breast and colon cancers. They exhibited moderate to good inhibitory activity against the above cell lines and compound **9** was found to be the most active one from these two series. Further studies showed that cancer cell growth inhibition by compounds **22** and **23** could be in part due to the inhibition of tubulin polymerization, with the IC_{50} values of 4.70 and 5.25 μM, respectively.

Keywords: Hydrazinecarboxamides, triazolo benzothiadiazines, anticancer activity, tubulin polymerization.

1. INTRODUCTION

The chemotherapy currently available for cancer treatment is associated with limitations including normal cell toxicity and tumor cell resistance. To overcome these challenges, new and effective cytotoxic agents with novel mode of action are urgently needed. Drug discovery and development of potent anticancer agents based on the lead candidates have attracted much attention of many medicinal chemists [1-2].

Benzene sulfonamides represent an important class of therapeutic agents in the current drug design and discovery efforts. Moreover, both aryl and hetero arylsulfonamides are known to possess anticancer activity and their mode of action is different from the traditional anticancer agents [3-5]. They act as carbonic anhydrase inhibitors [6], cyclooxygenase inhibitors [7] and arrests cell cycle in G1 phase [8], recently some compounds from this series have reached the clinical studies [9]. On the other hand, sulfonylurea derivative such as sulofenur (LY 186641) (**1**, Fig. **1**) has been clinically evaluated in several solid tumors models [10, 11] because of apparent lack of toxicity to proliferating normal tissues [12, 13]. 1,2,4 Benzothiadiazine 1,1-dioxide ring system and 10-hydroxy-3,10-dihydro-2*H*-benzo[*e*]imidazo[1,2-*b*][1,2,4]thidiazine 5,5 dioxide (**2**) exhibit potent anticancer and antiviral activities by inhibition of ribonucleotide reductase [14,15]. These benzothiadiazine analogs have shown to exhibit antitumor activity by inhibition of cyclin dependent kinases (CDKs) [16-18] and tubulin polymerization [19]. Various derivatives of 1,2,4-triazolo1,2,4-benzodithiazines (**3**) and 1,2,4-triazolo benzothiadiazines possess anticancer activity [20-24].

In the last decade triazole derivatives have attracted much interest for the development of potent anticancer agents [25-27]. 1,2,4-Triazole-3,5-diamine analog is reported as a novel and potent anticancer CDK inhibitor [28]. Further, triazole derivatives possess anticancer activity by inhibition of tubulin polymerization [29]. We have shown that various heterocyclic compounds including triazolobenzothiadiazine ring system are potential pharmacophores against various cancer cell lines [22-24, 30-34]. We were interested in exploring the alteration in benzodiathiazine ring system without changing the triazoloanilino moiety. In this report, we present the design, synthesis and *in vitro* evaluation of triazolobenzothiadiazine and hydrazinecarboxamides as anticancer agents based on our earlier studies [22-24] on this scaffold (Fig. **2**). Further, the effect of substitutents on the nitrogen of benzothiadiazine ring system and anilino ring has been investigated.

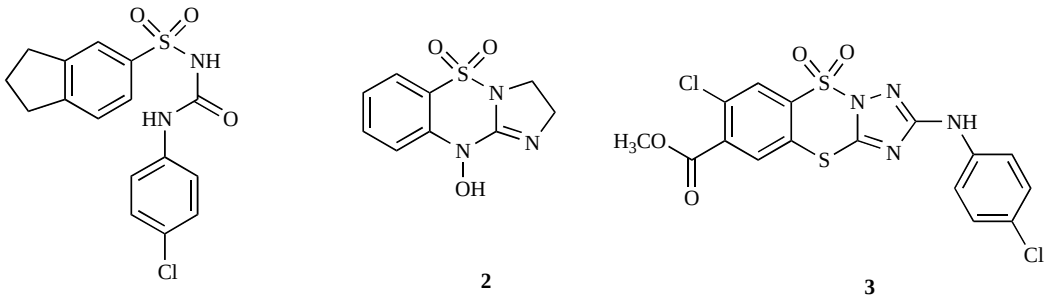
2. RESULTS AND DISCUSSION

2.1. Chemistry

The preparation of the starting materials, 3-hydrazino-4-methyl/phenyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**4a** and **b**) was accomplished by the synthetic sequences as previously reported [34] (Scheme **1**). The synthesis of *N*1-phenyl-2-(4-phenyl/methyl-1,1-dioxo-1,4-dihydro-1λ^6,2,4-benzothiadiazin-3-yl)-1-hydrazinecarboxamide (**5-14**) were obtained by reaction of **4a** and **b** with different aryl isocyanates in dry benzene at room temperature. Next, 2-anilino-10-phenyl/methyl-5,10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo [1,5-*b*][1,2,4]thiadiazine- 5,5-dione derivatives (**15-23**) were obtained by refluxing compounds **5-14** in phosphorous oxychloride [32] (Scheme **1**).

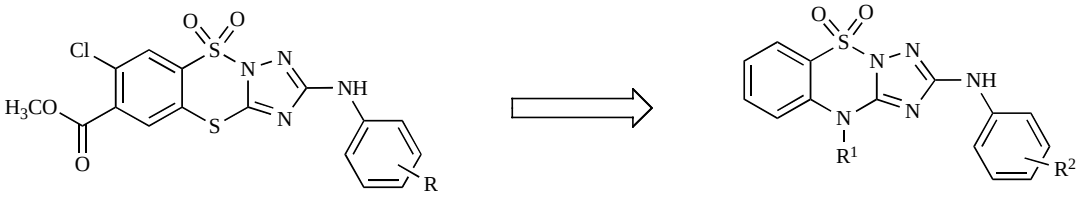
*Address correspondence to this author at the Division of Organic Chemistry, Indian Institute of Chemical Technology, Hyderabad 500 607, India; Tel: +91-40-27193157; Fax: +91-40-27193189;
E-mail: ahmedkamal@iict.res.in

1573-4064/11 $58.00+.00



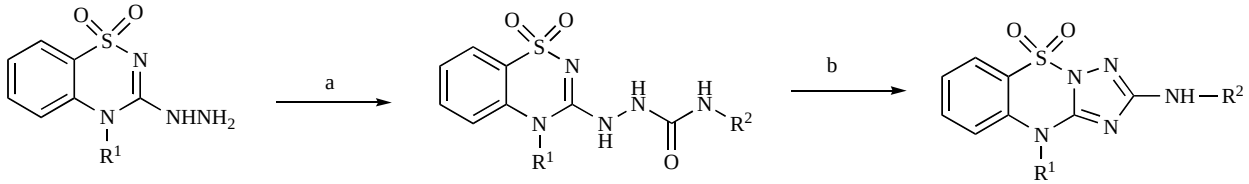
(**1, LY-186641**) **2** **3**

Fig. (1). Chemical structures of sulfonyl urea derivative (**1**, LY-186641), imidazo benzothiadiazine derivative (**2**) and triazolo dithiadiazine derivative (**3**).



3 **15-23**

Fig. (2). Design of triazolobenzothiadiazines.



4a: $R^1 = CH_3$
4b: $R^1 = Ph$ **5-14** **15-23**

Scheme1. Reagents and conditions: (a) aryl isocyanates, benzene, r.t.; (b) $POCl_3$, 110 ^{o}C, 3-4 h.

2.1. Anticancer Activity

All the synthesized compounds have been evaluated for their anticancer activity against 6 different human cancer cell lines (A-549, IGR-OV-1, PC-3, T-47-D, MCF-7, and CoLo-205. Overall, 10 hydrazinocarboxamide derivatives and 9 triazolobenzothiadiazine analogues were synthesized; their structures and corresponding activities are presented in Scheme **1**, Tables **1** and **2**. The test compounds were added at a single concentration (100 μM) and results for each compound were reported as percentage test cell growth inhibition compared with untreated control cells using sulforhodamine B (SRB) method [35]. Paclitaxel, mitomycin, adriamycin and 5-fluorouracil were used as positive controls in this assay. Some of the compounds have exhibited potential growth inhibitory effect in almost all the cell lines. Hydrazinocarboxamide analogues (**6**-**10**, **14**) displayed better growth inhibitory potency than their corresponding triazolobenzothiadiazine (**16**-**18**, **20**-**23**) analogues. However, compounds **5**, **11**-**13**, **15** and **19** did not show growth inhibitory effect (data not shown) at the concentration used in the assay. Compound **9** was found to be the most active from these series, which showed 99% growth inhibition against prostate cancer cell line and strong inhibition in most of the cell lines. Compound **8** and its corresponding triazolo analogue **18** also exhibited strong inhibitory effect (>80%) in most cases. Compounds with 4-methoxy substituent on phenyl rings of both triazolo (**5** and **15**) and carboxamido moieties (**11** and **20**) were found to be inactive. Interestingly, substitution of *meta* or *para* chloro on the phenyl ring has enhanced the inhibitory effect in compounds **6**, **16**, **7** and **17**, where R^1 = Ph, however, it showed less effect in compounds **12**, **21**, **13** and **22** where R^1 = Me. Similarly, 3,5-di(trifluoromethyl) substituents also increased the inhibitory effect in compounds **8** and **18** where R^1 = Ph and showed less effect in compounds **14** and **23**, where R^1 = Me.

2.2. Inhibition of Tubulin Polymerization

Since these new compounds possess a triazole moiety, it is interesting to investigate their effects on tubulin polymerization. One possible explanation of compounds showing anticancer activity is the inhibition of tubulin polymerization to form functional microtubules as it is observed with antimitotic agents such as nocodazole and colchicines. The polymerization of purified tubulin was investigated by monitoring the fluorescence at 360/420 nm excitation/emission using Varioscan multimode plate reader at 37 ^{o}C with and without the compounds at 3 μM (final concentration) which was diluted in PEM buffer from the stock solution concentration of 1mM in DMSO. Amongst the molecules examined, **22** and **23** suppressed the tubulin polymerization in comparison to the positive control compound (podophylltoxin). The compounds **22, 23** also demonstrated a dose

Table 1. Various Hydrazinocarboxamide and Triazolobenzothiadiazine Analogues (5-23)

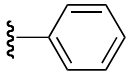
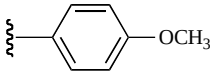
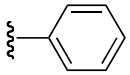
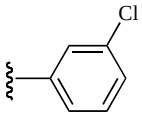
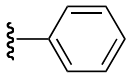
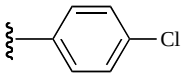
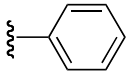
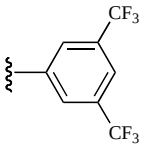
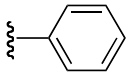
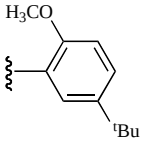
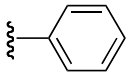
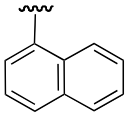
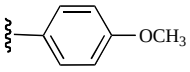
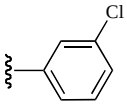
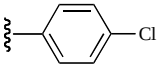
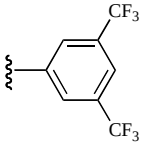
Compd	R^1	R^2
5, 15		
6, 16		
7, 17		
8, 18		
9		
10, 19		
11, 20	-CH_3	
12, 21	-CH_3	
13, 22	-CH_3	
14, 23	-CH_3	

Table 2. Percentage of Growth Inhibition for Selected Cancer Cell Lines by Hydrazinocarboxamide and Triazolobenzothiadiazine Compounds at 100 μM Concentrations

Compd	Lung A-549	Ovary IGR-OV-1	Prostate PC-3	Breast T-47-D	Breast MCF-7	Colon CoLo-205
6	80	65	72	55	74	74
7	73	56	72	55	64	62
8	80	83	95	53	81	94
9	91	71	99	79	90	87

Table 2. contd….

Compd	Lung A-549	Ovary IGR-OV-1	Prostate PC-3	Breast T-47-D	Breast MCF-7	Colon CoLo-205
10	-[a]	53	67	50	64	62
14	-[a]	52	-[a]	-[a]	-[a]	-[a]
16	77	50	72	56	74	-[a]
17	69	52	59	62	66	-[a]
18	84	88	82	64	85	94
20	-[a]	60	-[a]	-[a]	-[a]	-[a]
21	64	69	46	-	53	-
22	14	54	29	16	31	10
23	47	-	37	-	43	-
Paclitaxel[c]	61	63	-[b]	-[b]	-[b]	-[b]
Mitomycin[c]	-[b]	-[b]	67	-[b]	-[b]	-[b]
Adriamycin[d]	-[b]	-[b]	-[b]	71	63	-[b]
5-Fluorouracil[c]	-[b]	-[b]	-[b]	-[b]	-[b]	79

[a] Growth inhibition <50%, [b] not tested, [c] Inhibition at 10 μM concentration, [d] Inhibition at 1 μM concentration.

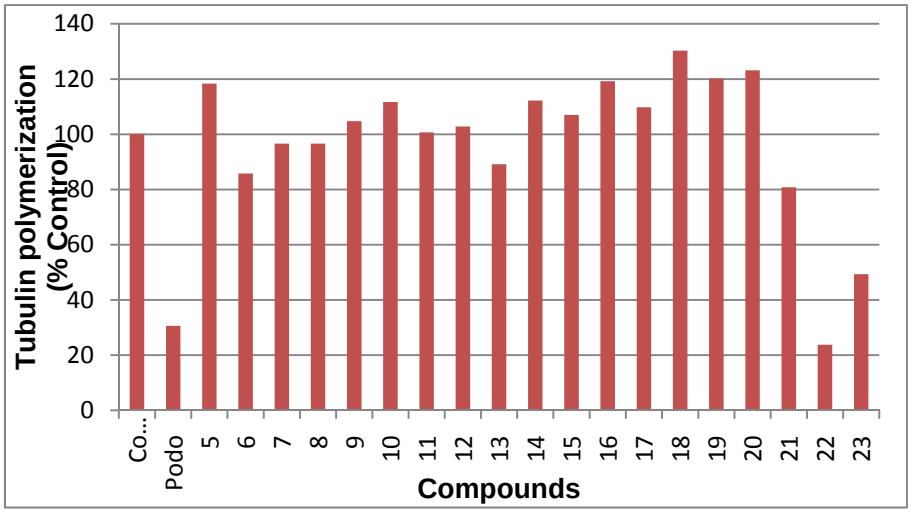


Fig. (3). Effect of compounds at 3 μM concentrations on tubulin polymerization.

dependent tubulin inhibition with IC_{50} values of 4.70 and 5.25 μM, respectively. Although compounds **8**, **9** and **18** exhibited strong growth inhibitory effect against most of the cancer cell lines, but were found to be inactive in possessing anti-tubulin activity. In contrast, compounds **22** and **23**, which showed better inhibitory effect on tubulin, were found to exhibit mild to moderate growth inhibitory effect on different cancer cell lines. Probably, the compounds **8**, **9** and **18** mediate cytotoxic effects by targeting pathways other than tubulin polymerization. Further structural modification of these lead compounds and studies on other targets are likely to provide new insights into the mechanism of cancer cell cytotoxicity by these compounds.

3. CONCLUSIONS

In summary, the synthesis and screening of anticancer activity for novel series of a *N*1-phenyl-2-(4-phenyl/methyl-1,1-dioxo-1,4-dihydro-1λ^6,2,4-benzothiadiazin-3-yl)-1-hydrazinecarboxamide (**5-14**) and 2-anilino-10-phenyl/methyl-5,10-dihydro-5 λ^6-benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4] thiadiazine-5,5-dione derivatives (**15-23**) were investigated. Compounds **6, 7, 8, 9, 18** showed higher potency against all the cell lines tested, and compound **9** was found to be the most active from this series. Compounds **22** and **23** have shown significant tubulin inhibitory activity. These preliminary results demonstrate that the potent compounds from these series may further be optimized for better anticancer leads.

4. EXPERIMENTAL SECTION

4.1. General

Chemistry: All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lanchester (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H and ^{13}C NMR spectra were recorded on Gemini Varian-VXR-unity (200, 400, 500 MHz) or Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI^{+} software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an Electrothermal melting point apparatus, and are uncorrected.

4.2. General Procedures

4.2.1. *N*1-(4-Methoxyphenyl)-2-(1,1-dioxo-4-phenyl-1,4-dihydro-1λ^{6},2,4-benzothiadiazin-3-yl)-1-hydrazinecarboxamide (5) The title compound was obtained from 3-hydrazino-4-phenyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**24**, 290 mg, 1 mmol) was taken in dry benzene to this 4-methoxy phenyl isocyanate (179 mg, 1.2 mmol) was slowly added and kept for overnight solid substance was formed filtered and washed with ether dried to form pure substances.

Yield: 363 mg, 83%; mp 181-184 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (bs, 2H); 8.17 (bs, 1H); 7.83 (d, *J* = 7.4 Hz,1H); 7.77-7.64 (m, 3H); 7.63-7.43 (m, 3H); 7.41-7.26 (m, 3H); 6.84 (d, *J* = 8.3 Hz, 2H); 6.32 (d, *J* = 8.3 Hz, 1H); 3.70 (s, 3H); LRMS (ESI) *m/z* 461 $[M+Na]^{+}$.

4.2.2. *N*1-(3-chlorophenyl)-2-(1,1-dioxo-4-phenyl-1,4-dihydro-1λ^{6},2,4-benzothiadiazin-3-yl)-1-hydrazinecarboxamide (6) The title compound was obtained from 3-hydrazino-4-phenyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**24**, 290 mg, 1 mmol) and 3-chloro phenyl isocyanate (184 mg, 1.2 mmol) as described for **5**.

Yield: 380 mg, 86%; mp 152-154 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (bs, 1H); 8.45 (bs, 1H); 7.83 (d, *J* = 7.2 Hz,1H); 7.77-7.64 (m, 5H); 7.60-7.45 (m, 3H); 7.37 (t, *J* = 7.2 Hz, 1H); 7.28 (t, *J* = 9.0 Hz, 1H); 7.06-6.97 (m, 1H); 6.33 (d, *J* = 9.0 Hz ,1H); LRMS(ESI) *m/z* 442 $[M^{+}]$.

4.2.3. *N*1-(4-Chlorophenyl)-2-(1,1-dioxo-4-phenyl-1,4-dihydro-1λ^{6},2,4-benzothiadiazin-3-yl)-1-hydrazinecarboxamide (7) The title compound was obtained from (**24**, 290 mg, 1 mmol) and 4-chloro phenyl isocyanate (184 mg, 1.2 mmol) as described for **5**.

Yield: 393 mg, 89%; mp 208-210 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.54 (bs, 1H); 8.36 (bs, 1H); 7.82 (d, *J* = 7.4 Hz, 1H); 7.77-7.63 (m, 2H); 7.63-7.42 (m, 5H); 7.41-7.21 (m, 4H); 6.32 (d, *J* = 8.5 Hz, 1H); LRMS (ESI) *m/z* 442 $[M^{+}]$.

4.2.4. *N*1-[3,5-Di(trifluoromethyl)phenyl]-2-(1,1-dioxo-4-phenyl-1,4-dihydro-1λ^{6},2,4-benzo thiadiazin-3-yl)-1-hydrazinecarboxamide (8) The title compound was obtained from (**24**, 290 mg, 1 mmol) and 3,5-di(trifluoromethyl) phenyl isocyanate (306 mg, 1.2 mmol) as described for **5**.

Yield: 441 mg 81%; mp 169-171 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (bs, 1H);8.14 (bs, 1H); 7.95 (dd, *J* = 7.5, 1.5 Hz, 1H); 7.81-7.70 (m, 4H); 7.61-7.54 (m, 2H); 7.48-7.31 (m, 3H); 6.49-6.43 (m, 2H); LRMS (ESI) *m/z* 544 $[M^{+}]$.

4.2.5. *N*1-[4-(*tert*-Butyl)-2-methoxyphenyl]-2-(1,1-dioxo-4-phenyl-1,4-dihydro-1λ^{6},2,4-benzo thiadiazin-3-yl)-1-hydrazinecarboxamide (9) The title compound was obtained from (**24**, 290 mg, 1 mmol) and 4-(*tert*-butyl)-2-methoxyphenyl isocyanate (205 mg, 1.2 mmol) as described for **5**.

Yield: 410 mg, 83%; mp 220-222 ^{o}C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6) δ 8.19 (bs, 1H); 8.02 (bs, 1H); 7.90 (bs, 1H); 7.79-7.53 (m, 6H); 7.39-7.10 (m, 2H); 6.96 (dd, *J* = 8.1, 2.2 Hz, 1H); 6.77-6.62 (m, 2H); 6.37 (d, *J* = 8.8 Hz, 1H); 3.74 (s, 3H); 1.26 (s, 9H); LRMS (ESI) *m/z* 517 $[M+Na]^{+}$.

4.2.6. *N*1-(2-naphthyl)-2-(1,1-dioxo-4-phenyl-1,4-dihydro-1λ^{6},2,4-benzothiadiazin-3-yl)-1-hydrazinecarboxamide (10) The title compound was obtained from (**24**, 290 mg, 1 mmol) and 2-naphthyl isocyanate (203 mg, 1.2 mmol) as described for **5**.

Yield: 407 mg, 89%; mp 247-249 ^{0}C; ^{1}H NMR (300 MHz DMSO-d_6) δ 8.81 (bs, 1H); 8.68 (bs, 1H); 8.49 (s, 1H); 8.17 (d, *J* = 7.9 Hz, 1H); 7.91 (dd, *J* = 9.1, 2.1 Hz, 1H); 7.86 (d, *J* = 7.2 Hz ,1H); 7.78-7.66 (m, 4H); 7.65-7.43 (m, 6H); 7.41-7.32 (m, 1H); 6.33 (d, *J* = 8.3 Hz, 1H).; LRMS(ESI) *m/z* 481 $[M+Na]^{+}$.

4.2.7. *N*1-(4-Methoxyphenyl)-2-(4-methyl-1,1-dioxo-1,4-dihydro-1λ^{6},2,4-benzothiadiazin-3-yl)-1-hydrazinecarboxamide (11) The title compound was obtained from (**25**, 226 mg, 1 mmol) and 4-methoxy phenyl isocyanate (179 mg, 1.2 mmol) as described for **5**.

Yield: 342 mg, 91%; mp 226-228 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 9.73 (bs, 1H); 8.80 (s, 1H); 8.39 (s, 1H); 7.91-7.57 (m, 3H); 7.56-7.30 (m, 3H); 7.00-6.88 (m, 2H); 3.79 (s, 3H); 3.65 (s, 3H); LRMS (ESI) *m/z* 399 $[M+Na]^{+}$.

4.2.8. *N*1-(3-Chlorophenyl)-2-(4-methyl-1,1-dioxo-1,4-dihydro-1λ^{6},2,4-benzothiadiazin-3-yl) -1-hydrazinecarboxamide (12) The title compound was obtained from (**25**, 226 mg, 1 mmol) and 3-chloro phenyl isocyanate (184 mg, 1.2 mmol) as described for **5**.

Yield: 331 mg, 87%; mp 252-253 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.71 (bs, 1H); 9.11 (bs, 1H); 8.58 (bs, 1H); 7.80-7.66 (m, 4H); 7.55 (d, *J* = 8.9 Hz, 1H); 7.42 (t, *J* = 8.9, 7.2 Hz, 1H); 7.30 (t, *J* = 7.2, 8.9 Hz, 1H); 7.02 (d, *J* = 7.2 Hz, 1H); 3.57 (s, 3H); LRMS (ESI) *m/z* 403 $[M+Na]^{+}$.

4.2.9. *N*1-(4-Chlorophenyl)-2-(4-methyl-1,1-dioxo-1,4-dihydro-1λ^{6},2,4-benzothiadiazin-3-yl) -1-hydrazinecarboxamide (13) the title compound was obtained from (**25**, 226 mg, 1 mmol) and 4-chloro phenyl isocyanate (184 mg, 1.2 mmol) as described for **5**.

Yield: 343 mg, 90%; mp 248-249 ^{o}C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.77 (bs, 1H); 9.09 (s, 1H); 8.55 (s, 1H); 7.86-7.70 (m, 2H); 7.69-7.50 (m, 3H); 7.49-7.29 (m, 3H); 3.62 (s, 3H); LRMS (ESI) *m/z* 403 $[M+Na]^+$.

4.2.10. *N*1-[3,5-Di(trifluoromethyl)phenyl]-2-(4-methyl-1,1-dioxo-1,4-dihydro-1λ^6,2,4-benzothiadiazin-3-yl)-1-hydrazinecarboxamide (14) The title compound was obtained from (**25** 226 mg, 1 mmol) and 3,5-di(trifluoromethyl) phenyl isocyanate (306 mg, 1.2 mmol) as described for **5**.

Yield: 415 mg, 86%; mp 274-276 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.77 (bs, 1H), 8.96 (s, 1H); 8.24 (s, 2H), 7.80-7.68 (m, 2H), 7.65 (s, 1H); 7.56 (d, *J* = 8.5 Hz 1H); 7.43 (t, *J* = 7.6 Hz 1H), 3.62 (s, 3H); LRMS (ESI) *m/z* 505 $[M+Na]^+$.

4.3.1. 2-(4-Methoxyanilino)-10-phenyl-5,10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4]thiadiazine-5,5-dione (15) The title compound (**15**) was obtained from by reaction of corresponding urea compound **5** (438 mg, 1 mmol) with $POCl_3$ (10 mL) and then reflux the reaction mixture at 110 ^{o}C for 3 h. Then the reaction mixture was poured in crushed ice and quenched with $NaHCO_3$ and extracted in ethyl acetate (4x25 mL) from the ice cold aqueous layer and dried over anhydrous Na_2SO_4. The resulting products (**15**) were purified by recrystallization from methanol.

Yield: 340 mg, 81%; mp 196-198 ^{o}C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.15 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.73-7.56 (m, 3H), 7.55-7.31 (m, 5H), 6.87 (d, *J* = 8.4 Hz, 2H), 6.73 (d, *J* = 8.4 Hz, 1H), 6.54 (s, 1H), 3.79 (s, 3H); LRMS (ESI) *m/z* 421 $[M+1]^+$.

4.3.2. 2-(3-Chloroanilino)-10-phenyl-5,10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4]thiadiazine-5,5-dione (16) The title compound was obtained from **6** (442 mg, 1 mmol) and $POCl_3$ (10 mL) as described for **15**.

Yield: 360 mg, 85%; mp 171-173 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (bs, 1H), 7.83 (d, *J* = 7.2 Hz, 1H); 7.78-7.64 (m, 4H), 7.62-7.46 (m, 3H); 7.44-7.24 (m, 3H), 7.02 (t, *J* = 7.2 Hz, 1H), 6.33 (d, *J* = 8.9 Hz, 1H), LRMS (ESI) *m/z* 447 $[M+Na]^+$.

4.3.3. 2-(4-Chloroanilino)-10-phenyl-5,10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4]thiadiazine-5,5-dione (17) The title compound was obtained from **7** (442 mg, 1 mmol) and $POCl_3$ (10 mL) as described for **15.**

Yield: 348 mg, 82%; mp 210-212 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.14 (bs, 1H); 8.25 (d, *J* = 7.6 Hz, 1H); 7.80-7.61 (m, 6H), 7.56 (d, *J* = 8.9 Hz, 2H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 8.9 Hz, 2H), 6.71 (d, *J* = 8.5 Hz, 1H); LRMS (ESI) *m/z* 447 $[M+Na]^+$.

4.3.4. 2-[3,5-di(trifluoromethyl)anilino]-10-phenyl-5, 10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4] thiadiazine-5,5-dione (18) The title compound was obtained from **8** (544 mg, 1 mmol) and $POCl_3$ (10 mL) as described for **15**.

Yield: 426 mg, 81%; mp 260-263 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.79 (bs, 1H); 8.27 (d, *J* = 7.7 Hz, 1H); 8.13 (s, 2H), 7.81-7.61 (m, 6H), 7.58 (s, 1H), 7.51 (t, *J* = 7.7 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H); LRMS (ESI) *m/z* 526 $[M^+]$.

4.3.5. 2-(2-Naphthylamino)-10-phenyl-5,10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4]thiadiazine-5,5-dione (19) The title compound was obtained from **10** (458 mg, 1 mmol) and $POCl_3$ (10 mL) as described for **24**.

Yield: 383 mg, 87%; mp 269-272 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.30(s, 1H); 8.34-8.04 (m, 2H), 7.92-7.35 (m, 13H), 6.77(d, *J* = 8.8 Hz, 1H); LRMS (ESI) *m/z* 441 $[M+1]^+$.

4.3.6. 2-(4-Methoxyanilino)-10-methyl-5,10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4]thiadiazine-5,5-dione (20) The title compound was obtained from **11** (376 mg, 1 mmol) and $POCl_3$ (10 mL) as described for **15**.

Yield: 293 mg, 82%; mp 256-258 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.84-7.67 (m, 1H), 7.54-7.23 (m, 3H), 6.90 (d, *J* = 8.8 Hz, 2H), 6.58 (d, *J* = 1.5 Hz, 1H), 3.81 (s, 3H), 3.78(s, 3H); LRMS (ESI) *m/z* 358 $[M+1]^+$.

4.3.7. 2-(3-Chloroanilino)-10-methyl-5,10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo[1,5-*b*] [1,2,4] thiadiazine-5,5-dione (21) The title compound was obtained from **12** (483 mg, 1 mmol) and $POCl_3$ (10 mL) as described for **15**.

Yield: 313 mg, 86%; mp 250-252 ^{o}C;^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12 (d, *J* = 8.0 Hz, 1H), 8.02-7.80 (m, 1H), 7.76-7.71 (m, 1H), 7.66-7.42 (m, 3H), 7.25 (t, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 3.85 (s, 3H); LRMS (ESI) *m/z* 362 $[M^+]$.

4.3.8. 2-(4-Chloroanilino)-10-methyl-5,10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo[1,5-*b*] [1,2,4] thiadiazine-5,5-dione (22) The title compound was obtained from **13** (483 mg, 1 mmol) and $POCl_3$ (10 mL) as described for **15**.

Yield: 326 mg, 90%; mp 304-306 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16-8.06 (m, 1H), 8.00-7.79 (m, 1H), 7.70-7.41 (m, 4H), 7.25 (d, *J* = 8.6 Hz, 2H), 3.85 (s, 3H); LRMS (ESI) *m/z* 362 $[M^+]$.

4.3.9. 2-[3,5-Di(trifluoromethyl)anilino]-10-methyl-5,10-dihydro-5λ^6-benzo[*e*][1,2,4]triazolo [1,5-*b*][1,2,4]thiadiazine-5,5-dione (23) The title compound was obtained from **14** (482 mg, 1 mmol) and $POCl_3$ (10 mL) as described for **15**.

Yield: 375 mg, 86%; mp 300-302 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.74 (bs, 1H), 8.28-8.14 (m, 1H); 7.94 (t, *J* = 7.5 Hz, 1H), 7.79-7.64 (m, 1H), 7.61 (s, 3H), 7.52 (t, *J* = 7.5 Hz , 1H), 3.85 (s, 3H); LRMS(ESI) *m/z* 464 $[M+1]^+$.

In vitro Cytotoxicity Evaluation

The synthesized compounds (**5-23**) have been evaluated for their *in vitro* cytotoxicity in selected human cancer cell lines of lung (A-549), ovary (IGR-OV-1), prostate (PC-3), breast (T-47-D and MCF-7) and colon (CoLo-205) origin. A protocol of 48 h continuous drug exposure has been used and a sulforhodamine B (SRB) protein assay [35] has been used to estimate cell growth.

Tubulin Polymerization Assay

The assay was performed employing the fluorescence based tubulin polymerization assay kit (BK011) obtained from Cytoskeleton Inc. The reaction mixture contained PEM

buffer [[80mM PIPES (pH-6.9), 1mM MgCl2, 1mM EGTA, 10% glycerol], Tubulin (2mg/ml), and GTP (1mM) in the presence or absence of test compound (3μM) at 37 ^{o}C. Tubulin polymerization was measured by increase in fluorescence (excitation and emission wavelengths of 360 nm and 420 nm respectively) using Varioscan multimode plate reader at. Flouresence units were recorded at every 60 sec intervals for up to 1 h. Podophyllotoxin was used as a positive control in each assay.

The IC_{50} value was defined as the drug concentration required to inhibit 50% of tubulin assembly compared to controls. The reaction mixture for these experiments include: tubulin (2 mg/mL) in PEM buffer, GTP (1mM), in the presence or absence of test compounds at 1.5, 3, 4.5 and 6 μM concentrations, different concentrations of podophyllotoxin was used as positive control in this study. Polymerization was monitored by increase in the flouresence at 360/420 nm of exitation/emission using Varioscan multimode plate reader at 37 ^{o}C. Flouresence was monitored at every 1 min for 1 h.

ACKNOWLEDGMENTS

The authors Y.V.V.S and M.A thank UGC, New Delhi, for the award of research fellowship.

REFERENCES

[1] Baguley, B. C. Multiple drug resistance mechanisms in cancer. *Mol. Biotechnol*., **2010**, *46*, 308-16.

[2] Mansi, L.; Thiery-Vuillemin, A.; Nguyen, T.; Bazan, F.; Calcagno, F.; Rocquain, J.; Demarchi, M.; Villanueva, C.; Maurina, T.;Pivot, X. Safety profile of new anticancer drugs. *Expert Opin. Drug Saf*., **2010**, *9*, 301-17.

[3] Brzozowski, Z.; Saczewski, F.; Slawinski, J.; Bednarski, P. J.; Grunert, R.; Gdaniec, M. Synthesis, structural characterization, and *in vitro* antitumor activity of novel *N*-(6-chloro-1,1-dioxo-1,4,2-benzodithiazin-3-yl) aryl sulfonamides. *Bioorg. Med. Chem*., **2007**, *15*, 2560-72.

[4] Ghorab, M. M.; Ragab, F. A.; Hamed, M. M. Design, synthesis and anticancer evaluation of novel tetrahydroquinoline derivatives containing sulfonamide moiety. *Eur. J. Med. Chem*., **2009**, *44*, 4211-17.

[5] Wilkinson, B. L.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Poulsen, S. A. Inhibition of carbonic anhydrases with glycosyltriazole benzene sulfonamides. *J. Med. Chem.*, **2008**, *51*, 1945–53.

[6] Gitto, R.; Agnello, S.; Ferro, S.; DeLuca, L.; Vullo, D.; Brynda,J.; Mader, P.; Supuran, C. Y.; Chimi, A. Identification of 3,4-dihydroisoquinoline-2(1*H*)-sulfonamides as potent carbonic anhydrase inhibitors: Synthesis, biological evaluation, and enzyme-ligand X-ray studies. *J. Med. Chem*., **2010**, *53*, 2401-08.

[7] Renard, J. F.; Arslan, D.; Garbacki, N.; Pirotte, B.; de Leval, X. Pyridine analogues of nimesulide: Design, synthesis, and *in Vitro* and *in Vivo* pharmacological evaluation as promising cyclooxygenase 1 and 2 inhibitors. *J. Med. Chem.*, **2009**, *52*, 5864–71.

[8] Kim, Y. H.; Shin, K. J.; Lee, T. G.; Kim, E.; Lee, M. S.; Ryu, S. H.; Suh, P. G. G2 arrest and apoptosis by 2-amino-*N*-quinoline-8 yl benzenesulfonamide (QBS), a novel cytotoxic compound. *Biochemical Pharmacology*, **2005**, *69*, 1333–41.

[9] Dittrich, C.; Dumez, H.; Calvert, H.; Hanauske, A.; Faber, M.; Wanders, J.; Yule, M.; Ravic, M.; Fumoleau, P. Phase I and pharmacokinetic study of E7070, a chloroindolyl-sulfonamide anticancer agent, administered on a weekly schedule to patients with solid tumors. *Clinical Cancer Research*, **2003**, *9*, 5195–04.

[10] Howbert, J. J.; Grossmann, C. S.; Crowell, T. A.; Rieder, B. J.; Harper, R. W.; Kramer, K. E.; Tao, E. V.; Aikins, J.; Poore, G. A.; Riezel, S. M.; Grindey, G. B.; Shaw, W. W.; Todd, G. C. Novel agents effective against solid tumors: the diarylsulfonylureas. Synthesis, activities, and analysis of quantitative structure-activity relationships. *J. Med. Chem*., **1990**, *33*, 2393-07.

[11] Houghton, P. J.; Bailey, F. C.; Germain, G. S.; Grindey, G. B.; Witt, B. C.; Houghton J. A. *N*-(5-Indanyl sulfonyl)-*N*'-(4-chlorophenyl)urea, a novel agent equally cytotoxic to nonproliferating human colon adenocarcinoma cells. *Cancer Res*, **1990**, *50*, 318-22.

[12] Hainsworth, J. D.; Hande, K. R.; Satterlee, W. G.; Kuttesch, J.; Johnson, D. H.; Grindey, G.; Jackson, L. E.; Greco, F. A phase I clinical study of *N*-[(4-Chlorophenyl)amino]carbonyl-2,3-dihydro-1*H*-indene-5-sulfonamide (LY186641). *Cancer Res*, ***1989***, *49*, 5217-20.

[13] Brien, M. E.; Hardy, J.; Tan, S.; Walling, J.; Peters, B.; Hatty, S.; Wiltshaw, E. A. phase II study of sulofenur, a novel sulfonylurea, in recurrent epithelial ovarian cancer. *Cancer Chemother. Pharmacol.*, **1992**, *30*, 245-48.

[14] Chern, J. W.; Rong, J. G. 1,2,4 benzothiadiazine 1,1-dioxide. V Synthesis of built-in hydroxuguanidine tricycles as potential anticancer agents. *Tetrahedron Lett*., **1991**, *32*, 2935-38.

[15] Chern, J. W.; Liaw, Y. C.; Chen, C. S.; Rong, J. G.; Huang, C .L.; Chan, C H.; Wang, A. A. H. Studies on 1,2,4-benzothiadiazine 1,1-dioxides VII and quinazolinones IV: Synthesis of novel built-In hydroxyguanidine tricycles as potential anticancer agents. *Heterocycles*, **1993**, *36*, 1091-03.

[16] Kubo, A.; Nakagawa, K.; Varma, R. K.; Conrad, N. K.; Cheng, J. Q.; Lee, W. C.; Testa, J. R.; Johnson, B. E.; Kaye, F. J.; Kelley, M. J. The p16 status of tumor cell lines identifies small molecule inhibitors specific for cyclin-dependent kinase 4. *Clin. Cancer Res*., 1999, *5*, 4279-86.

[17] Huwe, A.; Mazitschek, R.; Giannis, A. Small molecules as inhibitors of cyclin-dependent kinases. *Angew. Chem. Int. Ed*., **2003**, *42*, 2122-38.

[18] Kelly, M. J. Nakagawa, K.; Dent, B. R. Cyclin dependent kinase (CDK) 4 inhibitors and their use for treating cancer. US Patent 6630464, 2003.

[19] Jiang, B.; Hesson, D. P.; Dusak, B. A.; Dexter, D. L.; Kang, G. J.; Hamel, E. Synthesis and biological evaluation of 2-styrylquinazolin-4(3*H*)-ones, a new class of antimitotic anticancer agents which inhibit tubulin polymerization. *J. Med. Chem*., **1990**, *33*, 1721-28.

[20] Pomarnacka, E.; Gdaniec, M. Synthesis and anticancer activity of 2-amino-8-chloro-5,5-dioxo[1,2,4]triazolo[2,3-*b*][1,4,2]benzodithiazine derivatives. *Bioorg. Med. Chem*., **2003**, *11*, 1259-67.

[21] Pomarnacka, E.; Bednarski, P. J.; Reszka, P.; Dziemidowicz-Borys, E.; Bienczak, A.; Werel, W.; Halasa, R. Synthesis and biological activity of new 2-amino-8-chloro-5,5-dioxo[1,2,4]triazolo[2,3-*b*][1,4,2]benzodithiazines. *Eur. J. Med. Chem.*, **2006**, *41*, 633-39.

[22] Kamal, A.; Khan, M. N. A.; Reddy, K. S.; Srikanth, Y. V. V.; Sridhar, B. Synthesis, structural characterization and biological evaluation of novel [1,2,4]triazolo[1,5-*b*][1,2,4]benzothiadiazine-benzothiazole conjugates as potential anticancer agents. *Chem. Biol. Drug. Des*., **2008,** *71*, 78-86.

[23] Kamal, A.; Khan, M. N. A.; Srikanth, Y. V. V.; Rajesh, S. V. C. R. N. C. Synthesis and biological evaluation of mercapto triazolo-benzothiadiazine linked aminobenzothiazoles as potential anticancer agents. *Chem. Biol. Drug. Des*., **2009**, *73*, 687-93.

[24] Kamal, A.; Khan, M. N. A.; Srikanth, Y. V. V.; Reddy, K. S.; Juvekar, A.; Sen, S.; Kurian, N.; Zingde, S. Synthesis, DNA-binding ability and evaluation of antitumour activity of triazolo[1,2,4] benzothiadiazine linked pyrrolo[2,1-*c*] [1,4]benzodiazepine conjugates. *Bioorg. Med. Chem.*, **2008**, *16*, 7804-10.

[25] He, R.; Chen, Y.; Ougolkov, A. V.; Zhang, J. S.; Savoy, D. N.; Billadeau, D. D.; Kozikowski, A. P. Synthesis and biological evaluation of triazol-4-ylphenyl-bearing histone deacetylase inhibitors as anticancer agents. *J. Med. Chem.*, **2010**, *53*, 1347-1356.

[26] Zhai, X.; Zhao, Y. F.; Liu, Y. J.; Zhang, Y.; Xun, F. Q.; Liu, J.; Gong, P Synthesis and cytotoxicity studies of novel [1,2,4]triazolo [1,5-*a*]pyrimidine-7-amines. *Chem Pharm bull (Tokyo)*., **2008**, *56*, 941-45.

[27] Pachuta-Stec, A.; Rzymowska, J.; Mazur, L.; Mendyk, E.; Pitucha, M.; Rzaczyńska, Z. *Eur. J. Med. Chem*., **2009**, *44*, 3788-93.

[28] Lin, R.; Connolly, P. J.; Huang, S.; Wetter, S. K.; Lu, Y.; Murray, W. V.; Emanuel, S. L.; Gruninger, R. H. Fiemtes-Pesquera, A. R.; Rugg, C. A.; Middleton, S. A.; Jolliffe, L. K. Acyl-1*H*-[1,2,4] triazole-3,5-diamine analogues as novel and potent anticancer cyclin-dependent kinase inhibitors: Synthesis and evaluation of biological activities. *J. Med. Chem*., **2005**, *48*, 4208-11.

[29] Zhang, Q.; Peng, Y.; Wang, X. I.; Keenan, S. M.; Arora, S.; Welsh, W. J. Highly potent triazole-based tubulin polymerization inhibitors. *J. Med. Chem.*, **2007**, *50*, 749-55.

[30] Kamal, A.; Reddy, K. S.; Khan, M. N. A.; Rajesh, V. C. R. N. C. S.; Ramaiah, M. J.; Pushpavalli, S. N. C. V. L.; Srinivas, C.; Bhadra, M. P.; Chourasia, M.; Sastry, G. N.; Juvekar, A.; Zingde, S.; Barkume, M. Synthesis, DNA-binding ability and anticancer activity of benzothiazole/benzoxazole–pyrrolo[2,1-*c*][1,4]benzodiazepine conjugates. *Bioorg. Med. Chem*., **2010**, *18*, 4747-61.

[31] Kamal, A.; Sreekanth, K.; Kumar, P. P.; Shankaraiah, N.; Balakishan, G.; Ramaiah, M. J.; Pushpavalli, S. N.; Ray, P.; Bhadra, M. P. Synthesis and potential cytotoxic activity of new phenanthrylphenol-pyrrolobenzodiazepines. *Eur. J. Med. Chem.*, **2010**, *45*, 2173-81.

[32] Kamal, A.; Bharathi, E. V.; Ramaiah, M. J.; Dastagiri, D.; Reddy, J. S.; Viswanath, A.; Sultana, F.; Pushpavalli, S. N.; Srivastava, H. K.; Sastry, G. N.; Bhadra, M. P.; Juvekar, A.; Sen, S.; Zingde, S. Quinazolinone linked pyrrolo[2,1-c][1,4]benzodiazepine (PBD) conjugates: Design, synthesis and biological evaluation as potential anticancer agents. *Bioorg. Med. Chem.*, **2010**, *18*, 526-42.

[33] Kamal, A; Bharathi, E. V.; Ramaiah, M. J.; Reddy, J. S.; Dastagiri, D.; Viswanath, A.; Sultana, F.; Pushpavalli, S. N.; Bhadra, M. P.; Juvekar, A.; Sen, S.; Zingde, S. Synthesis, anticancer activity and apoptosis inducing ability of anthranilamide-PBD conjugate. *Bioorg. Med. Chem. Lett*., **2010**, *20*, 3310-3313.

[34] Kamal, A.; Khan, M. N. A.; Reddy, K. S.; Rohini, K.; Sastri, G. N.; Sateesh, B.; Sridhar, B. Synthesis, structure analysis, and antibacterial activity of some novel 10-substituted 2-(4-piperidyl/phenyl)-5,5-dioxo[1,2,4]triazolo[1,5-*b*][1,2,4]benzothiadiazine derivatives. *Bioorg. Med. Chem. Lett*., **2007**, *47*, 5400-05.

[35] Vichai, V.; Kirtikara, K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat. Protoc*., **2006**, *1*, 1112-16.

Received: November 03, 2011 Revised: March 02, 2011 Accepted: March 09, 2011