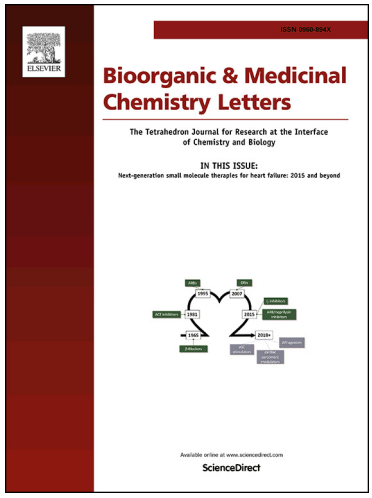
Accepted Manuscript



Novel benzothiazole hydrazine carboxamide hybrid scaffolds as potential *in vitro* GABA AT enzyme inhibitors: Synthesis, molecular docking and antiepileptic evaluation

Sadaf Jamal Gilani, Mohd. Zaheen Hassan, Syed Sarim Imam, Chandra Kala, Surya Prakash Dixit

PII: S0960-894X(19)30293-8
DOI: https://doi.org/10.1016/j.bmcl.2019.05.007
Reference: BMCL 26427

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 29 January 2019
Revised Date: 2 May 2019
Accepted Date: 5 May 2019

Please cite this article as: Jamal Gilani, S., Zaheen Hassan, Mohd., Sarim Imam, S., Kala, C., Prakash Dixit, S., Novel benzothiazole hydrazine carboxamide hybrid scaffolds as potential *in vitro* GABA AT enzyme inhibitors: Synthesis, molecular docking and antiepileptic evaluation, *Bioorganic & Medicinal Chemistry Letters* (2019), doi: https://doi.org/10.1016/j.bmcl.2019.05.007

Novel benzothiazole hydrazine carboxamide hybrid scaffolds as potential *in vitro* GABA AT enzyme inhibitors: Synthesis, molecular docking and antiepileptic evaluation

Sadaf Jamal Gilani[a*], Mohd. Zaheen Hassan[b], Syed Sarim Imam[c], Chandra Kala[c], Surya Prakash Dixit[d]

[a]College of Pharmacy, Jouf University, Aljouf, Sakaka, KSA.
[b]College of Pharmacy, King Khalid University, Abha, KSA.
[c]Glocal School of Pharmacy, Glocal University, Saharanpur, UP, India
[d]KIET School of Pharmacy, Ghaziabad, UP, India.

Corresponding Author:
Dr. Sadaf Jamal Gilani
Assistant Professor
Department of Pharmaceutical Chemistry
College of Pharmacy, Jouf University
Aljouf, Sakaka, KSA
E-mail: gilanisadaf@gmail.com
sjjilani@ju.edu.sa
Tel.: +966 507686858

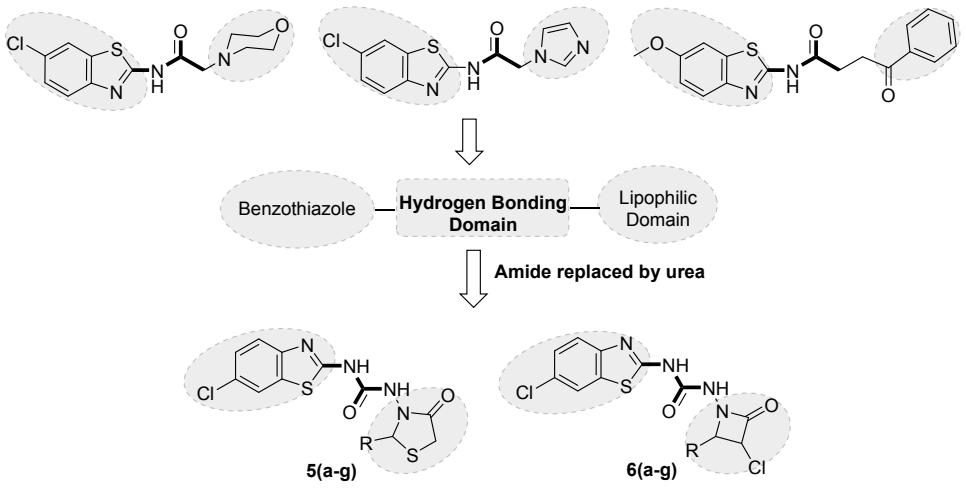
Abstract:

In the present study, a series of newer benzothiazole derivatives containing thiazolidin-4-one (**5a-g)** and azetidin-2-one (**6a-g)**, were synthesized by the cyclization of benzothiazolyl arylidene hydrazine carboxamide derivatives with thioglycolic acid and chloroacetyl chloride, respectively. Results of *in vivo* anticonvulsant screening revealed that compounds having 2,4-dicholoro (**5c** and **6c**) and 4-nitro substituent (**5g**) at the phenyl ring have promising anticonvulsant activities without any neurotoxicity. Selected compounds were also evaluated for their *in vitro* GABA AT inhibition. The results indicated that compound **5c** (IC_{50} 15.26 μM) exhibited excellent activity as compared to the standard drug vigabatrin (IC_{50} 39.72 μM) suggesting the potential of these benzothiazole analogues as new anticonvulsant agents.

Keywords: Benzothiazole, Antiepileptic, γ-aminobutyric acid aminotransferase (GABA AT), Molecular docking.

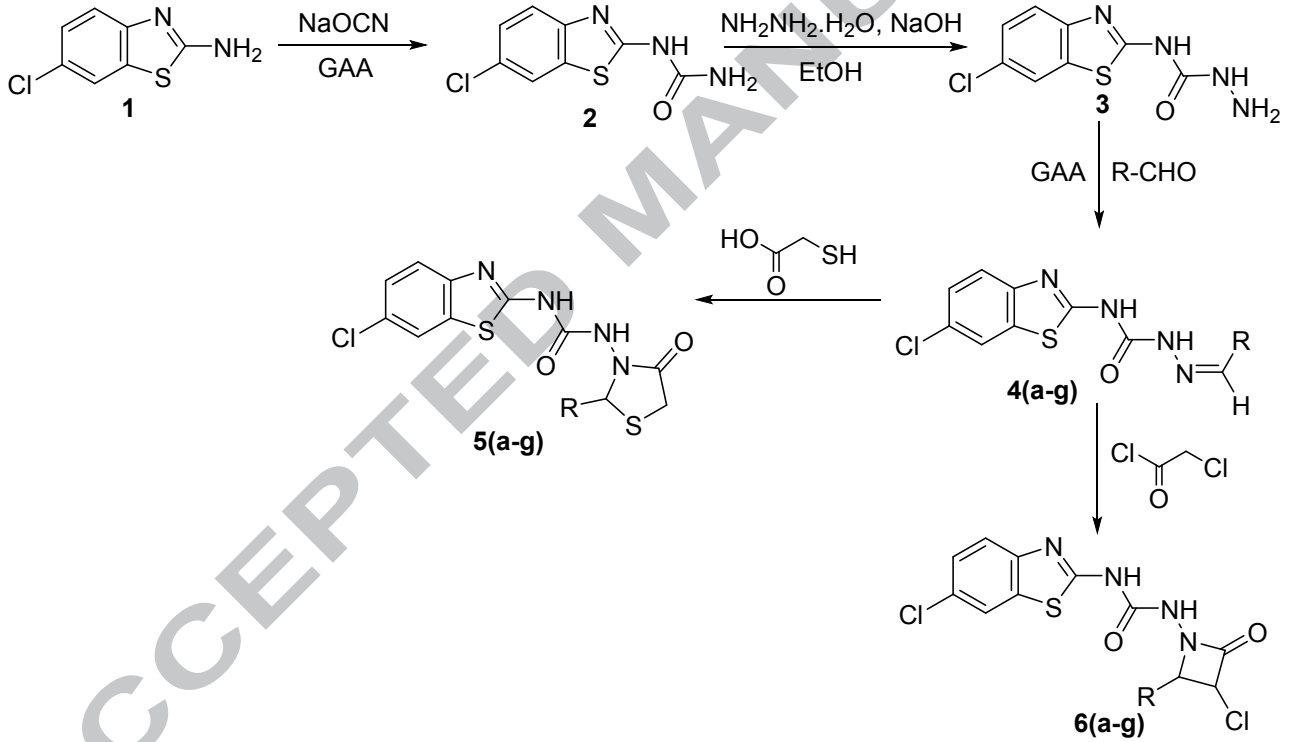
Epilepsy is characterized by recurrent abnormal electrical activity in the brain which affects majority of people all over the world [1,2]. Although, several newer classes of anticonvulsant drugs have emerged in the previous 20 years, but complete treatment of epilepsy is still a long way[3,4]. Most of these anti-epileptic drugs (AEDs) are ineffective against the 30% refractory epileptic patients and possess severe side effects *viz*., motor impairment, continuous pain in head, biliousness, loss of appetite, unsteady movements and staggering gait, liver damage, weariness, digestive disorder and gingival enlargement etc[5]. The restriction with the available conventional drugs leads to the evolution of novel drugs for convulsion [6].

Previously we have reported various heterocyclic compounds of nitrogen containing moieties with potential pharmacological activities. The promising results of these studies prompted us to further extend our synthesis of heterocyclic compounds and evaluation against the epileptic stimuli[7-9]. Encouraged by the antiepileptic profile of riluzole which is a benzothiazolamine derivative, recently research on benzothiazole as anticonvulsants has gained great momentum. Many of the reported benzothiazole analogues exhibited promising protective activity comparable to standard AEDs against mice [10-12]. Furthermore, thiazolidin-4-one[13] and azetidin-2-one[14] are also important class of heterocyclic compounds possessing diverse pharmacological properties including anticonvulsant activity. In our search for new compounds with anticonvulsant activity, the present series has been designed as a hybrid molecule containing benzothiazole and thiazolidin-4-one/azetidin-2-one connected through amide linkage. Previously, it has been observed that molecular hybridization of two lipophilic moieties connected through hydrogen bonding domain (HBD) *viz* amide linkage had promising anticonvulsant effects as it fulfilled the pharmacophoric requirements of anticonvulsant putative receptors [15,16]. In perspective of the above and in continuation of our interest in the benzothiazole derivatives, the present manuscript gives an account of the synthesis and anticonvulsant activity of some new hybrid analogues containing benzothiazole and thiazolidin-4-one/azetidin-2-one ring systems connected through amide linkage **(Scheme 1)**.



Scheme 1. 6-Chlorobenzothiazole derivatives containing thiazolidinone and azetidinone analogues connected through urea linkage.[14,15]

Titled compounds benzothiazolyl derivatives **5(a-g)** and **6(a-g)** were synthesized by following the reactions as given in **Scheme 2**. Intermediates arylidene hydrazine carboxamide **4(a-g)**[17] were prepared by reacting chlorobenzothiazol hydrazine carboxamide (**3**) with substituted benzaldehyde in alcohol. The product undergoes cyclization by reacting with thioglycolic acid to form thiazolidine-4-one derivatives **5(a-g)**[18]. Formation of the azetidin-2-one derivatives **6(a-g)**[19] was also accomplished in one step by adding chloroacetyl chloride and triethylamine to arylidene hydrazine carboxamide **4(a–g)** in dioxane. The formation of compound was ascertained by by IR, ^{1}H NMR, and LRMS[13,20].



Scheme 2. Protocol for the synthesis of titled compounds **5&6(a-g)**

All the pharmacological screenings were carried out using adult male albino mice having weight of 20±2 g. The present study was carried out by following the protocols accepted by the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) of KIET School of Pharmacy, Ghaziabad (UP) (Registration number & date of registration: 1099/c/07/CPCSEA, dated 27.07.2009). Animals were housed in groups of 4-5 in an atmospheric temperature (25±2ºC) and were permitted free access to food and water. Animals were not disturbed unnecessarily and treated gently. Previously sterilized instruments were used for drug preparation and animal injection. The anticonvulsant screening of the synthesized compounds was assessed by

following the standard protocols of Antiepileptic Drug Development (ADD) program by NINDS (National Institute of Neurological Disorders and Stroke), US[21-24]. At two pretreatment time intervals (0.5 h and 4 h), all the synthesized compounds were administered intraperitoneally into mice at doses of 30, 100 and 300 mgkg^{-1}. **Table 1** shows the results of the *in vivo* anticonvulsant activity of the synthesized moieties.

Table 1: Anticonvulsant profile and rotarod toxicity of the synthesized compounds **5 & 6(a-g)**

Compound	R	***i.p.* injection in mice[a]**						
		MES		**scPTZ**		**Neurotoxicity**		C Log P[c]
		0.5h	4h	0.5h	4h	0.5h	4h	
5a	Ph	300	300	300	300	-[b]	-	3.639
5b	2-ClPh	100	100	300	300	-	-	4.352
5c	2,4-Cl_2Ph	30	100	100	100	-	-	5.065
5d	2-CH_3Ph	300	300	-	-	300	300	4.088
5e	2-CH_3COOPh	300	300	-	-	-	-	2.988
5f	4-CH_3OPh	300	-	-	-	-	-	3.558
5g	4-NO_2Ph	30	100	100	100	-	-	3.382
6a	Ph	300	300	-	-	-	-	3.448
6b	2-ClPh	300	300	-	-	-	-	4.161
6c	2,4-Cl_2Ph	30	100	100	100	-	-	4.874
6d	2-CH_3Ph	300	300	-	-	300	300	3.897
6e	2-CH_3COOPh	300	300	-	-	-	-	2.797
6f	4-CH_3OPh	300	300	-	-	-	-	3.367
6g	4-NO_2Ph	100	100	300	300	-	-	3.191
Phenytoin	-	30	30	-	-	100	100	2.08*
Carbamazepine	-	30	100	100	100	100	300	2.38*
Valproic acid	-	-	-	300	-	-	-	2.76*
Ethosuximide	-	-	-	300	-	-	-	0.39*

[a]The data indicate the minimum dose (mgkg^{-1}) whereby bioactivity was demonstrated in half or more of the mice (six in each group). [b]A dash indicates the absence of activity. [c]CLogP was calculated using software ACD/log P; *Reported Clog P

Substitution with different electronic properties are attempted on the phenyl ring attached with the thiazolidin-4-ones and azetidin-2-ones to study their effects on anticonvulsant activity. Among the thiazolidin-4-ones (**5a-g**), all compounds showed some extent of protection in maximal electroshock screening (MES), which confirmed their therapeutic efficacy against convulsion at a deferent dose level. All the compounds showed quick onset and longer duration of protection except compound **5f**. Compounds **5c** (2,4-Cl_2Ph) and **5g** (4-NO_2Ph) showed protection of hind limb extension at a lower dose of 30 mgkg^{-1} after 0.5 h exhibiting their promising nature having quick onset. Compound **5b** (2-ClPh) revealed average protection at a dose of 100 mgkg^{-1} whereas, rest of the compounds were least effective at the optimum dose of 300 mgkg^{-1}. In subcutaneous pentylenetetrazole (scPTZ) test, compounds **5c** (2,4-Cl_2Ph) and **5g** (4-NO_2Ph) elevated seizure threshold at a dose of 100 mgkg^{-1} whereas **5a** (Ph) and **5b** (2-ClPh) exhibited protection at a maximum dose of 300 mgkg^{-1}. Thus, compounds **5c** and **5g** showed protections against both MES and scPTZ tests indicating their broad spectrum of anticonvulsant activity.

Azetidin-2-one derivatives (**6a-g**) also showed promising results against the maximal electroshock screening by preventing the hind limb tonic extension at different dose of 30-300 mgkg^{-1}. Only compound **6c** (2,4-Cl_2Ph) showed protection at a lower dose of 30 mgkg^{-1}. In the scPTZ test, only compounds **6c** (2,4-Cl_2Ph) and **6g** (4-NO_2Ph) effectively increases the convulsive threshold at a dose of 100 mgkg^{-1}. The anticonvulsant efficacy of **5c**, **5g** and **6c** were comparable to the standard drug carbamazepine in MES test effective at dose of 30 mgkg^{-1} (after 0.5 h), 100 mgkg^{-1}(after 4.0 h) and scPTZ test at a dose of 100 mgkg^{-1} (after 0.5 and 4.0 h). In the rotarod neurotoxicity test, all the compound except **5d** (2-CH_3Ph) and **6d** (2-CH_3Ph) were devoid of any sign of motor impairment at a higher dose of 300 mgkg^{-1}.

Agents effective against the scPTZ models are known to act through γ-aminobutyric acid (GABA) modulatory mechanisms [25,26]. Therefore, some selected compounds **5c**, **5g** and **6c** effective against the scPTZ were also evaluated for their whole brain GABA estimation to further get insights of their mechanism of seizure protective actions. Compared to the control animals ($p < 0.01$), whole brain GABA estimations revealed **(Fig.1)** that oral administration of compound **5c** for one week elevated the brain GABA level remarkably (by 1.5 fold). Thus, the anticonvulsant actions of these benzothiazolyl derivatives might be through increased GABA neurotransmission.

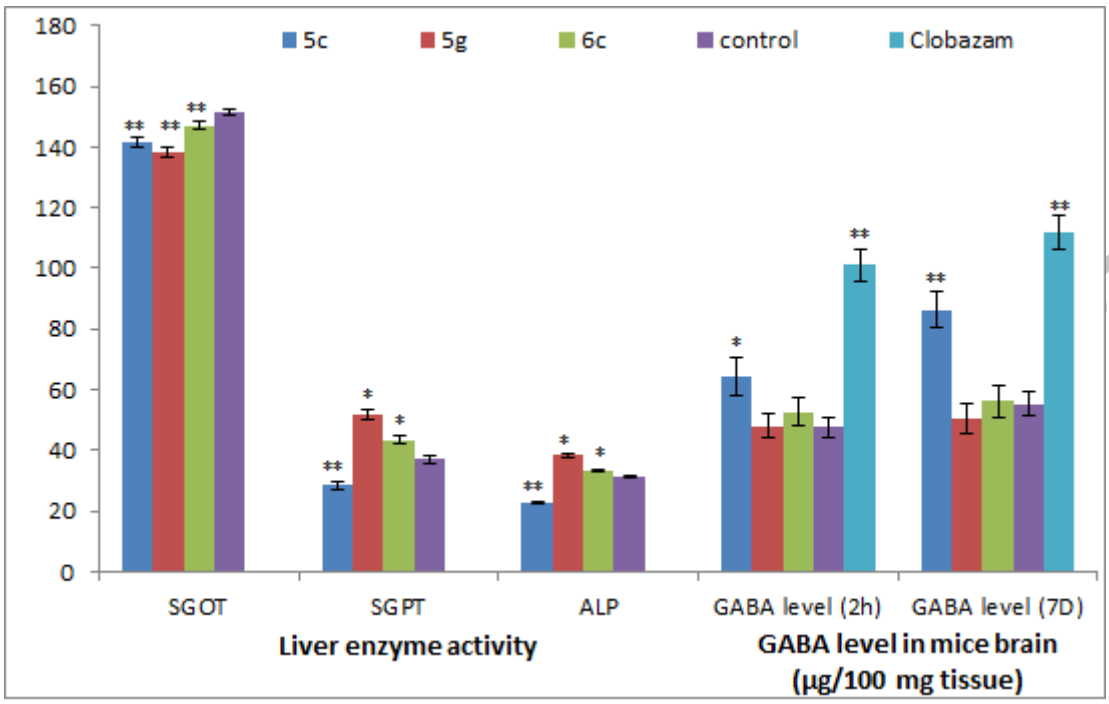


Fig. 1. Liver enzyme levels and GABA levels of compounds 5c, 5g and 6c treated mice.Each value represents the mean±SEM of six mice, significantly different from the control at *p < 0.05; **p < 0.01 (Student's t test).

In order to reveal more about GABA modulatory action of benzothiazolyl derivatives, *in-vitro* enzymatic assay[27] was performed. These newly synthesized compounds were also evaluated *in vitro* at γ-aminobutyric acid aminotransferase (GABA AT) activity to determine the IC_{50} value. GABA AT is a pyridoxal 5′-phosphate-dependent enzyme responsible for the breakdown of inhibitory neurotransmitter GABA. All the three analogues exhibited encouraging inhibitory results in the micromolar range. The median inhibitor potency (IC_{50}) of most promising compound **5c** was found to be 15.26 µM as compared to the standard drug vigabatrin showing median inhibitory concentration 39.72 µM **(Table 2)**. These results suggested that the increased concentration of GABA might be through the inhibition of GABA AT enzyme. Moreover, the results of *in vitro* inhibitions are consistent with the *in vivo* seizure protective efficacies of the benzothiazolyl analogues.

Table 2. *In silico* and *in vitro* GABA AT enzyme inhibition

Compound	**Docking score**	**Predicted inhibition constant (Ki)**	**Experimental inhibition constant (IC_{50}, µM)**
5c	-6.40	90.10µM	15.26±0.48
5g	-2.30	75.23mM	58.13±0.65
6c	-2.58	79.16mM	51.36±0.45
Vigabatrin	-2.803	82.76mM	39.72 ±0.47

An attempt has also been made to confirm the *in vitro* GABA AT inhibition of benzothiazole derivatives through *in silico* molecular docking studies on GABA AT enzyme. The X-ray crystal structures of GABA AT was retrieved form the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) having PDB ID 1OHV and 96% resemblance with the human brain enzyme. Results of molecular docking studies showed that compounds **5c, 5g** and **6c** had binding free energies from -2.30 to 6.40 kcal/mol. Compound **5c** showed minimum docked energy of -6.40 among the three mentioned analogues. **Fig. 2A-B** depict the docked conformations of the ligands **5c** bound to the active site of GABA AT. The oxo group of thiazolidinone seems to have an crucial role in strong hydrogen bonding with the active site residue Lys329. The residue Lys 329 is in close proximity and is probably the general base catalyst for the proton transfer reaction and drug vigabatrin inhibit this enzyme by covalent bond formation with Lys-329. Lipophilicity also appears to play crucial role in **5c** inhibitory activity, as 2,4-dichlorophenyl and thiazolidinone rings oriented to the more lipophilic area of GABA-AT binding site and formed π-π interactions with Ile72 and Phe189, respectively. The results of binding studies revealed that compound **5c** binds strongly in a mode very similar to standard drug vigabatrin **(Table 3)**.

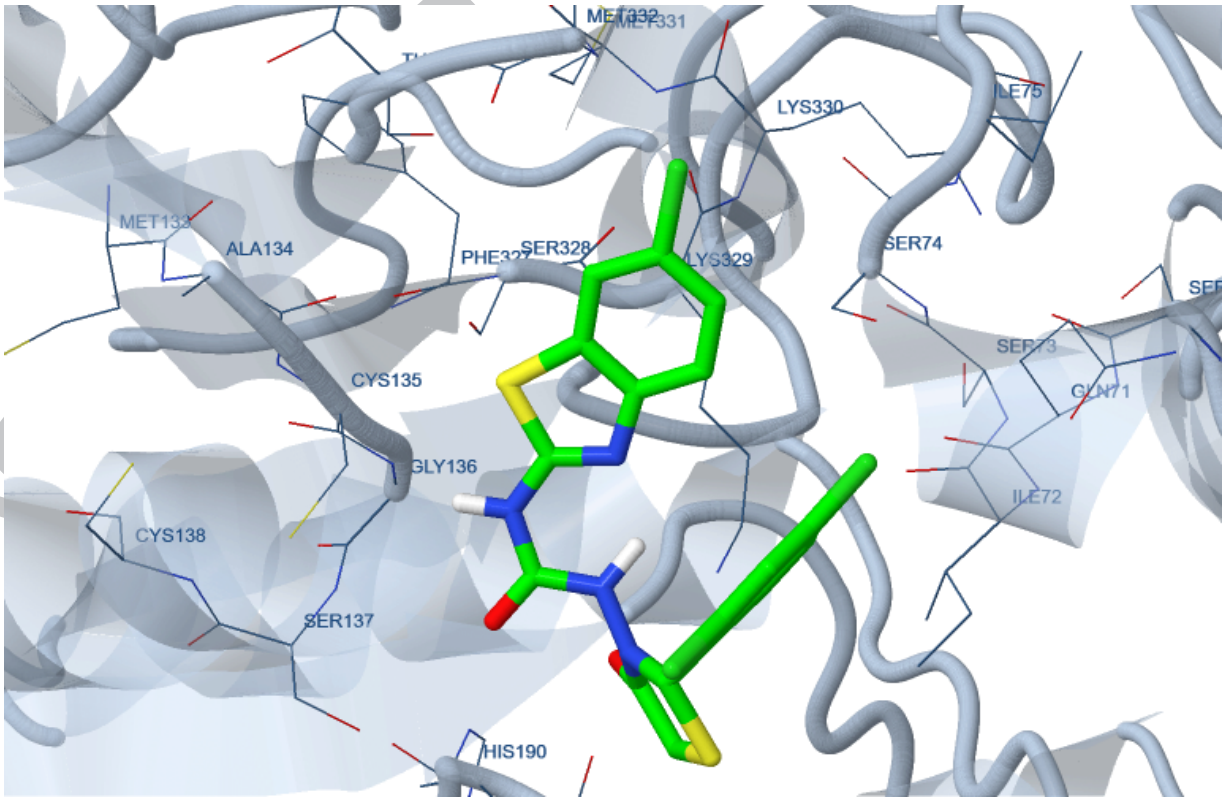


Fig 2A. Compound **5c** at the active site of GABA AT enzyme

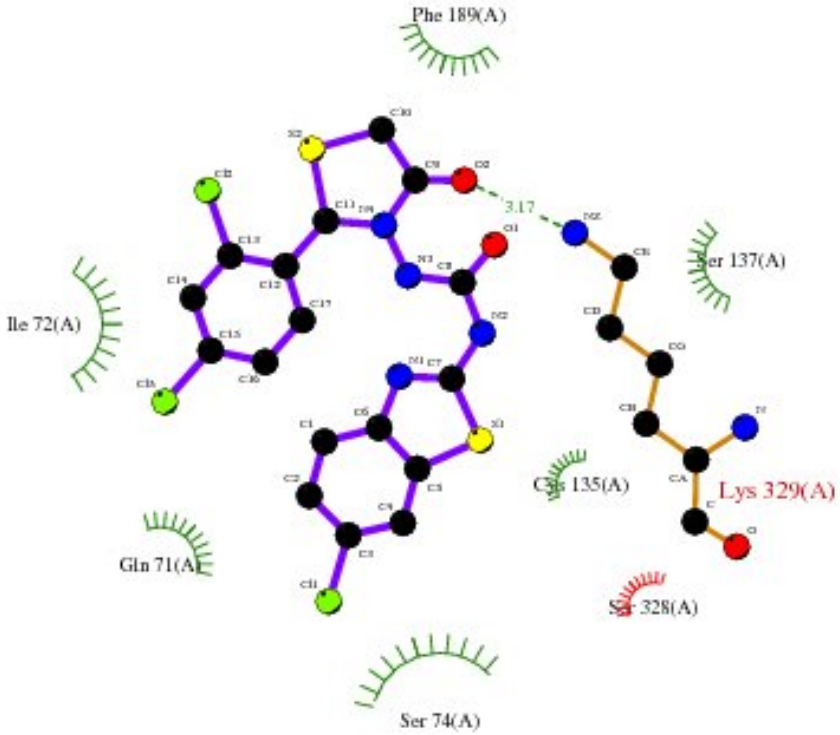


Fig 2B. 2D binding modes of compound **5c**

Table 3. Interactions of compound **5c** at the active site of GABA AT

Hydrogen bonds	Halogen-bond	Polar	Hydrophobic	Other
LYS329	SER 74	SER137	ILE72	CYS135
	GLN71		PHE189	SER328

As major problem associated with the clinically available antiepileptic drugs are their liver toxicity[7,8] therefore, the most promising compounds **5c**, **5g** and **6c** were also analyzed for their hepatotoxic profile. Repeated dose of the test compounds was administered orally in a dose of 30mg/kg daily for one months. At the end of the study, the levels of serum SGOT, SGPT and alkaline phosphatase were examined and any significant alterations in these enzymes were considered as liver toxicity. As shown in Fig.1, compound **5c** significantly decreased the levels of all enzymes ($P < 0.01$) as compared to the control group. However, compounds **5g** and **6c** moderately increased the levels of SGPT and ALP enzymes ($P < 0.05$) and decreased the levels of SGOT ($P < 0.01$). The liver histopathological studies of compound **5c** treated animals showed no remarkable diseased conditions as compared to control group. Section of liver showed normal hepatocytes with clearing of cytoplasm **(Fig. 3A-B)**. Thus, compound **5c** was not only effective against the MES and scPTZ test but also a safer agent devoid of any sign of neurotoxicity and liver toxicity.

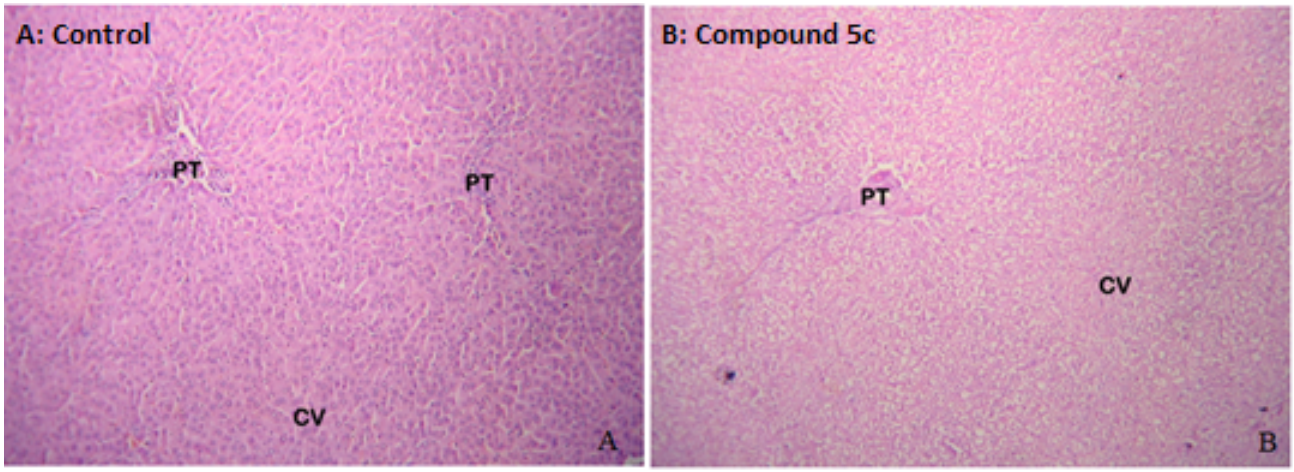


Fig. 3. A: Section of control liver showed normal hepatic parenchyma. PT=Portal Triad CV=Central Vein (100x); B: Section of compound 5c treated liver showed normal hepatocytes with clearing of cytoplasm. PT=Portal Triad CV=Central Vein (100x)

In conclusion, various benzothiazole analogues containing thiazolidin-4-one and azetidin-2-one connected through hydrogen bonding domain linkage were synthesised and examined for their antiepileptic activity. The results of antiepileptic screen exhibited that all the synthesized benzothiazole derivatives **5(a-g) and 6(a-g)** exhibit good to moderate anticonvulsant activity in mice. Three compounds **5c**, **5g** and **6c** showed promising anticonvulsant activity. The promising results could be due to the presence of electron withdrawing substituents *viz*. 2,4-Cl_2 **(5c)** and 4-NO_2 **(5g)** groups linked to the benzene moiety at second position of the thiazolidin-4-one ring, and 2,4-$C_6H_3Cl_2$ **(6c)** attached to the β-lactam ring. The promising results of *in vitro* and *in silico* studies clearly indicates that, the increased GABA concentration in brain is due to inhibition of GABA AT enzyme. Moreover, no overt toxicity was observed for the synthesized compounds in the various toxicity studies. Therefore, hybridization of the benzothiazole with thiazolidin-4-one/azetidin-2-one moieties provided a new opportunity for possible modification of anticonvulsant pharmacophoric requirements and future exploitation.

Acknowledgements

The authors are thankful to the Head of the Department, Pharmaceutical Chemistry for providing laboratory and animal facilities.

Conflict of interest : None

References and notes

1. Binder, D.K.; Scharfma, H. E. Recent Advances in Epilepsy Research, 1st ed.; Springer Science; US. **2004**, pp. 1811.
2. Manford, M. Recent advances in epilepsy. *J. Neurol.* **2017**, *264*, 1811–1824.

3. Shorvon, S.; Perucca, E.; EngelJr J. The Treatment of Epilepsy, 3rd ed.; Wiley-Blackwell; Oxford, **2009**, pp. 123.
4. Dars, M. D.; Bladin, P. F.; Eadie, M. J.; Millet, D. In Epilepsy: A comprehensive Text-book; Engel, J.; Pedley, T., Ed.; Lippincott Williams and Wilkins, Philadelphia, **2007**,Vol.1, pp.13.
5. Perucca, E. *Br. J. Clin. Pharmacol*. **1996**, *42*, 531-543.
6. Gilani, S. J.; Alam O.; Khan S A.; Siddiqui N.; Kumar H. *Der Pharm. Lett.* **2009**, *1 (2)*, 1-8.
7. Gilani, S. J.; Khan S.A., Siddiqui. *Bioorg & Med. Chem. Lett*. **2010**, *20*, 4762–4765.
8. Gilani, S. J., Khan, S. A. *Med. Chem. Res*. **2013**, *22*, 3316–3328.
9. Alam, O., Mullick, P., Verma, S. P, Gilani, S. J., Khan, S. A., Siddiqui, N., Ahsan, W. *Eur. J. Med. Chem.* **2010**,*45*, 2467-2472.
10. Gilani, S.J.; Khan S.A.; Verma S.P.; Mullick P.; Alam, O.; Siddiqui, N. *J. Enzyme Inhib. Med. Chem.* **2011**, *26 (3),* 332–340.
11. Murtuja, S.; Shaquiquzzaman, M.; Amir M. *Lett. Drug Des. Dis.* **2018**, *15*, 398-405.
12. Gagoria, J.; Verma, P.K.; Khatkar, A. *Cent Nerv Syst Agents Med Chem*. **2015**, *15*, 11-6.
13. Gilani, S J.; Nagarajan K.; Dixit S P.; Taleuzzaman M.; Khan S A. *Arab. J Chem*. **2016**, 9 *(2)*, S1523-S1531.
14. Hasan, H.; Akhter, M.; Akhter, W.; Ali, I.; Zaheen, M.; Ahsan, I.; Mahmood, D. *Med. Chem. Res*. **2011**, *20*, 1357–1363.
15. Hassan, M. Z.; Khan, S. A.; Amir, M. *Eur. J. Med. Chem.* **2012**, *58*, 206-213.
16. Amir, M., Asif, S., Ali, I., Hassan, M. Z. *Med. Chem. Res.* **2012**, *21*, 2661-2670.
17. General method for the synthesis of 2-substituted benzylidene-*N*-(6-chlorobenzothiazol-2-yl)hydrazinecarboxamide 4(a-g): Equimolar methanolic solution of *N*-(6-chlorobenzothiazol-2-yl)hydrazine carboxamide (0.01 mol) and substituted benzaldehyde (0.01 mol) were taken. To this solution, 1-2 drops of glacial acetic acid was included. For 5-6 hr this solution was then refluxed on a water bath. After completion of the reaction, the solution was kept aside to cool. After cooling, the solution was poured on the crushed ice and recrystallized from methanol.
18. General method for the synthesis of 1-(6-chlorobenzothiazol-2-yl)-3-(4-oxo-2-substituted phenylthiazolidin-3-yl)urea 5(a–g) : The reaction mixture of 2-substituted benzylidene-*N*-(6-chlorobenzothiazol-2-yl) hydrazinecarboxamide (0.01 mol) and thioglycollic acid (0.01 mol) was boiled on an oil bath for 7hr at 120-125 ºC with precaution. To the cooled solution sodium bicarbonate (10%) was added. Finally, the product was filtered, dried and recrystallized from methanol–dioxane in the ratio of 4:1.

19. General method for the synthesis of 1-(3-Chloro-2-oxo-4-substituted phenylazetidin-1-yl)-3-(6-chlorobenzothiazol-2yl)urea 6(a–g): Stirred mixture of chloroacetyl chloride (0.012 mol) and triethyl amine (0.012 mol) in dioxane (10 mL) maintained at 0–5 ºC, a solution of 2-substituted benzylidene-*N*-(6-chlorobenzothiazol-2-yl) hydrazine-carboxamide (0.01 mol) in dioxane (15 mL) was added. The combination was constantly stirred for six hour and kept aside for two days at 37 ºC. Finally, it was treated with cold water, washed and filtered. The solution was recrysrtallized from methanol to get the pure titled compound.
20. Analytical data of the selected compound: Compound **5c**: IR (KBr, ν cm^{-1}): 3210 (N-H), 3114 (C-H aromatic), 1734 (C=O thiazolidinone), 1663 (C=O), 1538 (C-C aromatic), 1433 (C-N benzothiazole), 836 (C-Cl), 683 (C-S-C thiazolidinone), 614 (C-S-C benzothiazole); ^{1}H NMR (300 MHZ, DMSO-d_6): δ 11.82 (s,1H,CONH), 8.00 (s,1H,NH), 7.68-7.71 (m, 6H, Ar-H), 2.47 (s, 2H, CH_2 thiazolidinone); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 174.4, 168.7, 153.7,151.2,135.5, 134.2, 132.3, 131.4,130.4, 129.7, 126.9, 125.9, 121.3,118.4,100.7, 58.8, 35.3 (CH_2 thiazolidinone); MS: *m/z*: 477[M^++2], 475[M^+], 473[M^+-2]
21. Anticonvulsant Screening Project, Antiepileptic Drug Development Program. National Institute of Health, DHEW Publ (NIH): US, **1978**; pp. 78–1093.
22. Siddiqui, N.; Alam, M.S.; Sahu, M.; Naim, J.M.; Shaharyar, M.; Alam, O. *Bioorg. Chem.* **2017**, *71*, 230-243.
23. Sahu,M.; Siddiqui, N.; Sharma,V.; Wakode, S. *Bioorg. Chem.* **2018**, *77*, 56-67.
24. Krall, R.L.; Penry, J.K.; White, B.G.; Kupferbeng, H.J.; Swinyard, E.A. *Epilepsia*. **1978**, *19*, 409-428.
25. Okada, R.; Negishi, N.; Nagaya, H. *Brain Res.* **1989**, *480*, 383-387.
26. Swinyard, E.A.; Woodhead, J.H.; White, H.S.; Franklin, M.R. In Antiepileptic Drugs; Levy, R.H.; Mattson, R.H.; Melrum, B.; Penry, J.K.; Dreifuss, F.E., Ed., Raven Press: New York, **1989**, pp. 85-102.
27. Bansal, S.K.; Sinha, B. N.; Khosa, R. L. *Med. Chem. Res*. **2013**, *22*. 134–146.
28. Amir, M.; Ali,I., Zaheen, H.M. *Med. Chem*. **2013**, *9*, 571.

Legends for Figures

Scheme 1 : 6-Chlorobenzothiazole derivatives containing thiazolidinone and azetidinone analogues connected through urea linkage

Scheme 2 : Protocol for the synthesis of titled compounds **5&6 (a-g)**

Figure 1 : Fig. 1. Liver enzyme levels and GABA levels of compounds 5c, 5g and 6c treated mice. Each value represents the mean±SEM of six mice, significantly different from the control at *p < 0.05; **p < 0.01 (Student's t test).

Figure 2A-B: Compound **5c** at the active site of GABA AT enzyme
2D binding modes of compound **5c**

Figure 3: A: Section of control liver showed normal hepatic parenchyma. PT=Portal Triad CV=Central Vein (100x); B: Section of compound 5c treated liver showed normal hepatocytes with clearing of cytoplasm. PT=Portal Triad CV=Central Vein (100x)

Highlights

- Compounds **5c**, **5g** & **6c** have optimum *in-vivo* antiepileptic activities without neurotoxicity.
- GABA modulatory effects-**5c**, **5g** & **6c** showed encouraging inhibitory results against GABA AT.
- Docking studies-**5c** binds strongly in a mode similar to standard drug vigabatrin.
- Results of *in vitro* inhibitions are consistent with *in vivo* seizure protection.
- Toxicity studies-No overt toxicity was observed for synthesized compounds.

Graphical abstract