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Synthesis and anticonvulsant activity of some new thiazolo[3,2-*a*][1,3]diazepine, benzo[*d*]thiazolo[5,2-*a*][12,6]diazepine and benzo[*d*]oxazolo[5,2-*a*][12,6] diazepine analogues

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Anticonvulsant activity

ABSTRACT

A new series of 6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepines (**9**–**12**), benzo[*d*]thiazolo[5,2-*a*][12,6]diazepines (**19**–**21**) and benzo[*d*]oxazolo[5,2-*a*][12,6]diazepine (**24**) analogues were synthesized and evaluated for their anticonvulsant activity. Compounds (*E*)-2-bromo-6,7-dihydro-thiazolo[3,2-*a*][1,3] diazepine-8(5*H*)-thione (**12**), 3-chloro-benzo[*d*]thiazolo[5,2-*a*][12,6]diazepin-10-one (**20**), and 4-chloro-benzo[*d*]oxazolo[5,2-*a*][12,6] diazepin-10-one (**24**) showed 100% protection against PTZ- and bicuculline-induced seizures; 70%, 33%, 70% protection against MES-induced tonic extension; and 70%, 66%, 100% protection against picrotoxin-induced convulsions, respectively. Compounds **12**, **20**, and **24** proved to act as $GABA_A$ receptor agonists, with ED_{50} values of 252, 380, 251 mg/kg; TD_{50} values of 398, 417, 355 mg/kg; PI values of 1.58, 1.09, 1.41; LD_{50} values of 380, 617, 537 mg/kg and TI values of 1.51, 1.62, 2.14, respectively.

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1. Introduction

GABA (γ-aminobutyric acid) is the principal inhibitory transmitter of many CNS pathways regulating numerous neurological functions including convulsions, anxiety and sleep activity. GABA acts on the $GABA_A$ chloride ion channel [1,2]. Molecular biology studies have demonstrated that several different receptor subunits combine to form the $GABA_A$ receptor complex with a number of receptor subtypes such as benzodiazepine receptor (BzR) [3–6]. Ligands acting at the BzR allosteric binding site of the $GABA_A$ receptor modulate the action of GABA on chloride ion flux. They show a wide variety of pharmacological actions ranging from full agonistic effects such as sedative/hypnotic, anxiolytic and anticonvulsant activities; to inverse agonistic effects such as anxiogenic, and proconvulsant activities [7]. Several classes of chemical compounds such as benzodiazepines, steroids and barbiturates bind to the $GABA_A$ chloride ion channel complex. The azepine derivatives such as imidazo[2,1-*b*]thiazepine (**1**, Chart 1), exhibited affinity for BzR. Their profile of action was characterized as partially agonistic at the BzRs on the basis of the GABA shift [8].

Thiazolo-[3,2-*a*][1,3]diazepine, and their analogues represent a new class of ultra-short acting hypnotics [9]. The ultra-short acting activity of ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-*a*][1,3]diazepin-3-carboxylate (HIE-124, **2**, Chart 1), overcomes many of the disadvantages and problems that usually associate the use of conventional intravenous anaesthetic agents. Compound **2** undergoes liver esterases enzymatic hydrolysis producing the free acid **3** (Chart 1), which is rapidly cleared out of the brain, terminating the hypnotic activity [9–14].

In the present study, a new series of 6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepine (**9**–**12**) was synthesized as bioisosteres of the imidazo[2,1-*b*]thiazepine nucleus; and evaluated for their anticonvulsant activity. The 3-ethyl carboxylate moiety of **2** was eliminated as an attempt to slow down its rapid metabolic clearance out of the brain (compounds **9**–**12**). Meanwhile, lipid solubility of the thiazolo[3,2-*a*][1,3]diazepine nucleus was increased either by the introduction of bromine atom (compounds **10** and **12**) or the thiation of the 8-oxo function (compounds **11** and **12**). The thiazole

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moiety of **2** was replaced by benzo-thiazole or benzo-oxazole heterocycles, producing benzo[*d*]thiazolo[5,2-*a*][12,6]diazepine (**19**–**21**) and the benzo[*d*]oxazolo[5,2-*a*][12,6] diazepine analogue **24**, to evaluate the effect of such isosteric replacement on the anticonvulsant activity.

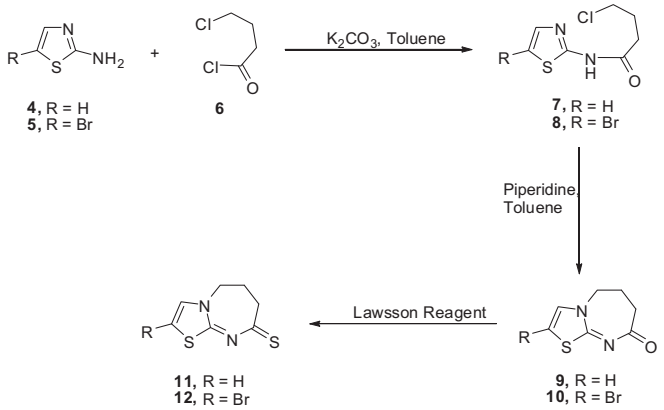
2. Chemistry

The reaction of 2-aminothiazole (**4**) or 2-amino-5-bromo-thiazole (**5**) with 4-chlorobutyryl chloride (**6**) in toluene at room temperature afforded 4-chloro-*N*-(thiazol-2-yl)-butanamide (**7**) or *N*-(5-bromothiazol-2-yl)-4-chlorobutanamide (**8**) which were cyclized upon reflux with piperidine/toluene to give (*E*)-6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepin-8(5*H*)-one (**9**) or (*E*)-2-bromo-6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepin-8(5*H*)-one (**10**). Com pound **9** and **10** were thiated by refluxing with Lawsson reagent in dioxane to afford (*E*)-6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepine-8(5*H*)-thione (**11**) and (*E*)-2-bromo–6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepine-8(5*H*)-thione (**12**), (Scheme 1).

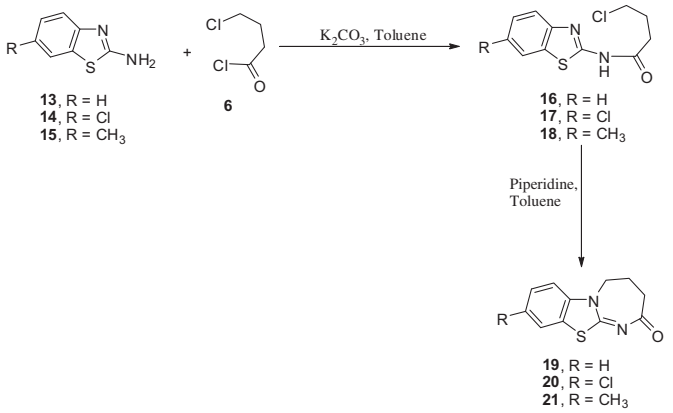
2-Amino-6-substituted-benzo[*d*]thiazoles (**13**–**15**) were allowed to react with 4-chlorobutyryl chloride (**6**) in toluene at room temperature afforded 4-chloro-*N*-(6-substituted-benzo[*d*]thiazol-2-yl)butanamides (**16**–**18**) in good yields. Compounds **16**–**18** were cyclized upon refluxing in piperidine/toluene to give 3-substituted-benzo [*d*]thiazolo[5,2-*a*][12,6]diazepin-10-one (**19**–**21**), Scheme 2. Meanwhile, 2-amino-5-chloro-benzo[*d*]oxazole (**22**) was allowed to react with 4-chlorobutyryl chloride (**6**) in toluene at room temperature afforded 4-chloro-*N*-(5-chloro-benzo[*d*]oxazol-2-yl) butanamide (**23**) in reasonable yield. Compound **23** was cyclized upon refluxing in piperidine/toluene to give 4-chloro-benzo[*d*]oxazolo [5,2-*a*][12,6]diazepin-10-one (**24**), Scheme 3.

3. Pharmacology

Pentylenetetrazole (PTZ) seizure threshold test is well-known to evaluate the potential anticonvulsant activity of compounds under investigation [15]. PTZ was used at dose level of 83 mg/kg, s.c.; this dose is the minimum dose that induces 100% clonic convulsions. Test compounds were used at different dose levels i.p. Sodium valproate was used as positive control at dose level of 200 mg/kg (1.38 mmol/kg, s.c.) 30 min after the administration of test compounds. At 15 and 25 min after i.p. administration of each of the test compounds at three dose levels, the inability of mice to climb up backwards in a glass tube of 25 cm length and 3 cm inner diameter within 30 s was recorded and taken as a measure of neurological deficits [16]. Normal mice climb up in 5–10 s. Motor activity was measured using activity metre at zero and 30 min after



Scheme 1. Synthesis of the target compounds **9**–**12.**



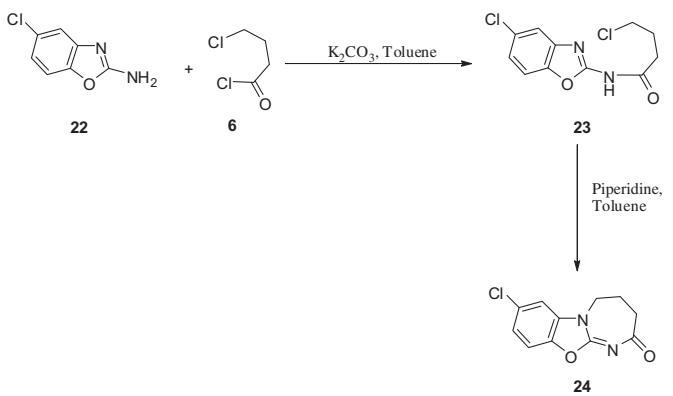
Scheme 2. Synthesis of the target compounds **19**–**21.**

administration of test compounds, taking zero time as 100%, while hypnotic effect of test compounds was performed using Writing Reflex method.

4. Results

Compounds **9**, **10**, **11**, **12**, **19**, **20**, and **24** were submitted for anticonvulsant activity evaluation. Results showed that compounds **9** and **11** caused 33% and 50% mortality within 7–11 min before even PTZ injection was administered, while compounds **10** showed marginal anticonvulsant activity. Compounds **12**, **20,** and **24** showed noticeable sedative effect in addition to 100% protection against PTZ-induced seizures (Table 1).

Compounds **12**, **20**, and **24** produced 70%, 33% and 70% protection against MES-induced tonic extension seizure, respectively. These finding suggested that those compounds have the ability to prevent the spread of seizure discharge throughout neuronal tissues and to raise seizure threshold. Compounds **9**, **10**, **11** and **19** proved inactive towards MES-induced seizure. Picrotoxin (Pic.) is a chloride channel blocker producing hyperpolarization and clonic convulsions. Compounds **12**, **20,** and **24** produced 70%, 66% and 100% protection against Pic.-induced convulsions, respectively, suggesting that those compounds may act as $GABA_A$ receptor agonist by increasing chloride influx via brain chloride channels. Bicuculline (Bic.) is a $GABA_A$ receptor blocker when given to mice, it induces clonic convulsions. All tested compounds produced 100% protection against Bic.-induced convulsions suggesting that those compounds may act directly as $GABA_A$ receptor agonist or indirectly by increasing GABA synthesis or its release as a brain inhibitory neurotransmitter (Table 2).



Scheme 3. Synthesis of the target compound **24.**

Table 1
Anticonvulsant activity of the new synthesized compounds against PTZ-induced convulsions.

Compd	Dose (mmol/kg)	% Protection[b]	% Chimney[c]	% Motor activity[d]	% Hypnotic[e]	% Mortality	CLogP
9[a]	1.5	0.0	0.0	0.0	0.0	33	1.19
	0.75	nt	nt	nt	nt	nt	
	0.375	nt	nt	nt	nt	nt	
10	1.5	30	80	12	30	0.0	2.05
	0.75	0.0	40	13	0.0	0.0	
	0.375	0.0	10	15	0.0	0.0	
11[a]	1.5	0.0	100	11	0.0	50	1.62
	0.75	0.0	30	26	0.0	10	
	0.375	0.0	10	30	0.0	0.0	
12	3.0	100	100	92	40	0.0	2.48
	1.5	100	25	84	14	nt	
	0.75	50	0.0	52	0.0	nt	
19	1.5	70	0.0	0.0	0.0	0.0	1.93
	0.75	0.0	nt	nt	nt	nt	
	0.375	0.0	nt	nt	nt	nt	
20	3.0	100	100	67	20	0.0	2.64
	1.5	50	25	33	0.0	0.0	
	0.75	25	0.0	20	0.0	0.0	
24	3.0	100	100	80	17	0.0	2.21
	1.5	67	50	50	0.0	0.0	
	0.75	33	17	33	0.0	0.0	
Sod. valproate	1.38	100	100	nt	nt	0.0	2.76

[a] Compound **9** and **12** showed 33 and 50% mortality, respectively before PTZ injection.
[b] Compounds showed anticonvulsant activity against PTZ-induced convulsions.
[c] Chimney test at 15 and 25 min after I.P. administration of test compounds.
[d] Motor activity was measured using activity metre at zero and 30 min after administration of test compounds, taking zero time as 100%.
[e] Hypnotic effect of tested compounds was performed using Writing Reflex.

A dose-response curve for the remarkably active anticonvulsant agents **12**, **20,** and **24** were performed along with the median effective (ED_{50}), median sedative (TD_{50}), protective index (PI), the acute toxicity determination (LD_{50}) and therapeutic index [17]. Results are shown in Table 2. Compounds **12**, **20,** and **24** showed ED_{50} values of 252, 380, 251 mg/kg; TD_{50} values of 398, 417, 355 mg/kg; PI (95% CL) values of 1.58, 1.09, 1.41; LD_{50} (95% CL) values of 380, 617, 537 mg/kg and TI (95% CL) values of 1.51, 1.62, 2.14, respectively.

5. Discussion

Correlation of the obtained anticonvulsant results and structure variation necessitate the comparison with the parent ultra-short acting hypnotic compound (*E*)-ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-*a*][1,3]diazepine-3-carboxylate (HIE-124, **2**) [9]. Removal of the 3-ethyl carboxylate function of **2**, produced compounds (*E*)-6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepin-8(5*H*)-one (**9**) and (*E*)-6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepine-8(5*H*)-thione (**11**) with total loss of hypnotic activity. Introduction of bromine atom to position 2- of **9** (CLogP 1.19) produced (*E*)-2-bromo-6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepin-8(5*H*)-one (**10**, CLogP 2.05) with the rise of a new type of CNS activity which is the anticonvulsant potency (30% protection against PTZ-induced convulsion). It seemed that lipophilicity plays a role in enhancing such activity as evidenced by the increase of anticonvulsant potency to 100% protection upon thiation of the 8-ketonic function of **10** to produce (*E*)-2-bromo-6,7-dihydro-thiazolo[3,2-*a*][1,3]

Table 2
Dose-response curve, ED_{50}, TD_{50}, PI, LD_{50} and TI for compounds **12**, **20** and **24**.

Compd[a]	Dose (mmol/kg)	Dose (mg/kg)	% PTZ	% Chim[b]	% MES[c]	% Pic[d]	% Bic[e]	ED_{50}[f] (mg/kg)	TD_{50}[f] (mg/kg)	PI[g]	LD_{50} (mg/kg)	TI[h]
12	3.0	505	100	100	70	70	100	252	398	1.58	380	1.51
	1.5	252	100	25	40	66	50	(195–324)	(309–513)		(302–478)	
	0.75	126	50	0.0	10	20	10					
	0.37	63	25	0.0	0.0	0.0	0.0					
20	3.0	759	100	100	33	66	100	380	417	1.09	617	1.62
	1.5	380	50	25	0.0	20	66	(295–490)	(324–537)		(479–794)	
	0.75	190	25	0.0	0.0	0.0	10					
	0.37	95	0.0	0.0	0.0	0.0	10					
24	3.0	710	100	100	70	100	100	251	355	1.41	537	2.14
	1.5	355	67	50	10	40	50	(195–324)	(275–457)		(427–661)	
	0.75	178	33	17	0.0	10	20					
	0.37	89	0.0	0.0	0.0	0.0	0.0					

[a] Each dose of selected compounds was tested using 10 animals and the percentage of animals protected was recorded and the anticonvulsant activity was calculated.
[b] Chim: Chimney test, was applied 15, and 25 min after the administration of each compound.
[c] MES: Maximal electroshock test, was applied 30 min after the administration of each compound.
[d] Pic:Picrotoxin (3.15 mg/kg, sc) was given 30 min after the administration of each compound.
[e] Bic: Bicuculline (1 mg/kg, sc) was given 30 min after the administration of each compound.
[f] ED_{50} and TD_{50}: Median effective and median sedative doses, respectively.
[g] PI: Protective index = TD_{50}/ED_{50}.
[h] TI: Therapeutic index = LD_{50}/ED_{50}.

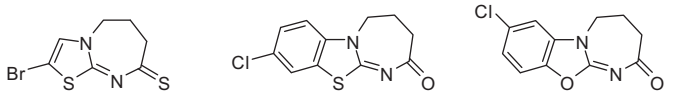
diazepine-8(5*H*)-thione (**12**, CLogP 2.48). The same analogy was noticed upon comparing (*E*)-7,8-dihydro-benzo[*d*]thiazolo[3,2-*a*][1,3]diazepin-9(6*H*)-ones (**19**, CLogP 1.93, 70% protection against PTZ-induced convulsions) and 3-Chloro-benzo [*d*]thiazolo[5,2-*a*][12,6]diazepin-10-one (**20**, CLogP 2.64, 100% protection). Isosteric replacement of the thiazole moiety with oxazole produced 4-chloro-benzo[*d*]oxazolo[5,2-*a*][12,6]diazepin-10-one (**24**, CLogP 2.21, 100% protection) Structure activity relationship (SAR) recommendations withdrawn from those findings revealed that (i) 3-ethyl carboxylate function connected to tetrahydro-thiazolo[3,2-*a*][1,3]diazepine as in **2** is essential for hypnotic activity, (ii) the removal of 3-ethyl carboxylate function of **2** will yield compounds with anticonvulsant activity, (iii) lipophilicity manipulate the magnitude of anticonvulsant potency of such chemical synthons.

6. Conclusion

In the present study, a new series of compounds belong to 6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepine, benzo[*d*]thiazolo[5,2-*a*][12,6]diazepine and benzo[*d*] oxazolo[5,2-*a*][12,6]diazepine nuclei were synthesized. The synthesized compounds were subjected to anticonvulsant activity evaluation. Four animal models were adopted, namely: pentylenetetrazole (PTZ), maximal electroshock (MES), picrotoxin (Pic), and bicuculline (Bic) induced convulsions. Compounds (*E*)-2-bromo-6,7-dihydro-thiazolo[3,2-*a*][1,3]diazepine-8(5*H*)-thione (**12**), 3-chloro-benzo[*d*]thiazolo[5,2-*a*][12,6]diazepin-10-one (**20**), and 4-chloro-benzo[*d*]oxazolo[5,2-*a*][12,6]diazepin-10-one (**24**) showed 100% protection against PTZ-induced seizures (Fig. 1). Compounds **12**, **20,** and **24** produced 70%, 33% and 70% protection against MES-induced tonic extension seizure, and 70%, 66% and 100% protection against picrotoxin-induced convulsions, respectively. All of the tested compounds produced 100% protection against bicuculline-induced convulsions. Those compounds proved to possess the ability to prevent the spread of seizure discharge throughout neuronal tissues and to raise seizure threshold. Those compounds may act as $GABA_A$ receptor agonist by increasing chloride influx via brain chloride channels or act directly as $GABA_A$ receptor agonist or indirectly by increasing GABA synthesis or its release as a brain inhibitory neurotransmitter.

7. Experimental protocols

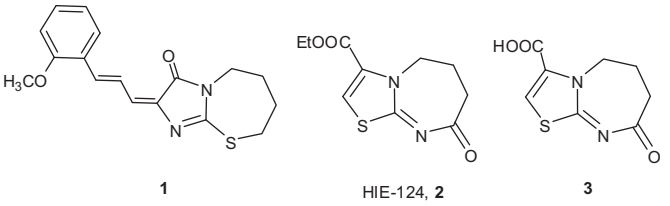
Unless otherwise specified all chemicals were of commercial grade, used without further purification and were obtained from Aldrich Chemical Co. (Milwaukee, WI). Solvents used for work ups were dried over $MgSO_4$, filtered and removed on a rotary evaporator. Elemental analyses were performed at College of Pharmacy, King Saud University-Central Laboratory. All of the new compounds were analyzed for C, H and N and agreed with the proposed structures within ±0.4% of the theoretical values. ^1H and ^{13}C NMR spectra obtained at 500 MHz. Bruker instrument, using TMS as internal standard. Thin layer and flash chromatography were performed using E. Merck Silica gel (230–400 mesh). Preparative thin layer chromatography was performed on Harrison model 7924 A chromatotron using Analtech silica gel GF rotors. Compounds **16** and **19** were previously reported [18]. Male Swiss albino mice (SWR) weighing 25–30 g, were obtained from the Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia and were housed in metabolic cages under controlled environmental conditions (25 °C and a 12 h light/dark cycle). Animals had free access to pulverized standard rat pellet food and tap water unless otherwise indicated. The protocol of this study has been approved by Research Ethics Committee of College of Pharmacy, King Saud University (KSU), Riyadh, Kingdom of Saudi Arabia (KSA). Test compounds were freshly dissolved in 99% DMSO. In preliminary screening, all tested compounds were used at dose level of 3.0, 1.5, 0.75 and 0.375 mmol/kg, i.p. Pentylenetetrazole (PTZ) was freshly dissolved in 0.9% NaCl and used at dose level of 83 mg/kg, s.c. Picrotoxin (Pic) was freshly dissolved in 1 ml 0.1 N warmed HCl and the final volume was made up with 0.9% NaCl. It has been used at dose level of 3.15 mg/kg s.c. and was given 30 min after the administration of test compounds. Bicuculline (Bic) was freshly dissolved in 1 ml 0.1 N warmed HCl and the final volume was made up with 0.9% NaCl. It has been used at dose level of 1 mg/kg sc and was given 30 min after the test compounds. Sodium valproate was freshly dissolved in 0.9% NaCl and used at dose level of 200 mg/kg s.c. 30 min after the administration of test compounds.



12 **20** **24**

Compd	Dose	% Anticonvulsant Activity				CLogP
	mmol/kg	PTZ	MES	Pic	Bic	
12	3.0	100	70	70	100	2.48
20	3.0	100	33	66	100	2.64
24	3.0	100	70	100	100	2.21

Fig. 1. Structures of the most active anticonvulsant agents.



1 HIE-124, **2** **3**

Chart 1. Literature cited active azepine analogues **1**, **2** and the inactive metabolic product **3**.

7.1. Chemistry

7.1.1. 4-Chloro-N-(thiazol-2-yl)-butanamide 7

A mixture of 2-aminothiazole (**4**, 4.0 g, 0.04 mol), 4-chlorobutyryl chloride (**6**, 11.3 g, 0.08 mol), and potassium carbonate (1.4 g, 0.01 mol) in toluene (100 ml) was stirred at room temperature for 24 h. The toluene was then evaporated under reduced pressure. The residue was then quenched with water, stirred, and filtered. The solid obtained was washed, dried and recrystallized from water to give the required product **7**; 92% yield, mp 125–127 °C, ^1H NMR ($CDCl_3$): δ 12.92 (s, 1H, NH), 7.54 (d, 1H, $J = 3.5$ Hz, thiazole-H), 7.04 (d, 1H, $J = 3.5$ Hz, thiazole-H), 3.71 (t, 2H, $J = 6.0$ Hz, CH_2), 2.81 (t, 2H, $J = 6.0$ Hz, CH_2), 2.31–2.26 (m, 2H, CH_2). ^{13}C NMR: δ 27.3, 32.8, 44.4, 113.6, 136.2, 160.1, 170.0. MS: m/z 113 (100%), $M = 204$ (20%), M + 2 = 206 (5%). ($C_7H_9ClN_2OS$) C, H, N.

7.1.2. N-(5-bromo-thiazol-2-yl)-4-chloro-butanamide 8

A mixture of 2-amino-5-bromothiazole hydrobromide (**5**, 1.3 g, 0.005 mol), 4-chlorobutyryl chloride (**6**, 1.1 ml, 0.01 mol), potassium carbonate (1.4 g, 0.01 mol) in toluene (50 ml) was stirred at room temperature for 24 h. The solvent was then evaporated under reduced pressure. The residue obtained was dissolved in chloroform and neutralized with ammonia solution. The organic layer was separated, dried over sodium sulphate and then evaporated to obtain the crude product which was recrystallized from toluene to

obtain the pure compound **8**; 94% yield, mp 153–155 °C ^{1}H NMR ($CDCl_3$): δ 12.37 (s, 1H, NH), 7.47 (s, 1H, thiazole-H), 3.65 (t, 2H, J = 6.5 Hz, CH_2), 2.60 (t, 2H, J = 6.5 Hz, CH_2), 2.03–2.05 (m, 2H, CH_2). ^{13}C NMR: δ 27.8, 32.4, 45.1, 101.9, 138.9, 158.8, 171.2. MS: m/z 179 (100%), M^+ = 283 (30%), M + 2 = 285 (8%). ($C_7H_8BrClN_2OS$) C, H, N.

7.1.3. (E)-6,7-dihydro-thiazolo [3,2-a][1,3]diazepin-8(5H)-one ***9***

A mixture of 4-chloro-*N*-(thiazol-2-yl)butanamide (**7**, 0.82 g, 0.004 mol) and piperidine (0.8 ml, 0.008 mol) in toluene (50 ml) was heated under reflux for 3 h. The reaction mixture was cooled, poured into water and stirred. Toluene was separated dried and evaporated to give a crude product which was purified by repeated silica gel and neutral alumina column chromatography eluting with $CHCl_3$/hexane (90:10 v/v); 56% yield, mp 75–77 °C ^{1}H NMR ($CDCl_3$): δ 7.35 (d, 1H, J = 3.5 Hz, thiazole-H), 6.91 (d, 1H, J = 7.5 Hz, thiazole-H), 4.05 (t, 2H, J = 7.5 Hz, CH_2), 2.58 (t, 2H, J = 7.5 Hz, CH_2), 2.18–2.12 (m, 2H, CH_2). ^{13}C NMR: δ 18.0, 31.6, 47.8, 113.6, 137.4, 157.7, 173.3. Ms: m/z 113 (100%), M^+ = 168 (42%), M + 2 = 170 (2%). ($C_7H_8N_2OS$) C, H, N.

7.1.4. (E)-2-bromo-6,7-dihydro-thiazolo [3,2-a][1,3]diazepin-8(5H)-one ***10***

A mixture of *N*-(5-bromo-thiazol-2-yl)-4-chloro-butanamide (**8**, 1.14 g, 0.004 mol) and piperidine (0.8 ml, 0.008 mol) in toluene (50 ml) was heated under reflux for 3 h. The reaction mixture was cooled, poured into water and stirred. Toluene was separated dried and evaporated to give a crude product which was purified by repeated silica gel and neutral alumina column chromatography eluting with $CHCl_3$/hexane (90:10 v/v); 45% yield, mp 118-20 °C. ^{1}H NMR ($CDCl_3$): δ 7.41 (s, 1H, thiazole-H), 4.16 (t, 2H, J = 7.5 Hz, CH_2), 2.40 (t, 2H, J = 7.5 Hz, CH_2), 2.24 (t, 2H, J = 7.5 Hz, CH_2). ^{13}C NMR: δ 18.2, 31.5, 47.3, 103.7, 138.2, 157.7, 173.6. MS: m/z 179 (100%), M^+ = 247 (65%), M + 2 = 249 (60%). ($C_7H_7BrN_2OS$) C, H, N.

7.1.5. (E)-6,7-dihydro-thiazolo [3,2-a][1,3]diazepine-8(5H)-thione 11

A mixture of (*E*)-6,7-dihydrothiazolo[3,2-*a*][1,3]diazepin-8(5*H*)-one (**9,** 0.67 g, 0.004 mol) and excess lawsson reagent was refluxed in dioxin (40 ml) for 3 h. The reaction mixture was cooled, poured into water and stirred. The solid was filtered and purified by silica gel column chromatography eluting with $CHCl_3$/hexane (95:5 v/v); 65% yield, mp 70–71 °C ^{1}H NMR ($CDCl_3$): δ 7.60 (d, 1H, J = 3.5 Hz, thiazole-H), 7.06 (d, 1H, J = 3.5 Hz, thiazole-H), 4.60 (t, 2H, J = 7.5 Hz, CH_2), 3.28 (t, 2H, J = 7.5 Hz, CH_2), 2.31–2.25 (m, 2H, CH_2). ^{13}C NMR: δ 20.3, 46.6, 56.7, 114.1, 137.2, 158.6, 199.7. Ms: m/z 113 (100%), M^+ = 184 (15%). ($C_7H_8N_2S_2$) C, H, N.

7.1.6. (E)-2-bromo-6,7-dihydro-thiazolo [3,2-a][1,3]diazepine-8(5H)-thione ***12***

A mixture of (*E*)-2-bromo-6,7-dihydro-thiazolo [3,2-*a*][1,3]diazepin-8(5*H*)-one (**10,** 1.0 g, 0.004 mol) and excess lawsson reagent was refluxed in dioxin (40 ml) for 3 h. The reaction mixture was cooled, poured into water and stirred. The solid was filtered and purified by silica gel column chromatography eluting with $CHCl_3$/hexane (95:5 v/v); 52% yield, mp 175–77 °C ^{1}H NMR (DMSO-d_6): δ 7.80 (s, 1H, thiazole-H), 4.47 (t, 2H, J = 7.5 Hz, CH_2), 3.21 (t, 2H, J = 7.5 Hz, CH_2), 2.21 (m, 2H, J = 7.5 Hz, CH_2). ^{13}C NMR: δ 200.5, 157.5, 138.4, 104.0, 56.2, 45.9, 19.8. Ms: m/z 179 (100%), M^+ = 263 (42%), M + 2 = 265 (38%). ($C_7H_7BrN_2S_2$) C, H, N.

7.1.7. N-(benzo[d]thiazol-2-yl)-4-chloro-butanamide ***16***

A mixture of 2-amino-benzo[*d*]thiazole (**13**, 6.0 g, 0.04 mol), 4-chlorobutyryl chloride (**6**, 11.3 g, 0.08 mol) and potassium carbonate (7.0 g, 0.05 mol) in toluene (100 ml) was stirred at room temperature for 24 h. The toluene was then evaporated under reduced pressure. The residue was then quenched with water, stirred, and filtered. The obtained solid was dissolved in chloroform and neutralized with ammonia solution. The organic layer was separated, dried over sodium sulphate and then evaporated to obtain the crude product which was used in the next reaction without further treatment [18].

7.1.8. 4-Chloro-N-(6-chloro-benzo[d]thiazol-2-yl)-butanamide ***17***

A mixture of 2-amino-6-chloro-benzo[*d*]thiazole (**14**, 7.4 g, 0.04 mol) 4-chlorobutyryl chloride (**6**, 11.3 g, 0.08 mol), and potassium carbonate (7.0 g, 0.05 mol) in toluene (100 ml) was stirred at room temperature for 24 h then continued as mentioned under **16**. The crude product was recrystallized from toluene to obtain the pure compound **17**; 89% yield, mp 135–137 °C ^{1}H NMR ($CDCl_3$): δ 11.83 (s, 1H, NH), 7.55 (s, 1H, Ar–H), 7.42–7.43 (m, 1H, Ar–H), 7.13–7.14 (m, 1H, Ar–H), 3.45–3.46 (m, 2H, CH_2), 2.50–2.52 (m, 2H, CH_2), 1.98–1.99 (m, 2H, CH_2). ^{13}C NMR: δ 27.4, 32.5, 44.1, 120.6, 121.5, 126.3, 128.5, 133.3, 147.4, 158.5, 171.4. MS: m/z 184 (100%), M^+ = 289 (20%), M + 2 = 290 (2%). ($C_{11}H_{10}Cl_2N_2OS$) C, H, N.

7.1.9. 4-Chloro-N-(6-methyl-benzo[d]thiazol-2-yl)-butanamide ***18***

A mixture of 2-amino-6-methyl-benzo[*d*]thiazole (**15**, 6.6 g, 0.04 mol), 4-chlorobutyryl chloride (**6**, 11.3 g, 0.08 mol), and potassium carbonate (7.0 g, 0.05 mol) in toluene (100 ml) was stirred at room temperature for 24 h then continued as mentioned under **16**. The crude product was used in the next reaction without further treatment.

7.1.10. 3-Substituted-benzo[d]thiazolo [5,2-a][12,6]diazepin-10-ones ***19–21***

A mixture of the butanamide analogues **16–18** (0.004 mol) and piperidine (0.8 ml, 0.008 mol) in toluene (50 ml) was heated under reflux for 3 h. The reaction mixture was cooled, poured into water and stirred. Toluene was separated dried and evaporated to give a crude product which was purified by repeated silica gel and neutral alumina column chromatography eluting with $CHCl_3$/hexane (90:10 v/v). **19**: 61% yield, mp 188–90 °C ^{1}H NMR ($CDCl_3$): δ 7.73–7.77 (m, 2H, Ar–H), 7.35–7.38 (m, 1H, Ar–H), 7.19–7.27 (m, 1H, Ar–H), 4.19–4.12 (m, 2H, CH_2), 2.65–2.68 (m, 2H, CH_2), 2.19–2.28 (m, 2H, CH_2). ^{13}C NMR: δ 18.1, 32.0, 48.2, 121.3, 121.4, 124.0, 126.1, 132.4, 148.6, 157.0, 174.3. MS: m/z 150 (100%), M^+ = 218 (15%), M + 2 = 220 (1%). ($C_{11}H_{10}N_2OS$) C, H, N [18];. **20**: 48% yield, mp 251–53 °C ^{1}H NMR ($CDCl_3$): δ 7.73–7.78 (m, 2H, Ar–H), 7.38–7.40 (m, 1H, Ar–H), 4.26 (t, 2H, J = 7.5 Hz, CH_2), 2.76 (t, 2H, J = 7.5 Hz, CH_2), 2.29–2.35 (m, 2H, CH_2). ^{13}C NMR: δ 18.1, 31.9, 48.2, 121.0, 122.1, 126.8, 129.4, 133.7, 147.3, 157.3, 174.4. MS: m/z 184 (100%), M^+ = 252 (20%), M + 2 = 254 (6%). ($C_{11}H_9ClN_2OS$) C, H, N. **21**: 39% yield, mp 218–20 °C ^{1}H NMR ($CDCl_3$): δ 7.73–7.75 (d, 1H, J = 8.0 Hz, Ar–H), 7.62 (s, 1H, Ar–H), 7.26–7.27 (d, 1H, J = 8.0 Hz, Ar–H), 4.28 (t, 2H, J = 7.5 Hz, CH_2), 2.75 (t, 2H, J = 7.5 Hz, CH_2), 2.49 (s, 3H, Ar–CH_3), 2.29–2.32 (m, 2H, CH_2). ^{13}C NMR: δ 18.1, 21.5, 32.0, 48.2, 120.9, 121.2, 127.6, 132.5, 134.0, 146.6, 156.4, 174.2. MS: m/z 164 (100%), M^+ = 232 (38%), M + 2 = 234 (1%). ($C_{12}H_{12}N_2OS$) C, H, N.

7.1.11. 4-Chloro-benzo[d]oxazolo [5,2-a][12,6]diazepin-10-one ***24***

A mixture of 2-amino-5-chloro-benzo[*d*]oxazole (**22**, 6.7 g, 0.04 mol), 4-chloro-butyryl chloride (**6**, 11.3 g, 0.08 mol), and potassium carbonate (7.0 g, 0.05 mol) in toluene (100 ml) was stirred at room temperature for 24 h then continued as mentioned under **16**. The crude product of 4-chloro-*N*-(5-chloro-benzo[*d*]oxazol-2-yl)butanamide (**23**) was used without further treatment. A mixture of **23** (1.1 g, 0.004 mol) and piperidine (0.8 ml, 0.008 mol) in toluene (50 ml) was heated under reflux for 3 h. The reaction mixture was cooled, poured into water and stirred. Toluene was separated dried and evaporated to give a crude product which was

purified by repeated silica gel and neutral alumina column chromatography eluting with $CHCl_3$/hexane (90:10 v/v). 54% yield, mp 230–2 °C ^{1}H NMR ($CDCl_3$): δ 7.50–7.51 (d, 1H, J = 1.5 Hz, Ar–H), 7.35–7.36 (d, 1H, J = 8.5 Hz, Ar–H), 7.14–7.16 (dd, 1H, J = 1.5, 8.5 Hz, Ar–H), 4.06 (t, 2H, J = 7.5 Hz, CH_2), 2.63 (t, 2H, J = 7.5 Hz, CH_2), 2.18–2.24 (m, 2H, CH_2). ^{13}C NMR: δ 18.3, 32.2, 47.7, 111.1, 118.9, 124.0, 130.2, 141.6, 147.3, 155.9, 172.9. MS: M^+ = 236 (16%), M + 2 = 238 (6%). ($C_{11}H_9ClN_2O_2$) C, H, N.

7.2. Anticonvulsant screening

7.2.1. Pentylenetetrazole (PTZ)-induced convulsion

Pentylenetetrazole (PTZ) seizure threshold test is one of well-known chemical tests used to evaluate the potential anticonvulsant activity of the tested compounds [15]. Each compound was tested in 6 mice and was given i.p. at four dose levels of 3.0, 1.5, 0.75 and 0.375 mmol/kg 30 min later, animals were received PTZ (83 mg/kg, s.c.) and observed for 30 min. A single 5 s episode of clonic spasms was taken as a threshold seizure. Compounds produced 100% protection against PTZ-induced seizures was further tested against maximal electroshock seizure (MES), picrotoxin and bicuculline-induced clonic convulsions, to explore the involvement of GABA-ergic receptors in their anticonvulsant activity.

7.2.2. Minimal neurotoxicity (Chimney test)

At 15 and 25 min after i.p. administration of each compound at four dose levels of 3.0, 1.5, 0.75 and 0.375 mmol/kg, the inability of mice to climb up backwards in a glass tube of 25 cm length and 3 cm inner diameter within 30 s was recorded and taken as a measure of neurological deficits [16]. Normal mice climb up in 5–10 s.

7.2.3. Effect of tested compounds against the maximal electroshock (MES)-induced convulsion

The maximal electroshock seizure (MES) is one of the electrical tests used to evaluate anticonvulsant activity [15]. In this test, MES that induced 100% maximal seizures was found to be 50 mA alternating current of 100 Hz frequency for 0.2 s, using ECT UNIT (model number 7801, UGO Basile, Varese, Italy). A 3.0 mmol/kg of test compounds which produced 100% protection against PTZ-induced seizures were injected i.p. 30 min later, mice were restrained by hand and subjected to electric shock through their ears, and released immediately following electrical stimulation, to permit observation of the maximal seizure. The maximal seizure typically consists of a short period of initial tonic flexion and a prolonged period of tonic extension (especially the hind limb). Protection was defined as complete absence of hind limb tonic extension.

7.2.4. Effect of tested compounds against picrotoxin-induced convulsion

A 3.0 mmol/kg of each compound which produced 100% protection against PTZ-induced seizures were injected i.p. in 6 mice. 30 min later, animals were injected s.c. with Pic (3.15 mg/kg) and observed for 30 min and the percentage of anticonvulsion of tested compounds was calculated [15].

7.2.5. Effect of tested compounds against bicuculline-induced convulsion

A 3.0 mmol/kg of each compound which produced 100% protection against PTZ-induced seizures were injected i.p. in 6 mice. 30 min later, animals were injected s.c. with Bic (1 mg/kg) and observed for 30 min and the percentage of anticonvulsion of tested compounds was calculated [15].

7.3. Determination of LD_{50} and dose-response curve

Several doses of compounds **12**, **20** and **24** which produced 70–100% protection against MES, Pic and Bic-induced convulsions were used to construct a dose-response curve. Also, LD_{50} for compounds **12**, **20** and **24** was determined using the Spearman–Karber method in which the doses were selected at equal logarithmic intervals [17].

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