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Synthesis and screening of novel N-benzo[d]thiazol-2-yl)-2-chloropropanamide derivatives as anticonvulsants

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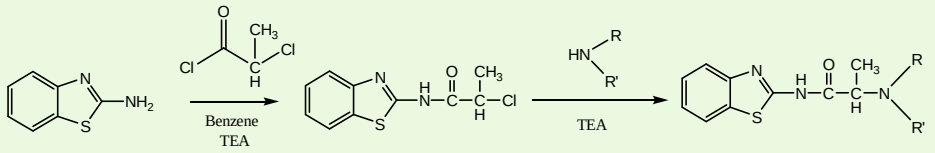
Abstract

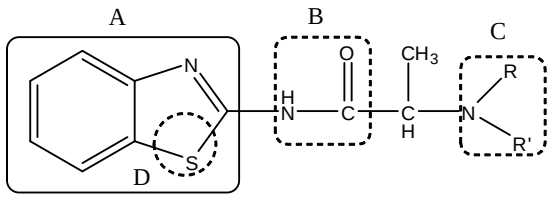
To probe the anticonvulsant activity connected with the benzo[d]thiazole 2-amine, a concatenation of some novel N-(benzo[d]thiazol-2-yl)-2-chlorpropanamide derivatives (A1-A7) were synthesized.These derivatives were characterized by IR, ^{1}H NMR, mass and elemental analysis. All the synthesized derivatives were evaluated for their anticonvulsant and neurotoxicity by using maximal electroshock (MES) method at 30, 100 and 300 mg/kg dose level using phenytoin as a standard drug showed auspicious anticonvulsant lead. It was found that the novel synthesized derivatives showed potent anticonvulsant activity.



1. Introduction

The chemistry and its bioactivity have been a fascinating area forever and ever in pharmaceutical chemistry. A multiplicity of heterocyclic derivatives consist nitrogen and sulphur atom were used as a distinctive and multifaceted stage for experimental drug design (Patel *et al*., 2010; Desai *et al*., 2021; Ram *et al*., 2022). Benzothiazole is solitary simplest dominant scaffolds that have received variable response due to its variegated molecular design and extraordinary properties (Ha-S-Koh *et al*., 2009; Samreen *et al*., 2022). It consists of thiazole ring fused with benzene ring and possesses manifold applications. The substituted benzothiazole derivatives are comparatively easy to prepare and possess characteristics pharmacological properties due to the presence of an inbuilt biologically active unit (Kumar *et al*., 2016). On exhaustive literature survey disclosed that benzothiazole analogs are connected with various pharmacological effects (Lieu *et al*., 2016). Convulsion is the recurrent serious neurological disorder in humans is specified by immoderate temporary neuronal discharges, affecting about 02 % population of the World (Yogeeswari *et al*., 2005; Krall *et al*., 1978). Epilepsy is the very familiar neurological disorder which affects about 02% population in all the countries (Arshad *et al*., 2014; Kitano *et al*., 1995). Therefore, there is a need to introduce safer, potent and also less toxic anticonvulsant drug (Khokra *et al*., 2019).

Experimental model was planned based on the entrenched anticonvulsant drugs according to which crucial attribute for the anticonvulsant activity are: (1) hydrophobic domain (A), (2) An electron donor system (D), (3) hydrogen bond donor (B) and (4) A distal amine residue (C) (Mallick *et al*., 2013).



A=Hydrophobic domain, D= Electron donor system, B= Hydrogen bonding domian, C = Distal amine residue.

In our investigation, novel derivatives were synthesized and evaluated for pharmacological activity to probe this hypothesis.

2. Material and Methods

All the chemical and solvents procured from Sigma Aldrich (India), S.D. Fine (India) and Merck (India) were used. The melting and boiling point of the synthesized derivatives were checked by using melting point apparatus (Scientech Company). Reactions completion were observed by thin layer chromatography by using solvent systems cyclohexane : ethyl acetate (1:1) and ethyl acetate : n-hexane (4:6). Spots were visualized using iodine vapors or UV

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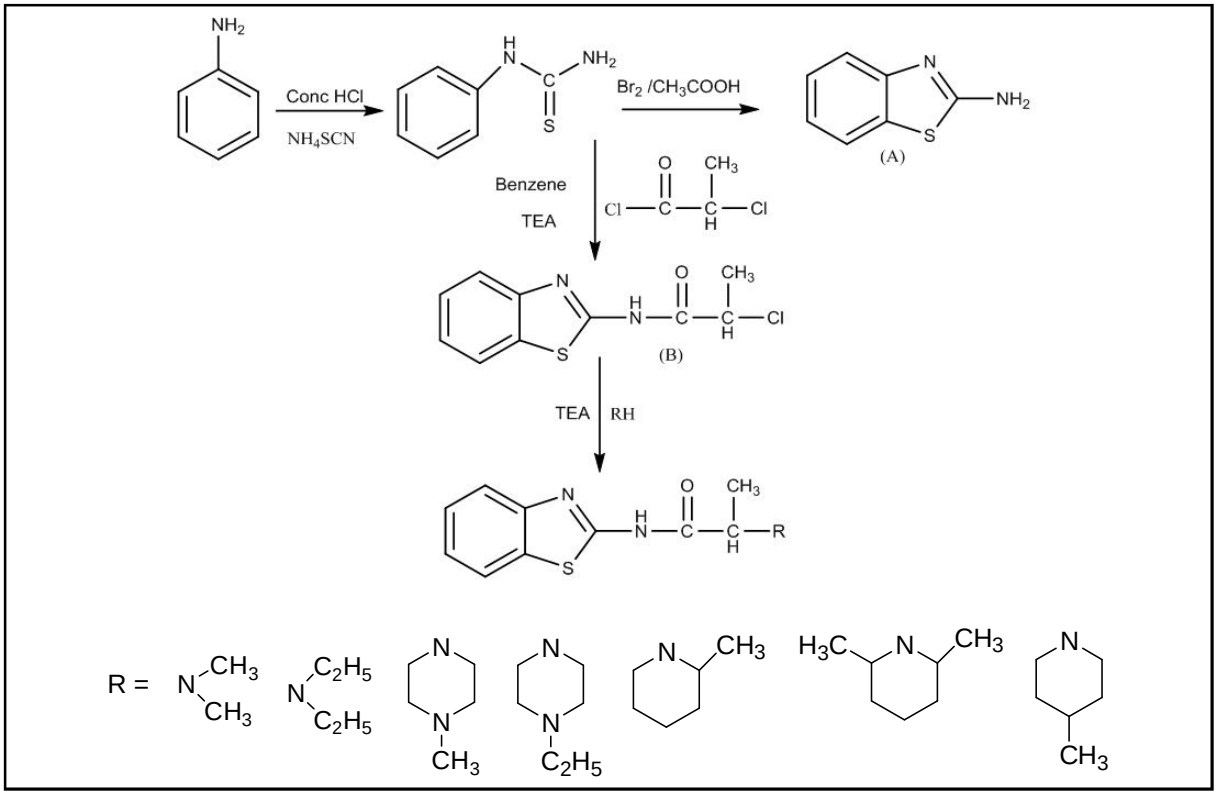
chamber. Infrared (IR) spectra were recorded using IR spectrophotometer (Perkin Elmer) (Das *et al*., 2022). ^{1}H NMR spectra were recorded by using ^{1}H NMR spectrometer (Brucker USA) in $CDCl_3$ with TMS as an internal standard on 300 and 400 MHz. Mass spectra was recorded in DART dried helium was used for ionization mode with JEOL-AccuTOF. Derivatives were refined using column chromatography (Merck silica gel, 60-120 mesh) and purity was observed by thin layer chromatography (Merck TLC plates).

2.1 Reaction scheme



2.2 Synthetic procedure

2.2.1 Benzo[d]thiazole 2-amine (A)

Benzo[d]thiazole 2-amine was synthesized by using equimolar quantity of aniline and ammonium thiocyanate (0.02 mol) mixed with ethanol containing 2 ml of conc. HCl. To this bromine in glacial acetic acid (0.05 mol) was added and refluxed for 1 h then it was cooled in ice cold water. Obtained precipitate was strained well, filtered and washed with ice cold water and dried. The product was recrystallized from rectified spirit (Mallick *et al*., 2013; Venkatesh *et al*., 2009). The purity of the synthesized compound was confirmed by TLC, using silica gel G by using solvent system chloroform : methanol : acetic acid (8:1:1). Yield 72%, m.p. 127°C. MS: (ESI+) m/z = 151 (M+1).

2.2.2 N-benzo [d] thiazol-2-yl)-2-chloropropanamide (B)

N-benzo [d] thiazol-2-yl)-2-chloropropanamide was synthesized by using equimolar quantity of benzo[d]thiazole 2-amine (0.05 mol) and triethylamine (0.05 mol) in dry benzene (30 ml). To this in ice cold condition dropwise 2-chloropropanoylchloride (0.05 mol) was added. Reaction mixture was then stirred for about 6 h and the separated amine hydrochloride was filtered off by using diethyl ether. The filtrate was again heated on a water bath for about 4-5 h, concentrated at reduced pressure and the separated solid was purified over the column of silica gel using chloroform as eluent. The compound was recrystallized by ethanol. The purity of the synthesized compound was checked by TLC, using silica gel G as stationary phase. The solvent system was used as chloroform : methanol : acetic acid (8:1:1). Yield 65 %, MS : (ESI+) m/z = 241 (M+1).

2.2.3 N-benzo [D]thiazol-2-Yl)-2-chloropropanamide derivatives (A1-A4)

Equimolar solution of N-benzo[d]thiazol-2-yl) 2-chloro-propanamide and various amines (dimethyl amines, diethylamine, 4-methyl piperazine and 4-ethyl piperazine) in presence of triethylamine were stirred for 6 h at 25°C. The synthesized compound was collected by using diethyl ether and ether was evaporated to get compound. The purity of the synthesized compound was checked by TLC by using solvent system chloroform : methanol : acetic acid (8:1:1).

2.2.4 N-benzo [d] thiazol-2-yl)-2-chloropropanamide derivatives (A5-A7)

Equimolar mixture of N-(benzo[d]thiazol-2-yl)propanamide and amines (2- methylpiperidine, 2,6 dimethyl piperidine and 4-methyl piperidine) (0.1 mole) were refluxed for 5-6 h in presence of DMF. The reaction mixture was cooled and poured into crushed ice. The compound is extracted from diethyl ether. The purity of the synthesized compound was checked by TLC, using silica gel G as

stationary phase. The solvent system was used as cyclohexane : ethyl acetate (8:2). Solid, mp 167°C, IR vmax (KBr/cm-1): 3404 (NH), 2976 (C-H_{str}, Ar), 1534 (C=N), 1636 (C=O), 1313 (C-S), 920, 746 (Ar-H bending vibration).

2.3 Anticonvulsant activity

All newly synthesized derivatives were estimated for anticonvulsant activity on Sprague Dawley (S.D.) rats using the maximal electroshock seizures (MES) method (Kumar *et al*., 2022). The outcomes from this study are shown in Table 2. The synthesized novel derivatives were getting protective against MES induced seizures at the dose 30, 100 and 300 mg/kg body weight (Losher *et al*., 1991). The preliminary pharmacological screening revealed that one compound (A6) showed maximum (68%) anticonvulsant protection and some of them (A1, A2, A3, A5 and A7) showed moderate anticonvulsant protection (52 %, 51%, 41%, 45% and 45%), whereas compound A4 showed minimum (8%) anticonvulsant protection. None of the derivative has shown neurotoxicity at the dose of 30 mg/kg body weight.

Standard drug: Phenytoin

Test compounds: All synthesized compounds 30 mg, 100 mg and 300 mg/kg body weight.

Control group: 1 % aqueous CMC suspension.

Equipment's: Electroconvulsiometer (Decibel Instrument, model no. 5832) and Rota rod apparatus (Medicraft, Model No. 519/E-30) (Kitano *et al.*, 1995; Krall *et al*., 1978; Losher *et al*., 1991).

2.4 Neurotoxicity study

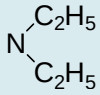
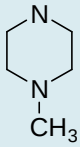
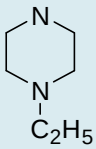
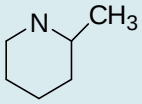
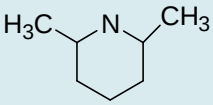
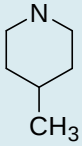
The screening of the newly synthesized derivatives interfering with motor coordination was evaluated by rotarod test. S.D. rat were trained to balance on the rotating rod, that rotates 6 rpm. Trained animals were feeded with test compounds. Neurological impairment (*e.g*., sedation, hyper excitability and ataxia) was determined by the incapacity of animal to maintain equilibrium on rotarod for 1 min in each of three successive trials (Kucukguzel *et al*., 2004).

The experimental animals were administered intraperitonially test compounds at different doses 30, 100 and 300 mg/kg body weight at different time interval. The data in the given table specified the minimum dose by virtue of activity was demonstrated in half or more of the animals. The experimental animals were tested 0.5 and 4 h after administration were made. ED_{50} is calculated using XLSOFT (Version 2012.2.01) on account of maximum effect observed at ½ hour (Choi *et al.*, 1996).

3. Results

All the synthesized molecules were confirmed by spectroscopic techniques (^{1}H NMR, IR, elemental analysis and mass spectroscopy) and melting point study.

Table 1: Physical date of synthesized compounds (A1-A7)

S.No.	Compound	R	Molecular formula	Molecular weight	Yield	R_f value
1.	A1		$C_{12}H_{15}N_3OS$	249	72%	0.70
2.	A2		$C_{14}H_{19}N_3OS$	277	70%	0.68
3.	A3		$C_{15}H_{20}N_4OS$	304	58%	0.65
4.	A4		$C_{16}H_{22}N_4OS$	318	61%	0.64
5.	A5		$C_{16}H_{21}N_3OS$	303	62%	0.71
6.	A6		$C_{17}H_{23}N_3OS$	317	68%	0.68
7.	A7		$C_{16}H_{21}N_3OS$	303	70%	0.70

3.1 Spectral characterization

N-(benzo[d]thiazol-2-yl)-2-(dimethyl-amino)-propanamide (A1)

Solid, m.p. 213ºC, IR v_{max} (KBr/cm^{-1}): 3394 (NH), 2934 (C-H$_{str}$, Ar), 1534 (C=N), 1631 (C=O), 1220 (C-S), 885, 770 (Ar-H bending vibration). ^{1}H NMR ($CDCl_3$) d = 12.486 (s, ^{1}H, -NH), 6.681-8.196 (m, 4H, Ar-H), 3.754 (q, 1H, -CH-CH_3), 2.260 (s, 3H, -N-CH_3), 1.483 (d, 3H, -CH_3). MS: (ESI+) m/z = 250 (M^+1).

N-(benzo[d]thiazol-2-yl)-2-(diethyl-amino)-propanamide (A2)

Solid, m.p. 237ºC, IR v_{max} (KBr/cm^{-1}): 3387 (NH), 2921 (C-H$_{str}$, Ar), 1532 (C=N), 1640 (C=O), 1308 (C-S), 884, 742 (Ar-H bending vibration). ^{1}H NMR ($CDCl_3$) d = 12.248 (s, 1H,-NH), 6.840-8.186 (m, 4H, Ar-H), 3.494 (q, 1H, -CH-CH_3), 2.83 (q, 2H,-CH_2-CH_3), 2.089 (d, 3H,-CH-CH_3), 1.154 (t, 2H, N-CH_2-CH_3). MS: (ESI+) m/z =277 (M^+), 278 (M^+1).

N-(benzo[d]thiazol-2-yl)-2-(4-methylpiperazin-1-yl)-propanamide (A3)

Solid, m.p. 187ºC, IR v_{max} (KBr/cm^{-1}): 3384 (NH), 3080 (C-H$_{str}$, Ar), 1541 (C=N), 1637 (C=O), 1259 (C-S), 852 (Ar-H bending vibration). ^{1}H NMR ($CDCl_3$) d = 12.426 (s, 1H,-NH), 7.280-8.168 (m, 4H, Ar-H), 3.682 (q, 1H, -CH-CH_3), 2.768 (s, 8H, piperazine), 2.269 (s, 3H,-N-CH_3), 1.282 (d, 3H, -CH_3).Anal. Calcd. for $C_{15}H_{20}N_4OS$: C, 59.18; H, 6.62; N, 18.41 %. Found: C, 59.33; H, 6.56; N, 18.11 %.

N-(benzo[d]thiazol-2-yl)-2-(4-ethylpiperazin-1-yl)-propanamide (A4)

Solid, m.p. 196ºC, IR v_{max} (KBr/cm^{-1}): 3394 (NH), 3055 (C-H$_{str}$, Ar), 1529 (C=N), 1643 (C=O), 1307 (C-S), 887 (Ar-H bending vibration). ^{1}H NMR ($CDCl_3$) d = 12.479 (s, 1H, -NH), 8.016 (m, 4H, Ar-H), 3.741 (q, 1H, -CH_3), 2.318 (q, 2H, -CH_2), 2.712 (s, 8H, piperazine), 1.022 (t, 3H, -CH_3). MS: (ESI+) m/z =318 (M^+), 319 (M^{+1}).

N-(benzo[d]thiazol-2-yl)-2-(2-methylpiperidin-1-yl)propanamide (A5)

Solid, m.p. 167ºC, IR v_{max} (KBr/cm^{-1}): 3404 (NH), 2976(C-H$_{str}$, Ar), 1534 (C=N), 1636 (C=O), 1313 (C-S), 920, 746 (Ar-H bending vibration). ^{1}H NMR ($CDCl_3$) d = 12.484 (s, 1H, -NH), 8.182 (m, 4H, Ar-H), 3.624 (q, 1H, -CH_3),2.523 (m, 2H, -piperidine),1.512 (m, 6H, piperidine),1.122 (d, 1H, -CH-CH_3).MS: (ESI+) m/z =303 (M^+).

N-(benzo[d]thiazol-2-yl)-2-(2,6 di-methylpiperidin-1-yl)propanamide (A6)

Solid, m.p. 176ºC, IR v_{max} (KBr/cm^{-1}): 3397 (NH), 3060(C-H$_{str}$, Ar), 1529 (C=N), 1640 (C=O), 1311 (C-S), 886, 741 (Ar-H bending vibration). ^{1}H NMR ($CDCl_3$) d = 11.912 (s, 1H, -NH),8.110 (m, 4H, Ar-H), 3.262 (q, ^{1}H, -CH_3),2.422 (m, 2H, -CH), 1.422 (m, 6H, piperidine),1.102 (d, ^{1}H, -CH_3). MS: (ESI+) m/z =317 (M^+).

N-(benzo[d]thiazol-2-yl)-2-(4-methylpiperidin-1-yl)propanamide (A7)

Solid, m.p. 161ºC, IR v_{max} (KBr/cm^{-1}): 3424 (NH), 3066(C-H$_{str}$, Ar), 1536 (C=N), 1620 (C=O), 1309 (C-S), 881, 726 (Ar-H bending vibration). ^{1}H NMR ($CDCl_3$) d = 12.324 (s, 1H, -NH), 7.982 (m, 4H, Ar-H), 3.112 (q, 1H, -CH_3), 2.521 (t, 2H, -CH_2), 1.597 (q, 1H, -CH_3). MS: (ESI+) m/z =303 (M^+), 304 (M^{+1}).

Table 2: MES activity and neurotoxicity of synthesized compounds

Compound	Dose (mg/kg^{-1})	MES		Neurotoxicity		ED_{50} (mgkg^{-1})	ED_{50} (m molkg^{-1})	Activity in comparison to phenytoin
		½ h	04 h	½ h	04 h			
A1	30	2/6	1/6	0/6	0/6	30.7	0.10	0.52
	100	5/6	4/6	3/6	3/6			
	300	5/6	5/6	4/6	4/6			
A2	30	3/6	2/6	0/6	0/6	30	0.10	0.51
	100	5/6	5/6	3/6	3/6			
	300	6/6	6/6	4/6	4/6			
A3	30	3/6	2/6	0/6	0/6	31.5	0.10	0.41
	100	4/6	5/6	3/6	3/6			
	300	6/6	6/6	4/6	4/6			
A4	30	2/6	2/6	0/6	0/6	143.6	0.05	0.08
	100	3/6	1/6	3/6	3/6			
	300	4/6	2/6	4/6	4/6			
A5	30	3/6	3/6	0/6	0/6	30	0.09	0.45
	100	5/6	4/6	3/6	2/6			
	300	6/6	6/6	4/6	3/6			
A6	30	4/6	4/6	0/6	0/6	18.5	0.05	0.68
	100	5/6	5/6	3/6	3/6			
	300	6/6	6/6	4/6	2/6			
A7	30	3/6	1/6	0/6	0/6	31.6	0.10	0.45
	100	4/6	3/6	3/6	1/6			
	300	5/6	5/6	4/6	3/6			
Phenytoin	10	3/6	3/6	0/6	0/6	10.3	0.041	01
	30	4/6	4/6	3/6	3/6			
	100	5/6	6/6	4/6	4/6			

4. Discussion

Since many therapeutically effective anticonvulsants feature a heteroatomic system with a phenyl ring and electron donor system, we developed and synthesized the compounds listed above in the current experiment. All synthesized compounds (A1-A7) fulfill the required pharmacophore have been achieved by reaction scheme 2.1.

All the titled compounds showed significant anticonvulsant activity except compound N-(benzo[d]thiazol-2-yl)-2-(4-ethylpiperazin-1-yl)-propanamide (A4), Compounds like N-(benzo[d]thiazol-2-yl)-2-(dimethyl-amino)-propanamide(A1), N-(benzo[d]thiazol-2-yl)-2-(diethyl-amino)-propanamide (A2), N-(benzo[d]thiazol-2-yl)-2-(4-methylpiperazin-1-yl)-propanamide (A3), N-(benzo[d]thiazol-2-yl)-2-(2-methylpiperidin-1-yl)propanamide (A5) and N-(benzo[d]thiazol-2-yl)-2-(4-methylpiperidin-1-yl)propanamide(A7) showed moderate activity and compound N-(benzo[d]thiazol-2-yl)-2-(2,6 di-methylpiperidin-1-yl)propanamide (A6) showed maximum activity.

5. Conclusion

All the novel synthesized compounds (A1-A7) were screened for anticonvulsant activity using phenytoin as standard drug. All the compounds were found active and satisfy the basic pharmacophore for anticonvulsant activity, *i.e.*, hydrophobic domain, electron donor system, hydrogen bonding domain and distal amine residue. It indicates that all these pharmacophore are necessary for the anticonvulsant activity.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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