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ResearchArticle

Synthesis, Characterization & Pharmacological evaluation of some newer Benzothiazole derivatives

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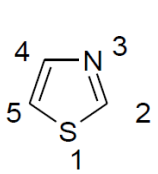
Abstract

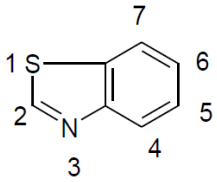
The objective of the present work is to develop safer new chemical entities that show good anticonvulsant effects and antimicrobial actions. The current work describes the synthesis of Benzothiazole derivatives with encouraging anticonvulsant activity against MES and scPTZ tests with interesting pattern of antimicrobial effects. The synthesized Benzothiazole derivatives could be considered as lead molecule for the development of therapeutic agents. Many polycyclic and fused ring systems containing the thiazolenucleus(I) are well known. The most important is bicyclic system wherein the second ring benzene is fused to the 4,5 position of thiazole ring i.e. Benzothiazole.(II)

Introduction

Epilepsy is widespread among the general population with over two million affected individuals in the United States. Epilepsy is not a single entity; it is a family of different recurrent seizure disorders that have in common the sudden, excessive and disorderly discharge of cerebral neurons. This results in abnormal movements or perceptions that are of short duration but that tend to recur. The site of the electrical discharge determines the symptoms that are produced. For example, epileptic seizures may cause convulsions if the motor cortex is involved. The seizures may include visual, auditory, or olfactory hallucinations if the parietal or occipital cortex plays a role. Drug therapy is the most widely effective mode of treatment for epilepsy. Seizures can be controlled completely in approximately 50% of epileptic patients, and meaningful improvement is achieved in at least one half of the remaining patients. Epilepsy is one of the most common disorders of the brain, affecting about 50 million individuals worldwide. Although 70-80% of all epileptics are adequately treated by current available drugs, seizure protection is often accompanied by numerous side effects including drowsiness, ataxia, gastrointestinal disturbances, gingival hyper plasia, hirsuitism and megaloblastic anaemia.Many polycyclic and fused ring systems containing the thiazole nucleus (I) are well known. The most important is bicyclic system wherein the second ring benzene is fused to the 4,5 position of thiazole ring i.e. Benzothiazole.(II)

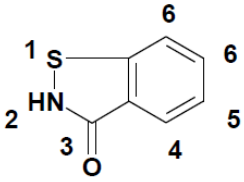


(I)Thiazole



(II)Benzothiazole

On changing the position of Nitrogen in 5-membered ring the another possible structure would be,(III) e.g. saccharin



(III) Benzothiazolin-3-one

In recent decades there has been constant interest in the chemistry of benzothiazole. Among compounds of this type substances with high and varied biological activity and a wide spectrum of practical qualities have been found (polymethine dyes, stabilizers of polymeric materials, antioxidants, optical sensitizers for photographic materials, etc.). Some of them were isolated from natural materials, e.g., the alkaloid luciferin [2-(2-benzothiazolyl)-^{2}thiazoline-4-carboxylic acid] and a bioluminescent [2-(5-hydroxy-2-benzothiazolyl)thiazole-4-carboxylic acid]. Benzothiazole and

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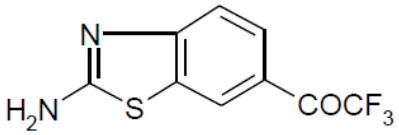
its many derivatives are particularly important. The history of Benzothiazole dates from 1878 when, by the action of PCl5 on phenylthiocarbamide, A.W.Hoffmann prepared a reactive chloro compound(2-chlorobenzothiazole) which was later reduced to Benzothiazole(II).He also prepared 2-phenylbenzothiazole by heating benzanilide with sulphur and, supported by analogies with the known imidazoles and benzoxazoles, correctly assigned structures to the compounds on the basis of their degradation to, and synthesis from *o*-aminothiophenol.[1] The tendency of formation of Benzothiazole ring system appears to be great, and ring closure occurs readily with a large variety of compounds. Thus on treating aromatic amines or their derivative with sulfur at high temperature (180-250˚C) frequently produces Benzothiazole in satisfactory yield (up to 75%).similarly various reactants under similar condition give[2];

Properties

Benzothiazole is colorless mobile liquid resembling quinolin in odour and is insoluble in water but dissolves in strong aqueous acid forming salts: hydrochloride, m.p.173˚; sulphate m.p.174˚; picrate m.p.168˚. Benzothiazolemethiodide and ethiodide have m.p.210˚and 139˚ respectively. Benzothiazole is a feeble base, the ring system is stable but fusion of benzothiazole with alkali opens the heterocyclic ring. Benzothiazole is nitrated at position 6 and sulphonated at position 4, 6 & 7.Bromine which forms a perbromide in chloroform reacts at 450˚ to form 2-bromo- benzothiazole[3]. Substitution in 2-position takes place with PCl5 and with sodamide to give the 2-chloro and 2-amino compound respectively, and at this position halogen substituents are mobile, methyl group is reactive and amino-group is diazotisable. On the other hand there is marked tendency in 2-amino-,2-hydroxy- and 2-mercapto-benzothiazoles to assume the tautomeric forms derived from 2,3-dihydrobenzothiazole. Benzothiazole-S-dioxide, m.p. 105˚-107˚, is said to be produced from *o*-formamidophenyl methyl sulphone and phosphorus oxychloride.

Pharmacological Activities

Benzothiazole derivatives are of particular interest within the realm of medicinal chemistry[4]. They possess selective antitumor activities, analgesic, anti-inflammatory, antiarthritic, antitumor, anti-tubercular, anti-convulsant, antibacterial, anti-fungal and anti-microbial properties etc.Compounds containing a thiophene moiety also possess a wide range of biological activities.[5-7] In 1950s, a number of 2-aminobenzothiazole were intensively studied as central muscle relaxant, Riluzole (6-trifluoromethoxy-2- benzothiazolamine) was found to interfere with Glutamate neurotransmission in biochemical electrophysiological behavior experiments[8-13].



Riluzole

Material & Methods

Reagents & Solvents

Most of the solvents were of LR grade and purified prior to use. chemicals were obtained from Central Drug House Pvt.Ltd., E.Merck and S.D.Fine Chemicals Ltd.

Equipments

The melting points of newly synthesized compounds were determined in open glass capillary and are uncorrected. All the Infra Red (IR) spectra were recorded in KBr on JASCO FT-IR 410 spectrophotometer. Proton Magnetic Resonance (^{1}H NMR)spectra were recorded on BRUCKER MODEL DRX-300 NMR spectrometer in CDCl3 and DMSO-d6 using Tetramethylsilane(TMS) as the internal reference.chemical shifts are reported in part per million (ppm;δ) and the signals are described as a singlet (s), doublet(d), triplet(t), quartet(q) and multiplet(m). All the reaction were monitored by Thin Layer Chromatography (TLC) by using Silica gel G (solvent toluene: Ethyl acetate :: 8:2).

Purification of organic Solvents

Commercially available grades of organic solvents are of adequate purity for use in such reaction provided that the presence of small quantities of water (the most wide spread impurity in all organic solvent) is not harmful to reactions. The commercially available grade for general uses are often impurity containing , however ,when the level of impurities including moisture, are acceptable for particular reaction and when large volume of such solvents are likely to be required , it is frequently more economical to purify the commercial grade.

Dry Chlorobenzene

Dry chloro benzene is frequenty required as a refluxing solvent.Chloro benzene is dried by pouring sodium wire inside the bottle containing solvents.

Ethanol

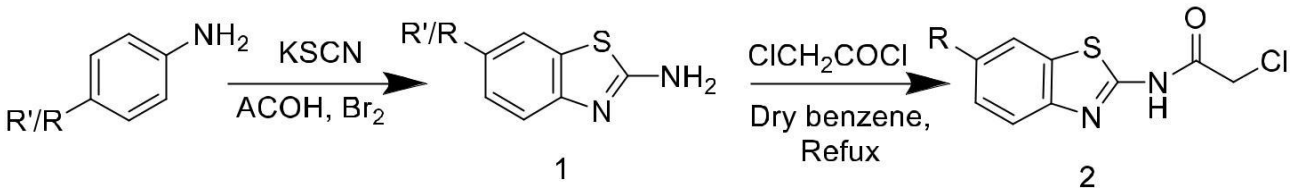
Ethanol of high degree of purity is frequently required in preparative organic chemistry. For some purpose ethanol of 99.5% purity is required. Rectified spirit is the constant boiling mixture, which, ethanol forms with water and usually contains 95.6% of ethanol by weight. Generally purification of alcohol is done by distillation method.

Absolute alcohol

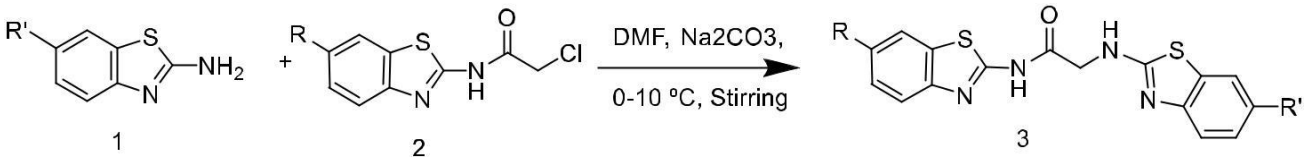
Rectified spirit (700 ml) was poured into 1L round bottomed flask and added 200g of calcium oxide , freshly ignited over Bunsen flame. Flask was fitted with double surface condenser carrying a calcium chloride guard tube. The mixture was refluxed for 6 hrs and allowed to stand overnight. Then ethanol was distilled , the first 20 ml was discarded.

Synthetic Scheme

I. SCHEME 1:



II. SCHEME 2:



Synthesis of titled compounds 3(a-o)

Procedure for the preparation of benzothiazol-2-amine (1) To a mixture of aniline derivatives (0.1 mole) and KSCN (0.1 mole) dissolved in 100 ml glacial acetic acid, Br2 (0.1mole) in glacial acetic acid (20 ml) was added drop wise with stirring while keeping the temperature below 10 ^{o}C throughout the addition. Finally the solution was filtered washed with water and recrystallize with ethanol.

Procedure for the preparation of N-(6-chlorobenzothiazol-2-yl)acetamide (2) 2-aminobenzothiazole (0.05 mole, 9.22g) and chloroacetylchloride (0.05 mole, 3.98 ml) in dry benzene was refluxed for 1 hr. The solution was filtered and residue was washed with benzene and then with sodium bicarbonate solution. Finally it was washed with distilled water and recrystallized from ethanol. Yield 80%.

General procedure for the preparation of N-(Benzo[d]thiazol-2-yl)-2-(benzo[d]thiazol-2-yl)amino) acetamide (3a-o) To substituted benzothiazolamine (1) (0.0019 mole) dissolved in dry DMF (30 ml) was added potassium carbonate (0.0019 mole, 0.274 ml). To this 2-N-(6-chlorobenzothiazol-2-yl)acetamide (2) (0.0019 mole, 500 mg) was added slowly and refluxed for 6 hrs. The solid thus obtained was filtered and washed with benzene and then with distilled water and recrystallized from ethanol.The synthesis of substituted Benzothiazole derivatives by the described above method remitted in products with good yield.

Table 1: Physical of data of substituted compounds

Compound Name	Com. No.	Mol. Wt.	R_f value	M.P(°C)
N-(Benzo[d]thiazol-2-yl)-2-((6-fluorobenzo[d]thiazol-2-yl)amino)acetamide	3a	358	0.6	211^{o}C
N-(Benzo[d]thiazol-2-yl)-2-((6-chlorobenzo[d]thiazol-2-yl)amino)acetamide	3b	374	0.55	204^{o}C
N-(Benzo[d]thiazol-2-yl)-2-((6-bromobenzo[d]thiazol-2-yl)amino)acetamide	3c	417	0.65	198^{o}C
N-(Benzo[d]thiazol-2-yl)-2-((6-methylbenzo[d]thiazol-2-yl)amino)acetamide	3d	354	0.62	222^{o}C
N-(Benzo[d]thiazol-2-yl)-2-((6-methoxybenzo[d]thiazol-2-yl)amino) acetamide	3e	370	0.58	202^{o}C
N-(6-chlorobenzo[d]thiazol-2-yl)-2-((6-fluorobenzo[d]thiazol-2-yl)amino)acetamide	3f	392	0.72	178^{o}C
N-(6-chlorobenzo[d]thiazol-2-yl)-2-((6-chlorobenzo[d]thiazol-2-yl)amino) acetamide	3g	407	0.65	208^{o}C
2-((6-bromobenzo[d]thiazol-2-yl)amino)-N-(6-chlorobenzo[d]thiazol-2-yl) acetamide	3h	451	0.60	211^{o}C
N-(6-chlorobenzo[d]thiazol-2-yl)-2-((6methylbenzo[d]thiazol-2-yl)amino) acetamide	3i	388	0.6	186^{o}C
=N-(6-chlorobenzo[d]thiazol-2-yl)-2-((6-methoxybenzo[d]thiazol-2-yl)amino) acetamide	3j	404	0.55	192^{o}C

2-((6-fluorobenzo[d]thiazol-2-yl)amino) N(6methylbenzo[d]thiazol-2-yl) acetamide	3k	372	0.70	232^{o}C
2-((6-chlorobenzo[d]thiazol-2-yl)amino)-N-(6-methylbenzo[d]thiazol-2-yl) acetamide	3l	388	0.75	228^{o}C
2-((6-bromobenzo[d]thiazol-2-yl)amino)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide	3m	431	0.68	184^{o}C
N-(6-methylbenzo[d]thiazol-2-yl)-2-((6-methylbenzo[d]thiazol-2-yl)amino) acetamide	3n	368	0.73	196^{o}C
2-((6-methoxybenzo[d]thiazol-2-yl)amino)-N-(6-methylbenzo[d]thiazol-2-yl) acetamide	3o	384	0.70	210^{o}C

Methodology For Anticonvulsant activity

MES Test

Each compound will be administered as an i.p. injection at dose level of 30 mg per kg and the anticonvulsant activity was assessed after 0.5 hr and 4 hr interval of administration. Maximal electroshock seizures were elicited in mice by delivering a 60 Hz, 50 mA electric stimuli for 0.2 sec via ear clip electrode[14]. The maximal electroshock seizures typically consists of a short period of tonic extension of the hind limbs and a final clonic episode. Blocking of the limb's tonic extensor component due to the drug treatment is taken as the end point[15-16].The reading was taken on Electroconvulsometer.

Pentylenetetrazole induced seizure (scPTZ) test

The subcutaneous pentylenetetrazole test was performed according to the known protocol. This method utilizes pentylenetetrazole (75 mg/kg) that produces seizures in > 95% of animals as a 0.5% solution subcutaneously in the posterior midline. The animals were observed for 30 min. Failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection.

Neurotoxicity screening (NT)

The minimal motor impairment was measured in mice by the rotarod test. The mice were trained to stay on an accelerating rotarod of diameter 3.2 cm that rotates at 10 rpm. Trained animals were given i.p. injection of the test compounds 30, 100 and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the trials.

Result of Anticonvulsant Activity

Table 2: Minimum inhibitory concentrations (MICs, µg/ml) of newly synthesized derivatives

Compound	R	R'	MIC (µg/ml) *S. aureus* NCTC 6571	MIC (µg/ml) *E. coli* ATCC 25922	MIC (µg/ml) *A. niger* ATCC 9029
3a	H	F	12.5	12.5	50
3b	H	Cl	100	100	>200
3c	H	Br	100	100	>200
3d	H	CH3	>200	>200	>200
3e	H	OCH3	>200	>200	>200
3f	Cl	F	25	25	50
3g	Cl	Cl	100	100	>200
3h	Cl	Br	100	100	>200
3i	Cl	CH3	>200	>200	>200

3j	Cl	OCH3	>200	>200		>200
3k	CH3	F	25	25		50
3l	CH3	Cl	100	100		>200
3m	CH3	Br	100	100		>200
3n	CH3	CH3	>200	>200		>200
3o	CH3	OCH3	>200	>200		>200
Ofloxacin			12.5	12.5		-
Ketoconazole			-	-		12.5

Table 3: Preliminary anticonvulsant screening of compounds 3a-o in mice (*i.p.* administration)

Compounds	**R**	**R'**	**MES**		**Sc-PTZ**		**Neurotoxicity**
			0.5 h	**4 h**	**0.5 h**	**4 h**	**0.5 h**
3a	H	F	1/4[a]	1/4	1/4[b]	1/4	0/4[c]
3b	H	Cl	1/4	1/4	0/4	1/4	0/4
3c	H	Br	1/4	1/4	1/4	1/4	0/4
3d	H	CH3	0/4	4/4	1/4	4/4	0/4
3e	H	OCH3	0/4	2/4	0/4	2/4	0/4
3f	Cl	F	1/4	1/4	0/4	0/4	0/4
3g	Cl	Cl	1/4	1/4	0/4	0/4	0/4
3h	Cl	Br	1/4	1/4	0/4	0/4	0/4
3i	Cl	CH3	0/4	4/4	0/4	4/4	0/4
3j	Cl	OCH3	0/4	2/4	0/4	2/4	0/4
3k	CH3	F	0/4	0/4	0/4	0/4	0/4
3l	CH3	Cl	0/4	0/4	0/4	0/4	0/4
3m	CH3	Br	1/4	1/4	1/4	1/4	0/4
3n	CH3	CH3	0/4	4/4	0/4	4/4	0/4
3o	CH3	OCH3	0/4	2/4	0/4	2/4	0/4
Phenytoin[c]	-	-	4/4	4/4	-[d]	-	-
Carbamazepine[c]	-	-	4/4	-	4/4	4/4	-

[a] Number of animals protecting the hind limb tonic extension over the total number tested at a dose of 30 mg/Kg b.wt. b Number of animals raising the seizure threshold over the total number tested at a dose of 100 mg/Kg b.wt. c Number of animals showing minimal motor impairment at a dose of 300 mg/Kg over the total tested animals. A dash (-) indicates activity performed.

Result & Discussion

The introduction of diverse bioactive groups at benzothiazolamine and their subsequent screening led to an understanding of electronic property, size and lipophilicity influence at benzothiazole nucleus. The amide linkage –NHCO- with delocalized electrons is a key determinant of anticonvulsant activity, which would be greatly influenced by the nature of adjoining substituent. Therefore, compounds 3(a-o) were synthesized to explore the effects of adding substituents to acetamide linkage at benzothiazole ring. In MES screening, all the synthesized compounds having acetamide linkage were shown to moderate protective action except. The potency order for protection of hind limb tonic extension was observed as methyl >methoxy> fluorine, chlorine, bromine. Out of these, compound 3d, 3i and 3n respectively, showed excellent anti-generalized tonic-clonic activity (100% protection) after 4h at 30mg/kg indicating that these compounds have slow onset and longer duration of action. compound 3e, 3j and 3o respectively, showed moderate anti-generalized tonic-clonic activity (50% protection) after 4h at 30mg/kg indicating that these compounds have slow onset and longer duration of action. In the scPTZ screen, compounds 3d, 3e, 3i, 3j, 3n and 3o elevated seizure threshold at dose of 100 mg/kg after 0.5 h. Results of scPTZ screen showed that the compounds were less effective against the absence seizure whereas more effective against the generalized tonic-clonic seizure. Results from the rotarod motor impairment test demonstrated that all the compounds did not show neurotoxicity at the maximum dose administered (300 mg/kg). There is no sign of any motor impairment and related CNS toxicity associated with these molecules.

Conclusion

These benzothiazole derivatives shows moderate anticonvulsant activities. These compounds are less effective against the absence seizure whereas more effective against the generalized tonic-clonic seizure. the Substitution with the lipophlic methyl group increases the anticonvulsant activity.the Potency order observed was methyl >methoxy> fluorine, chlorine, bromine.

Acknowledgement

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