Synthesis, Characterization And Evaluation Of 2-Imino Benzothiazole Derivatives As Anticonvulsant Agents

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ABSTRACT
Purpose - To synthesize potent benzothiazole derivatives screened to possess anti-convulsant activity as a way forward for treatment of epilepsy.
Method: The present work emphasizes on synthesis of new series of 2-amino-6-substituted benzothiazole (A-D) by using 4-substituted aniline and potassium thiocyanate in presence of bromine in glacial acetic acid which further treated with various substituted aromatic aldehydes in presence of glacial acetic acid to get the 2-imino-benzothiazole derivatives (2A to 2I). (6-Substituted-benzothiazol-2-yl)-(4-substituted-benzylidene)-amine derivatives obtained from the reactional sequence were injected intraperitoneally into mice and evaluated by the maximal electroshock (MES), neurotoxicity screen using rotarod at the dose of 30 mg/kg. Phenytoin was used as the standard for the comparison at the dose level of 25 mg/kg.
Results: The MES activity indicated significant anticonvulsant activity for all of the test compounds. However, they were found to be less potent when compared with the reference standard Phenytoin. Within the series A, B, C and D compounds of series B with substitution of -F at 6\textsuperscript{th} position of benzothiazole showed maximum potency.
Conclusion: All the compounds showed protections against seizures in the range 50-75\% indicative of the promising nature of the compounds against seizure spread. Thus it can be concluded that substitution of 6\textsuperscript{th}-position with fluoro, chloro, bromo and nitro groups (A-D) at the terminal benzothiazole ring is beneficial for anticonvulsant activity.

KEYWORDS
Benzothiazole, Schiff base, anticonvulsant, neurotoxicity, max. electroshock seizure (MES).

INTRODUCTION
Epilepsy has been recognized as a neurological disorder, affecting a large section of people both males and females across the world. Every year approximately 2,50,000 new cases are added to this figure. Many patients have seizures that are resistant to the available medical therapies. Newer drugs such as Topiramate, Zonisamide and Vigabatrin have emerged as promising anticonvulsants. Despite introduction of these new drugs women of child bearing age and chronic patients face specific problems of neurotoxicity, symptoms of depression and CNS related ailments. Therefore, it is essential to search for newer chemical entities for the treatment of epilepsy\textsuperscript{1}. Benzothiazole nucleus has resulted in a large number of compounds having diverse pharmacological activities viz. antibacterial\textsuperscript{2}, antifungal\textsuperscript{3}, antihelminthic\textsuperscript{4}, antitumour\textsuperscript{5}, anti-inflammatory\textsuperscript{6}, antitubercular\textsuperscript{7} etc.
Many anticonvulsants may be placed in the category of the Schiff bases. A common structural feature is the presence of conjugation, usually aromatic ring associated with the imine double bond, which increases the reduction potential of the iminium form. Iminium form is active against partial seizures\textsuperscript{8}. In the 2-imino benzothiazole derivatives, the pharmacophoric element is thought to be a lipophilic aryl ring. The attachment of a second aryl ring, designated as the distal ring to the proximal aryl ring, increases the van der waal’s bonding at the binding site and may also increase potency\textsuperscript{9}.

The chemistry and biological potencies of 2-amino benzothiazole as important class of compounds have been studied. This article is focused on exploring as there is very less understanding known about 2-amino benzothiazole having an imine group at the 2 position. So in the present study, 2-imino benzothiazole derivatives will be evaluated for their anticonvulsant properties.
MATERIALS AND METHODS

The chemicals required were obtained from Hi-media Chem. Ltd, SD-Fine Ltd. and Sigma Aldrich Pvt. Ltd. and were used as such. Melting points were determined using open capillary melting point apparatus and are uncorrected. Reactions were monitored by thin layer chromatography (TLC) on silica gel G plates using methanol : chloroform (0.3 : 9.7), methanol : chloroform (1:9) with iodine vapors in UV chamber for visualizing spots. After physical characterization, the compounds were subjected to spectral analysis. Proton Nuclear Magnetic resonance spectra were recorded on Bruker WM-300 (at 300 MHz) spectrometer and chemical shifts were reported in parts per million (δ value) from TMS (δ 0 ppm for 1H NMR) as an internal standard. Coupling constant were given in Hertz. Mass spectra were recorded on a JEOL-SX-102 instrument using ESI. Infrared spectra were taken on Perkin-Elmer AX-1 spectrometer and values are expressed in cm⁻¹.

Syntheses of 6-substituted-1,3-benzothiazole-2-amines (A-D)

Substituted anilines (0.01 mol) and potassium thiocyanate (0.01 mol) were dissolved in glacial acetic acid, cooled and stirred for 15 min. Cold bromine solution (0.01 mol, 3 mL in 10 mL acetic acid) was added dropwise. The mixture was continuously stirred for additional 3 h. Separated hydrochloride salt was filtered off, washed with acetic acid, dissolved in hot water and neutralized with aqueous ammonium solution (25%). The resulting precipitate was filtered off, washed with water and recrystallized from ethanol to get the desired compounds (A-D). The progress of reaction was monitored by TLC using methanol : chloroform (1:9).

Scheme 1

6-chloro-benzothiazol-2-ylamine (A)

Yield: 52%, melting range 184-186°C. IR (KBr pellet) (Ar) C=C str, 1458.0 cm⁻¹; C=N str, 1650.7 cm⁻¹; (Aromatic) N-H str, 3412.5 cm⁻¹. ESI m/z: 185.30 (M+ peak).

6-Fluoro-benzothiazol-2-ylamine (B)

Yield: 45%, melting range 178-180°C. IR (KBr pellet) (Ar) C=C str, 1449.9 cm⁻¹; C=N str, 1603.8 cm⁻¹; (Aromatic) N-H str, 3439.8 cm⁻¹.

6-Bromo-benzothiazol-2-ylamine (C)

Yield: 84%, melting range 164-166°C. IR (KBr pellet) (Ar) C=C str, 1444.58 cm⁻¹; C=N str, 1633.59 cm⁻¹; (Aromatic) N-H str, 3452.34 cm⁻¹.

6-Nitro-benzothiazol-2-ylamine (D)

Yield: 60%, melting range 198-200°C. IR (KBr pellet) (Ar) C=C str, 1572.8 cm⁻¹; C=N str, 1650.0 cm⁻¹; (Aromatic) N-H str, 3436.6 cm⁻¹. ESI m/z: 196.2 (M+ peak).

Syntheses of 2-imino-benzothiazole derivatives (2A-I)

The compound (0.01 mol) 6-substituted-benzothiazol-2-ylamine (A-D) and (0.01mol) substituted aromatic aldehydes were refluxed in ethanol (20 ml) for 9-10 hrs. The resultant solution was cooled and solid was precipitated out. The solid substance was filtered and recrystallised by ethanol. The progress of reaction was monitored by TLC using methanol : chloroform (0.3:9.7).
Scheme 2

(6-Chloro-benzothiazol-2-yl)-(4-Chloro-benzylidene)-amine (2A)
 Yield: 78%, melting range 200-202°C. IR (KBr pellet) (Aliphatic) C-H str, 2854.45 cm\(^{-1}\); C=N str, 1616.24 cm\(^{-1}\); (Aromatic) C=C str, 1558.38 cm\(^{-1}\). ESI m/z: 309.0025 (M+ peak). \(^1\)H NMR (CDCl\(_3\)): δ 7.425-7.88 (m, 3H), δ 7.816-7.978 (m, 4H), δ 9.035 (s, H).

(6-Chloro-benzothiazol-2-yl)-(4-dimethylamino-benzylidene)-amine (2B)
 Yield: 60%, melting range 206-208°C. IR (KBr pellet) (Aliphatic) C-H str, 2854.45 cm\(^{-1}\); C=N str, 1659.23 cm\(^{-1}\); (Aromatic) C=C str, 1529.45 cm\(^{-1}\). ESI m/z: 316.0870 (M+ peak). \(^1\)H NMR (CDCl\(_3\)): δ 7.425-7.88 (m, 3H), δ 7.816-7.978 (m, 4H), δ 9.035 (s, H).

(6-Chloro-benzothiazol-2-yl)-(4-methoxy-benzylidene)-amine (2C)
 Yield: 40%, melting range 130-132°C. IR (KBr pellet) (Aliphatic) C-H str, 2923.88 cm\(^{-1}\); C=N str, 1593.09 cm\(^{-1}\); (Aromatic) C=C str, 1515.94 cm\(^{-1}\). ESI m/z: 303.2934 (M+ peak). \(^1\)H NMR (CDCl\(_3\)): δ 3.1083 (s, 6H), δ 6.7145-7.3948 (m, 3H), δ 7.7489-7.8996 (m, 4H), δ 9.036 (s, H).

(6-Chloro-benzothiazol-2-yl)-(2-methyl-benzylidene)-amine (2D)
 Yield: 34%, melting range 168-170°C. IR (KBr pellet) (Aliphatic) C-H str, 3060.82 cm\(^{-1}\); C=N str, 1589.23 cm\(^{-1}\); (Aromatic) C=C str, 1529.45 cm\(^{-1}\). ESI m/z: 287.0598 (M+ peak). \(^1\)H NMR (CDCl\(_3\)): δ 3.115 (s, 3H), δ 7.298-7.484 (m, 4H), δ 7.812-8.205 (m, 3H), δ 9.365 (s, H).

(6-Fluoro-benzothiazol-2-yl)-(4-chloro-benzylidene)-amine (2E)
 Yield: 40%, melting range 192-194°C. IR (KBr pellet) (Aliphatic) C-H str, 2923.88 cm\(^{-1}\); C=N str, 1593.09 cm\(^{-1}\); (Aromatic) C=C str, 1515.94 cm\(^{-1}\). ESI m/z: 303.2934 (M+ peak). \(^1\)H NMR (CDCl\(_3\)): δ 3.115 (s, 3H), δ 6.725-6.753 (d, 2H), δ 7.128-7.186 (t, 1H), δ 7.466-7.493 (d, 1H), δ 8.823 (s, H).

(6-Fluoro-benzothiazol-2-yl)-(4-dimethylamino-benzylidene)-amine (2F)
 Yield: 66%, melting range 188-190°C. IR (KBr pellet) (Aliphatic) C-H str, 2923.88 cm\(^{-1}\); C=N str, 1593.09 cm\(^{-1}\); (Aromatic) C=C str, 1515.94 cm\(^{-1}\). ESI m/z: 291.332 (M+ peak). \(^1\)H NMR (CDCl\(_3\)): δ 3.115 (s, 3H), δ 7.298-7.484 (m, 4H), δ 7.812-8.205 (m, 3H), δ 9.365 (s, H).

(6-Fluoro-benzothiazol-2-yl)-(4-methoxy-benzylidene)-amine (2G)
 Yield: 54%, melting range 134-136°C. IR (KBr pellet) (Aliphatic) C-H str, 2837.09 cm\(^{-1}\); C=N str, 1560.30 cm\(^{-1}\); (Aromatic) C=C str, 1569.95 cm\(^{-1}\). ESI m/z: 287.0598 (M+ peak). \(^1\)H NMR (CDCl\(_3\)): δ 3.093-3.145 (s, 6H), δ 6.739-7.590 (m, 4H), δ 8.212-8.718 (m, 3H), δ 8.899 (s, H).

(6-Bromo-benzothiazol-2-yl)-(4-dimethylamino-benzylidene)-amine (2H)
 Yield: 78%, melting range 180-182°C. IR (KBr pellet) (Aliphatic) C-H str, 2837.09 cm\(^{-1}\); C=N str, 1595.02 cm\(^{-1}\); (Aromatic) C=C str, 1560.30 cm\(^{-1}\). ESI m/z: 287.0598 (M+ peak). \(^1\)H NMR (CDCl\(_3\)): δ 3.128 (s, 3H), δ 6.725-6.753 (d, 1H), δ 7.128-7.186 (t, 1H), δ 7.466-7.493 (d, 1H), δ 8.827 (s, H).

(6-Nitro-benzothiazol-2-yl)-(4-dimethylamino-benzylidene)-amine (2I)
 Yield: 70%, melting range 144-146°C. IR (KBr pellet) (Aliphatic) C-H str, 2921.96 cm\(^{-1}\); C=N str, 1664.45 cm\(^{-1}\); (Aromatic) C=C str, 1602.74 cm\(^{-1}\). ESI m/z: 327.1151 (M+ peak). \(^1\)H NMR (CDCl\(_3\)): δ 3.093-3.145 (s, 6H), δ 6.739-7.590 (m, 4H), δ 8.212-8.718 (m, 3H), δ 8.899 (s, H).
Pharmacology

Anticonvulsant activity- Anticonvulsant evaluation was undertaken using a reported procedure. Albino mice (swiss, 25-30) were used in groups of five each as experimental animals. The test compounds and standard drug were suspended in 0.5% methyl cellulose-water mixture and administered intraperitoneally. The animals were maintained on an adequate diet and allowed free access to food and water except during the short time they were removed from cages for testing. The animals were maintained at room temperature (25±2 °C).

Maximum electroshock seizure test (MES)- Maximum electroshock seizure was elicited with a 60 cycle alternating current of 50 mA intensity delivered for 0.25 sec. via ear clip electrodes. Maximal seizure typically consists of a short period of tonic extension of the hind limbs and final clonic episodes. Abolition of the hind limb tonic extensor component of the seizure is defined as protection and the results are expressed as % protection.

Neurotoxicity (NT)- The rotarod test was used to evaluate neurotoxicity. The animal was placed on a 3.2 cm diameter knurled rod rotating at 6 rpm. Normal mice can remain indefinitely on a rod rotating at this speed. Neurological toxicity is defined as the failure of the animals to remain on the rod for 1 min. Results are expressed as the number of animals exhibiting toxicity/ number of animals tested.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>MES screen protection (%)</th>
<th>Neurotoxicity screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A</td>
<td>56.27%</td>
<td>(-)</td>
</tr>
<tr>
<td>2B</td>
<td>70.32%</td>
<td>(-)</td>
</tr>
<tr>
<td>2C</td>
<td>67.87%</td>
<td>(-)</td>
</tr>
<tr>
<td>2D</td>
<td>64.36%</td>
<td>(-)</td>
</tr>
<tr>
<td>2E</td>
<td>58.03%</td>
<td>(-)</td>
</tr>
<tr>
<td>2F</td>
<td>75.45%</td>
<td>(-)</td>
</tr>
<tr>
<td>2G</td>
<td>72.09%</td>
<td>(-)</td>
</tr>
<tr>
<td>2H</td>
<td>56.05%</td>
<td>(-)</td>
</tr>
<tr>
<td>2I</td>
<td>51.20%</td>
<td>(x)</td>
</tr>
</tbody>
</table>

Please note:

Values are expressed as Mean ± SEM (N=5) (compared to Control group) by using One way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test P< 0.01*.

(-) absence of activity; × not tested

Fig 1: Graphical representation of anticonvulsant activity by Maximum electroshock seizure
RESULTS AND DISCUSSION

(6-Substituted-benzothiazol-2-yl)-(4-substituted -benzylidene)-amine derivatives obtained from the reactional sequence were injected intraperitoneally into mice and evaluated by the maximal electroshock (MES), neurotoxicity screen using rotarod at the dose of 30 mg/kg body mass and observations were carried out at 45 min after the compound administration. The result of anticonvulsant activity and neurotoxicity was presented in table 1. Phenytoin was used as the standard for the comparison at the dose level of 25 mg/kg. The MES activity indicated significant anticonvulsant activity for all of the test compounds. However, they were found to be less potent when compared with the reference standard Phenytoin. Within the series A, B, C and D compounds of series B with substitution of -F at 6th position of benzothiazole showed maximum potency. the 2F with -F at 6th position of benzothiazole moiety and -(CH3)2 at the 4th position of the phenyl ring was the most active due to increase in lipophilicity, which could further increase penetration through the blood brain barrier. Among the derivatives of A series, 2B was found to be most active, which has similar substitution at 4th position of phenyl ring as was in 2F. However the compounds 2H and 2I were less active than derivatives of B and A series. The highest activity of derivatives of series B may be attributed due to the presence of the fluoro group. In the neurotoxicity studies, none of the compounds displayed neurotoxicity since they successfully passed the rotarod test without any sign of motor impairment.

CONCLUSION

Various (6-Substituted-benzothiazol-2-yl)-(4-substituted -benzylidene)-amine derivatives were designed and synthesized starting from various substituted aromatic aldehydes. Structures of all the compounds were confirmed on the basis of spectral and elemental analyses. All the newly synthesized compounds were screened for their anticonvulsant activity and were compared with the standard drug Phenytoin. Interestingly, all the compounds showed protections against seizures in the range 50-75% indicative of the promising nature of the compounds against seizure spread. Compound 2F showed highest protection against MES induced seizures. The synthesis of number of 2-amino-6-substituted benzothiazole (A-D) derivatives as candidate anticonvulsant has been reported in this study. The results suggest that minor changes to structure can increase or decrease the activity. Thus it can be concluded that substitution of 6th-position with fluoro, chloro, bromo and nitro groups (A-D) at the terminal benzothiazole ring is beneficial for anticonvulsant activity.

REFERENCES