Ind. J. Pharm. Edu. Res., 2024; 58(1s):s80-s92.
https://www.ijper.org

Original Article

Synthesis, Characterization, *in silico* and *in vivo* Evaluation of Amino Acid Derived Schiff Bases of Quinoline-Benzimidazole Hybrids as Anti-epileptic Agents

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ABSTRACT

Aim/Background: Novel quinoline compounds containing benzimidazole have been synthesized in a number of different ways and screened for anti-epileptic potential through *in vivo* and *in silico* studies. **Materials and Methods:** The novel quinoline-benzimidazole hybrids were synthesized by using substituted carbaldehyde (1,2) and substituted benzimidazole (derived from amino acids) (3a-f). The synthesized derivatives were characterized by IR, ^{13}C-NMR, ^{1}H-NMR, and Mass spectroscopy and anti-convulsant activity was analyzed by *in silico* studies on the swiss albino mice using the ScPTZ model. Conclusion and **Results:** Compounds (1*H*-Benzoimidazol-2-yl)-substituted-(2-p-tolyloxy-quinolin-3-ylmethylene)-amine (4a-f) and (Benzoimidazol-2-yl)-substituted-(2-ethoxy-quinolin-3-ylmethylene)-amine (5a-f) were synthesized. The synthesized derivatives were characterized (IR, ^{13}C-NMR, ^{1}H-NMR, and mass spectrometry) and screened for their anticonvulsant potentiality by subcutaneous pentylenetetrazol method using carbamazepine. Additionally, the anti-epileptic potential of the produced compounds is confirmed by *in silico* study results that were obtained. The study also proved that the synthesized derivatives used aminobutyric acid ($GABA_A$) receptors. At a dose level of 30 mg/kg body weight, 4a, 4b, and 4d were found to be more effective than the other synthesized derivatives.

Keywords: Seizure, Anti-epileptic, Quinoline, Benzimidazole, Subcutaneous pentylenetetrazol.

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Received: 09-01-2023;
Revised: 27-03-2023;
Accepted: 14-08-2023.

INTRODUCTION

People of various races, ages, socio-economic backgrounds, and geographical areas are susceptible to epilepsy, one of the most prevalent brain diseases.[1] A chronic propensity to develop seizures as well as the neurobiological, psychological, cognitive, and social repercussions of recurrent seizures describe this brain disorder.[2] One of the oldest known medical conditions, epilepsy has been described in writing as early as 4000 BCE. A single seizure does not cause epilepsy (up to 10% of people worldwide have one seizure during their lifetime). Epileptic seizures are defined as two or more unannounced seizures. Epileptic seizures are frequent paroxysmal occurrences, stereotyped behavioral changes that represent the underlying brain mechanisms of the disease.[3] Seizures and epilepsy are often brought on by aberrant signals from neurons, a kind of brain cell, even though the underlying causes of epilepsy vary and are not entirely understood.[4] The list of clinical illnesses that can be distinguished from epilepsy by changes in behavior and/or awareness. Most of the time, the presence of the disease can be detected by a detailed medical history or by seeing a seizure. An etiologic agent can be identified in about 50% of cases, but the etiology is unexplained.[5] Anti-epileptic medications (AEDs) work through a variety of molecular mechanisms, including glutamate-mediated excitatory neurotransmission, voltage-gated ion channels (Na^+ and Ca^{2+}), and GABA-mediated inhibitory neurotransmission.[6] This allows seizure-related firing to be blocked without interfering with non-epileptic activity, which is necessary for normal signals between brain cells.[7,8] They can successfully manage epilepsy in many cases, it is estimated that 20 to 30% of individuals still experience seizures that are resistant to pharmacological therapy. Currently, existing Anti-epileptic Drugs (AEDs) have limited effectiveness and unfavorable side effects that make it difficult to manage patients.[9,10]

DOI: 10.5530/ijper.58.1s.8

According to previously published data, the potential pharmacological action of heterocyclic substances containing nitrogen and their derivatives has recently gained a lot of attention. To develop more powerful anti-epileptic drugs, researchers are constantly investigating heterocycles. It is well recognized that quinoline, a heterocyclic molecule, is a constituent of naturally occurring chemicals and that it is the main determinant of their pharmacological potential. The research on Quinoline synthesis as well as the pharmacological evaluation of quinoline and its derivatives are always developing in new directions.[11-13] Several studies revealed that scholars have been interested in quinoline scaffolds because they have a wide spectrum of biological and pharmacological effects[14] such as antibiotic,[15] antioxidant,[16] anti-proliferative,[17] anti-inflammation,[18] anti-malaria,[19] anti-convulsant,[20] etc., via several mechanisms of action such as growth inhibitors, angiogenesis inhibitors, apoptosis inducers, cell migration disruption, and nuclear receptor modulators. On the other hand, it has also been asserted that various pharmacological effects are caused by the presence of benzimidazole moieties in several compounds.[21,22] A good anti-epileptic medication needs to have the following properties, according to the conformational analysis of well-known anti-epileptic medications: pharmacophoric properties: an aryl hydrophobic region, a hydrogen donor/acceptor unit, distal hydrophobic, and an electron donor.[23,24]

The novelty of this research lies in the fact that the compounds synthesized are Schiff bases derived from amino acids and quinoline-benzimidazole hybrids. The use of amino acids as starting materials for the synthesis of Schiff bases is a relatively new approach and could provide compounds with improved pharmacological properties. Moreover, the quinoline-benzimidazole hybrid is a unique structural motif that has been reported to possess significant anticonvulsant activity. Combining this hybrid with amino acids to form Schiff bases could enhance the anti-epileptic activity of the resulting compounds. Finally, the *in vivo* evaluation of the synthesized compounds using animal models of epilepsy demonstrates their promising anti-epileptic activity. The authors also report that the compounds exhibit good safety profiles, which is an essential aspect of drug development. Overall, the article presents a novel approach to the design and synthesis of potential anti-epileptic agents, which could lead to the development of safer and more effective drugs for the treatment of epilepsy.

MATERIALS AND METHODS

Chemicals and Instruments Used

From a number of companies, chemical reagents and solvents of LR quality were acquired including Central Drug House (CDH), E. Merck India Ltd., FINAR, RenChem, etc. The compounds' purity was examined using TLC, melting point, and boiling determination. The silica gel G (160-120 lattice, CDH) was employed for TLC. Toluene, ethyl acetate, and formic acid (5:4:1) made up the mobile phase, while the visualizing agents were iodine vapor and UV light. Uncorrected melting points were found using a digital melting point instrument (LABINOIA-melting range apparatus). In the NIET Instrument Facility (Pharmacy Institute), IR spectra were captured using an FTIR (Perkin Elmer-spectrum 65FT-IR spectrometer).

Synthesis of 2-*p*-tolyloxy-quinoline-3-carbaldehyde (1)

2-*p*-tolyloxy-quinoline-3-carbaldehyde (1) was formed by Vilsmeier-Haack reactions from 2-chloro-quinoline-3-carbaldehyde by using DMF, K_2CO_3, and *p*-cresol as per the reported protocol as shown in (Scheme 1). The reported melting point of compounds is 300–304°C and the melting point was observed to be 302-306°C.[25,26]

Synthesis of 2-Ethoxy-quinoline-3-carbaldehyde (2)

2-Ethoxy-quinoline-3-carbaldehyde (2) was synthesized by using 2-chloro-quinoline-3-carbaldehyde by using KOH and ethanol as per the reported protocol as shown in (Scheme 2). The reported melting point of compounds is 270–274°C and the melting point was observed 272-276°C.[27]

Synthesis of substituted 1-(1*H*-benzimidazol-2-yl)-methenamines (3a-f)

O-phenylenediamine (5 mmol) and various amino acids (7 mmol) reacted together to yield 1-(1*H*-benzimidazol-2-yl)-substituted amine (2) as per the reported protocol[28-30] as shown in (Scheme 3). The melting points of reported intermediates are shown below in Table 1.

General methods for the synthesis of substituted (1*H*-Benzoimidazol-2-yl)-substituted methyl-(2-p-tolyloxy-quinolin-3-ylmethylene)-amine (4a-f)

The substituted benzimidazoles (3a-f) (1 mmol) were dissolved in 50 mL boiling distilled water. At room temperature, 1 mmol of the 2-p-tolyloxy-quinoline-3-carbaldehyde (1) was dissolved in DMF (10 mL). The suitable solutions 1 and 2 were then combined, and they were left to stir at room temperature for 15 min. Vacuum filtration was used to separate the separated solid (precipitate) product 4 from the ethanol.

Synthesis of (1*H*-Benzoimidazol-2-ylmethyl)-(2-p-tolyloxy-quinolin-3-ylmethylene)-amine (4a)

It was obtained as grayish solids, yield: 86%, m.p.: 134-138°C; IR (KBr), V_{max} (cm^{-1}): 3344 (N-H), 3069-2925 (C-H, str, Ar), 2888 (C-H, str, alkane), 1691 (C=N, str), 1616-1498 (C=C, Ar), 1213 (C-O), 1155 (C-N); ^1H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.724 (s, 1H, -C=N), 7.899-7.872 (d, 2H, Ar-H), 7.76-7.65 (m, 4H, Ar-H), 7.47-7.42 (m, 4H, Ar-H), 7.27-7.25 (d, 2H, Ar-H), 4.215 (s, 1H, Ar-H), 1.572 (s, 2H, =C-H), 1.256 (s,

3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 181.25, 151.10, 142.23, 137.58, 135.63, 134.15, 132.21, 131.22, 131.10, 130.94, 130.90, 129.23, 128.42, 127.94, 94.50, 60.11, 21.22; EI-MS (m/z): 392 (M^+); Anal. Calcd. For $C_{25}H_{20}N_4O$: C, 76.51; H, 5.14; N, 14.28; O, 4.08. Found: C, 76.60; H, 5.28; N, 14.35; O, 4.11.

Synthesis of [1-(1*H*-Benzoimidazol-2-yl)-ethyl]- (2-p-tolyloxy-quinolin-3-ylmethyle ne)-amine (4b)

It was obtained as creamish solids, yield: 78%, m.p.: 100-104°C; IR (KBr), V_{max} (cm^{-1}): 3348 (N-H), 3069-2921 (C-H, str, Ar), 2888 (C-H, str, alkane), 1692 (C=N, str), 1616-1420 (C=C, Ar), 1213 (C-O), 1148 (C-N); ^1H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.724 (s, 1H, -C=N), 7.899-7.872 (d, 2H, Ar-H), 7.76-7.65 (m, 4H, Ar-H), 7.47-7.42 (m, 4H, Ar-H), 7.27-7.25 (d, 2H, Ar-H), 7.24-7.18 (q, 1H, -CH-N), 4.215 (s, 1H, Ar-H), 1.572 (s, 3H, =C-H), 1.256 (s, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 181.28, 151.06, 142.21, 137.58, 135.60, 134.15, 132.21, 131.25, 131.10, 130.95, 130.90, 129.21, 128.45, 127.94, 94.50, 60.01, 21.21, 21.10; EI-MS (m/z): 406 (M^+); Anal. Calcd. For $C_{26}H_{22}N_4O$: C, 76.83; H, 5.46; N, 13.78; O, 3.94. Found: C, 76.87; H, 5.56; N, 13.85; O, 3.84.

Synthesis of 4-{2-(1*H*-Benzoimidazol-2-yl)-2-[(2-p-tolyloxy-quinolin-3-ylmethylene)-amino]-ethyl}-phenol (4c)

It was obtaineds as yellowish solid, yield: 80%, m.p.: 136-140°C; IR (KBr), V_{max} (cm^{-1}): 3410 (OH, str)3348 (N-H), 3069-2921 (C-H, str, Ar), 2886 (C-H, str, alkane), 1690 (C=N, str), 1618-1420 (C=C, Ar), 1215 (C-O), 1150 (C-N); ^1H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.724 (s, 1H, -C=N), 7.899-7.872 (d, 3H, Ar-H), 7.76-7.64 (m, 9H, Ar-H), 7.47-7.41 (m, 4H, Ar-H), 7.27-7.24 (d, 2H, Ar-H), 7.24-7.18 (q, 1H, -CH-N), 2.416 (s, 1H, C-H), 1.57- 1.56 (d, 1H, CH_2), 1.24 (s, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 181.27, 152.10, 142.20, 137.55, 135.68, 134.15, 132.24, 131.22, 131.10, 130.96, 130.90, 129.25, 128.41, 127.94, 94.51, 60.22, 50.01, 48.58, 44.05, 21.22; EI-MS (m/z): 498 (M^+); Anal. Calcd. For $C_{32}H_{26}N_4O_2$: C, 77.09; H, 5.26; N, 11.24; O, 6.42. Found: C, 77.60; H, 5.38; N, 11.47; O, 6.52.

Synthesis of [1-(1*H*-Benzoimidazol-2-yl)-2-methyl-propyl]- (2-p-tolyloxy-quinolin-3-ylmethylene)-amine (4d)

It was obtained as grayish solid, yield: 75%, m.p.: 130-134°C; IR (KBr), V_{max} (cm^{-1}): 3357 (N-H), 3035-2921 (C-H, str, Ar), 2868 (C-H, str, alkane), 1689 (C=N, str), 1616-1458 (C=C, Ar), 1212 (C-O), 1160 (C-N); ^1H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.724 (s, 1H, -C=N), 7.899-7.872 (d, 2H, Ar-H), 7.76-7.65 (m, 4H, Ar-H), 7.47-7.42 (m, 4H, Ar-H), 7.27-7.25 (d, 2H, Ar-H), 7.24-7.18 (q, 1H, -CH-N), 2.415 (s, 1H, C-H), 2.67-2.65 (q, 1H, Aliphatic), 1.572 (s, 3H, =C-H), 1.256 (s, 6H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 181.26, 151.11, 142.23, 137.58, 135.63, 134.15, 132.21, 131.22, 131.10, 130.94, 130.90, 129.25, 128.42, 127.94, 94.50, 60.05, 34.21, 21.22, 17.71; EI-MS (*m/z*): 434 (M^+); Anal. Calcd. For $C_{28}H_{26}N_4O$: C, 77.39; H, 6.03; N, 12.89; O, 3.68. Found: C, 77.50; H, 6.23; N, 12.94; O, 3.82.

Synthesis of [(1*H*-Benzoimidazol-2-yl)-phenyl-methyl]- (2-p-tolyloxy-quinolin-3-ylmethylene)-amine (4e)

It was obtained as white solid, yield: 72%, m.p.: 138-142°C; IR (KBr), V_{max} (cm^{-1}): 3344 (N-H), 3035-2921 (C-H, str, Ar), 2867 (C-H, str, alkane), 1689 (C=N, str), 1616-1458 (C=C, Ar), 1213 (C-O), 1159 (C-N); ^1H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.724 (s, 1H, -C=N), 7.899-7.872 (d, 5H, Ar-H), 7.76-7.65 (m, 9H, Ar-H), 7.47-7.42 (m, 4H, Ar-H), 7.27-7.25 (d, 2H, Ar-H), 7.24-7.18 (q, 1H, -CH-N), 2.415 (s, 1H, C-H); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 181.25, 151.10, 142.23, 137.58, 135.63, 134.15, 132.21, 131.22, 131.10, 130.94, 130.90, 129.23, 128.42, 127.94, 94.50, 60.09, 50.02, 48.59, 21.22; EI-MS (m/z): 468 (M^+); Anal. Calcd. For $C_{31}H_{24}N_4O$: C, 79.46; H, 5.16; N, 11.96; O, 3.41. Found: C, 79.56; H, 5.38; N, 11.67; O, 3.62.

Synthesis of [1-(1*H*-Benzoimidazol-2-yl)-2-phenyl-ethyl]- (2-p-tolyloxy-quinolin-3-ylmethylene)-amine (4f)

It was obtained as creamish solid, yield: 78%, m.p.: 132-136°C; IR (KBr), V_{max} (cm^{-1}): 3348 (N-H), 3069-2921 (C-H, str, Ar), 2888 (C-H, str, alkane), 1692 (C=N, str), 1616-1420 (C=C, Ar), 1213

Table 1: Melting point of reported intermediate compounds.

Compound	R	IUPAC name	M.P. (°C)
1	-	2-*p*-tolyloxy-quinoline-3-carbaldehyde [26]	302–306
2	-	2-chloro-3-ethoxy-quinoline [27]	272-276
3a	H	1-(1*H*-benzimidazol-2-yl)-methenamine [28]	102–106
3b	CH_3	1-(1*H*-benzimidazol-2-yl)-ethan-1-amine [28]	98–102
3c	C_7H_7O	4-[2-amino-2-(1*H*-benzimidazole-2-yl)-ethyl]-phenol [29]	202-206
3d	C_3H_8	1-(1*H*-benzimidazol-2-yl)-2-methylpropan-1-amine [30]	198-202
3e	C_6H_5	1-(1*H*-benzimidazole-2-yl)-1-phenylmethanamine [30]	276-280
3f	C_7H_7	1-(1*H*-benzimidazol-2-yl)-2-phenylethan-1-amine [28]	202-206

(C-O), 1148 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.724 (s, 1H, -C=N), 7.899-7.872 (d, 3H, Ar-H), 7.76-7.65 (m, 9H, Ar-H), 7.47-7.42 (m, 4H, Ar-H), 7.27-7.25 (d, 2H, Ar-H), 7.24-7.18 (q, 1H, -CH-N), 2.415 (s, 1H, C-H), 1.57-1.56 (d, 1H, CH_2), 1.24 (s, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 181.25, 151.10, 142.23, 137.58, 135.63, 134.15, 132.21, 131.22, 131.10, 130.94, 130.90, 129.23, 128.42, 127.94, 94.50, 60.11, 50.03, 48.57, 44.12, 21.22; EI-MS (m/z): 498 (M^+); Anal. Calcd. For $C_{32}H_{26}N_4O$: C, 79.64; H, 5.55; N, 11.75; O, 3.42. Found: C, 79.70; H, 5.68; N, 11.87; O, 3.52.

General method for the synthesis of substituted (1*H*-Benzoimidazol-2-yl)-substituted methyl-(2-p-tolyloxy-quinolin-3-ylmethylene)-amine (5a-f)

The substituted benzimidazoles (3a-f) (1 mmol) were dissolved in 50 mL boiling distilled water. At room temperature, 1 mmol of the 2-Ethoxy-quinoline-3-carbaldehyde (2) was dissolved in DMF (10 mL). The suitable solutions 1 and 2 were then combined, and they were left to stir at room temperature for 15 min. Vacuum filtration was used to separate the separated solid (precipitate) product 5 from the ethanol.

Synthesis of (1*H*-Benzoimidazol-2-ylmethyl)-(2-ethoxy-quinolin-3-ylmethylene)-amine (5a)

It was obtained as off white solid, yield: 82%, m.p.: 140-144°C; IR (KBr), V_{max} (cm^{-1}): 3357 (N-H), 3035-2921 (C-H, str, Ar), 2868 (C-H, str, alkane), 1689 (C=N, str), 1616-1458 (C=C, Ar), 1212 (C-O), 1160 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.72 (s, 1H, N=CH), 7.89-7.87 (d, 2H, Ar-H), 7.87-7.65 (m, 2H, Ar-H), 7.48-7.42 (m, 6H, Aliphatic), 7.27-7.25 (d, 2H, Ar-H), 7.24-7.16 (m, 2H, Ar-H), 2.41 (s, 2H, CH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 187.25, 163.23, 141.10, 137.58, 135.63, 134.15, 129.23, 128.42, 127.94, 94.50, 65.11, 60.11, 14.32; EI-MS (m/z): 330 (M^+); Anal. Calcd. For $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96; O, 4.84. Found: C, 72.60; H, 5.38; N, 16.87; O, 4.92.

Synthesis of [1-(1*H*-Benzoimidazol-2-yl)-ethyl]-(2-ethoxy-quinolin-3-ylmethylene)-amine (5b)

It was obtained as greyish solid, yield: 72%, m.p.: 142-146°C; IR (KBr), V_{max} (cm^{-1}): 3344 (N-H), 3035-2921 (C-H, str, Ar), 2867 (C-H, str, alkane), 1689 (C=N, str), 1616-1458 (C=C, Ar), 1213 (C-O), 1159 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.72 (s, 1H, N=CH), 7.89-7.87 (d, 2H, Ar-H), 7.87-7.65 (m, 2H, Ar-H), 7.48-7.42 (m, 9H, Aliphatic), 7.27-7.25 (d, 1H, Ar-H), 7.24-7.16 (m, 2H, Ar-H), 2.41 (s, 2H, CH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 187.25, 163.21, 141.10, 137.56, 135.63, 134.15, 131.10, 129.23, 128.42, 127.94, 94.50, 65.11, 59.11, 21.11, 14.32; EI-MS (m/z): 344 (M^+); Anal. Calcd. For $C_{21}H_{20}N_4O$: C, 73.23; H, 5.85; N, 16.27; O, 4.65. Found: C, 73.36; H, 5.98; N, 16.47; O, 4.82.

Synthesis of 4-{2-(1*H*-Benzoimidazol-2-yl)-2-[(2-ethoxy-quinolin-3-ylmethylene)-amino]-ethyl}-phenol (5c)

It was obtained as reddish brown solid, yield: 84%, m.p.: 148-152°C; IR (KBr), V_{max} (cm^{-1}): 3410 (OH, str)3348 (N-H), 3069-2921 (C-H, str, Ar), 2886 (C-H, str, alkane), 1690 (C=N, str), 1618-1420 (C=C, Ar), 1215 (C-O), 1150 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.72 (s, 1H, N=CH), 7.89-7.87 (d, 2H, Ar-H), 7.87-7.66 (m, 5H, Ar-H), 7.48-7.43 (m, 8H, Aliphatic), 7.27-7.25 (d, 2H, Ar-H), 7.24-7.16 (m, 2H, Ar-H), 3.894 (s, 1H, OH), 2.41 (s, 2H, CH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 187.25, 163.23, 141.10, 137.58, 135.63, 134.15, 131.10, 129.23, 128.42, 127.94, 94.50, 65.11, 59.13, 50.01-48.58, 44.05, 14.32; EI-MS (*m/z*): 436 (M^+); Anal. Calcd. For $C_{27}H_{24}N_4O_2$: C, 74.29; H, 5.54; N, 12.84; O, 7.33. Found: C, 74.60; H, 5.68; N, 12.97; O, 7.52.

Synthesis of [1-(1*H*-Benzoimidazol-2-yl)-2-methyl-propyl]- (2-ethoxy-quinolin-3-ylmethylene)-amine (5d)

It was obtained as dark brown solid, yield: 75%, m.p.: 138-142°C; IR (KBr), V_{max} (cm^{-1}): 3344 (N-H), 3069-2925 (C-H, str, Ar), 2888 (C-H, str, alkane), 1691 (C=N, str), 1616-1498 (C=C, Ar), 1213 (C-O), 1155 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.724 (s, 1H, -C=N), 7.899-7.872 (d, 2H, Ar-H), 7.76-7.65 (m, 2H, Ar-H), 7.47-7.42 (m, 2H, Ar-H), 7.27-7.25 (d, 2H, Ar-H), 7.24-7.18 (q, 1H, -CH-N), 2.415 (s, 1H, C-H), 2.67-2.65 (q, 3H, Aliphatic), 1.572 (s, 3H, =C-H), 1.256 (s, 6H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 187.25, 163.25, 141.11, 137.58, 135.63, 134.15, 131.10, 129.23, 128.42, 127.94, 94.50, 65.11, 59.13, 34.21, 17.03, 17.05, 14.32; EI-MS (m/z): 372 (M^+); Anal. Calcd. For $C_{23}H_{24}N_4O$: C, 74.17; H, 6.49; N, 15.04; O, 4.30. Found: C, 74.60; H, 6.38; N, 15.47; O, 4.52.

Synthesis of [(1*H*-Benzoimidazol-2-yl)-phenyl-methyl]- (2-ethoxy-quinolin-3-ylmethylene)-amine (5e)

It was obtained as brown solid, yield: 75%, m.p.: 138-142°C; IR (KBr), V_{max} (cm^{-1}): 3344 (N-H), 3035-2921 (C-H, str, Ar), 2867 (C-H, str, alkane), 1689 (C=N, str), 1616-1458 (C=C, Ar), 1213 (C-O), 1159 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.72 (s, 1H, N=CH), 7.89-7.86 (d, 2H, Ar-H), 7.88-7.65 (m, 4H, Ar-H), 7.48-7.42 (m, 6H, Aliphatic), 7.27-7.25 (d, 2H, Ar-H), 7.24-7.16 (m, 4H, Ar-H), 2.41 (s, 2H, CH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 187.25, 163.23, 141.09, 137.58, 135.63, 134.15, 131.10, 129.23, 128.42, 127.94, 94.50, 65.11, 59.11, 50.02, 48.59, 14.32; EI-MS (m/z): 406 (M^+); Anal. Calcd. For $C_{26}H_{22}N_4O$: C, 76.83; H, 5.46; N, 13.78; O, 3.94. Found: C, 76.90; H, 5.68; N, 13.97; O, 3.52.

Synthesis of [1-(1*H*-Benzoimidazol-2-yl)-2-phenyl-ethyl]- (2-ethoxy-quinolin-3-ylmethylene)-amine (5f)

It was obtained as yellowish brown solid, yield: 75%, m.p.: 138-142°C; IR (KBr), V_{max} (cm^{-1}): 3344 (N-H), 3069-2925 (C-H, str, Ar), 2888 (C-H, str, alkane), 1691 (C=N, str), 1616-1498 (C=C, Ar), 1213 (C-O), 1155 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm): 10.65 (s, 1H, N-H), 8.72 (s, 1H, N=CH), 7.89-7.87 (d, 2H, Ar-H), 7.87-7.65 (m, 5H, Ar-H), 7.48-7.42 (m, 8H, Aliphatic), 7.27-7.25 (d, 1H, Ar-H), 7.24-7.16 (m, 4H, Ar-H), 2.41 (s, 2H, CH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ(ppm): 187.25, 163.21, 141.10, 137.58, 135.63, 134.15, 131.10, 129.23, 128.42, 127.94, 94.50, 65.11, 59.14, 50.03, 48.57, 44.12, 14.32; EI-MS (m/z): 420 (M^+); Anal. Calcd. For $C_{27}H_{24}N_4O$: C, 77.12; H, 5.75; N, 13.32; O, 3.80. Found: C, 77.60; H, 5.88; N, 13.47; O, 3.92.

Pharmacology

Animals

The current investigation secured animal ethical approval from the CPCSEA-affiliated institution ethics committee (NIET, Pharmacy Institute) (Protocol NO. IAEC/NIET/2022/01/13). The Noida Institute of Engineering and Technology, Pharmacy Institute, in Greater Noida, Uttar Pradesh, central animal house facilities provided Swiss albino mice of either sex weighing 25–35 g were selected for this testing. In a laboratory environment with temperature control of (25±2°C) and a 12 hr light/dark cycle, five animals are kept in separate plastic cages. Animals were given unlimited access to drinking water fed a standard laboratory mouse chow diet, and had cage bedding made of rice husk that was free of dust. Before the experiment animals fasted for the night.

The usual treatments in scPTZ models were carbamazepine and phenytoin sodium. Pentylenetetrazol (PTZ) was administered to cause convulsions in albino mice during scPTZ screening. The prepared derivatives were given intraperitoneally in the amount of 10 µL/g of body weight using PEG-200 as a vehicle.

Acute Toxicity (LD_{50}) Study

An acute toxicity study aims to determine the test compound's lethal dose (LD_{50}). Eventually, animals were x divided x into three groups; x each group contains three animals, according to the dose recommended by the OECD 423 standards.[31] Each drug was given a different dose concentration of 5, 50, 300, 1000, and 2000 mg/kg. The Intraperitoneal route was used for the administration of the doses. During the first 4 hr of observation, the mortality rate in each group was recorded, as well as their gross behavioral activity and other changes. And they were examined for their impermanency. Following the delivery of the drug, each animal was monitored for 24 hr.

Subcutaneous pentylenetetrazol (scPTZ) induced seizure screening

Test substances with anticonvulsant action were given 0.5 hr before the scPTZ therapy, and protection was shown by the inability to observe a clonic spasm episode lasting 5 sec. The control group of mice received subcutaneous PTZ solutions (in PEG-200) at the convulsion's onset, severity, and posterior midline.[32,33]

Neurotoxicity Test

Using rotarod technology, the neurotoxicity of each test drug was assessed. The animal's inability to stay upright on the rotarod for at least 60 sec in each of the three trials that followed served as a sign of neurotoxicity.[34]

In silico Studies

ADME Parameters

ADME features can be predicted by using computational analysis of generated compounds. The % Absorption and TPSA, the number of rotatable bonds, the number of hydrogen donor and acceptor atoms, logP of synthesized compounds were all determined using the online calculators pkSCM (http://biosig.unimelb.edu.au/pkcsm/prediction) and Molinspiration (https://www.molinspiration.com/) respectively.[35]

Molecular docking study

AutoDock 4.2 software was used to perform ligand docking experiments of the compounds (4a-f) or (5a-f) into the gamma-aminobutyric acid aminotransferase ($GABA_A$) target. The crystal structures of the Gamma-aminobutyric acid aminotransferases ($GABA_A$) enzyme were retrieved x from the proteins data bank (PDB ID: 2COJ), which is a validated target for AEDs. Using the 'Discovery studio visualizer, the protein preparation was done in three steps: pre-processing, review and change, and refining. Water molecules are removed and hydrogen atoms are introduced in these phases. AutoDock x 4.2 was used to decrease the structure's energy. The default box was produced after running the receptor grid-generating program by clicking any ligand atom. Extra precision was used to dock the ligand into the grid formed by the protein (2COJ). The binding energy and interaction were used to evaluate the results.

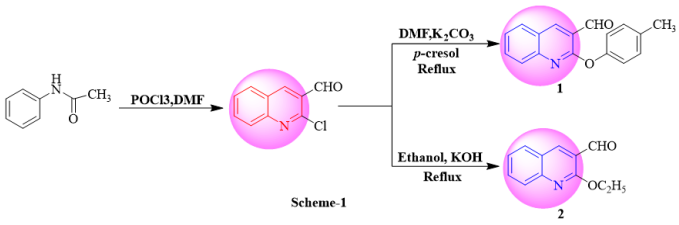
RESULTS AND DISCUSSION

Chemistry

The reaction scheme for the formation of titled compounds is outlined in (Schemes 1-3). In the first step, 2-p-Tolyloxy-quinoline-3-carbaldehyde (1) and 2-chloro-3-ethoxy-quinoline (2) were synthesized from 2-Chloroquinoline-3-Carbaldehyde by Vilsmeier-Haack reaction using K_2CO_3, p-cresol, DMF and KOH, ethanol. In the second step, 1-(1H-benzimidazol-2-yl) methenamine (3a-f) was prepared

by the cyclization reaction between various amino acids and *o*-phenylenediamine using ethanol and 4N HCl by Phillip's method. In the third step, titled derivative (4a-f) and (5a-f) were synthesized from 1-(1H-benzimidazol-2-yl)-methenamine (3a-f) react with 2-p-Tolyloxy-quinoline-3-carbaldehyde (1) and 2-Ethoxy-quinoline-3-carbaldehyde (2) by condensation respectively. Spectral and elemental analytical data were used to characterize every molecule that was produced.

Synthesis of substituted 2-Quinolines-3-Carbaldehyde (Scheme-1)

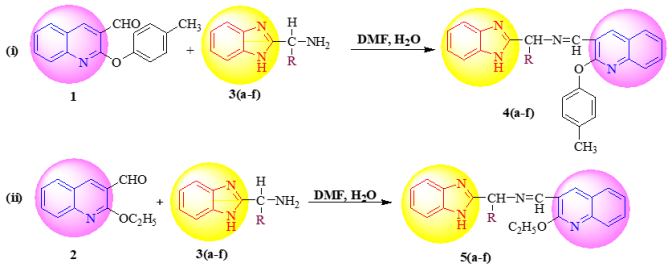


Scheme-1

Synthesis of Amino Acid Derived Benzimidazoles (Scheme-2)

Scheme-2

Synthesis of Amino Acid derived Schiff base bearing quinoline and benzimidazole (Scheme-3)



Pharmacological Studies

Acute toxicity (LD_{50}) study

According to OECD 423, studies on acute toxicity were conducted to determine the median lethal dose and the likelihood that produced compounds would have harmful effects. The dose of each compound was observed after intraperitoneal injection of all the prepared compounds. The dose concentration given was 30, 100, 200, and 300mg/kg, among Swiss albino mice. After the medication administration, each animal was watched for 24 hr. Table 2 of the data obtained clearly demonstrates that mice who were given a dosage concentration of 300 mg/kg display mortality along with uncontrollable behaviors such as undesirable jerky movements and body stiffness. Except for 4e and 5f, which displayed mild spasmodic behavior, no other indications or mortality have been documented in any groups at a dose concentration of 200 mg/kg when synthetic chemicals were administered. All animals survived and maintained their health at dose concentrations of 30 and 100 mg/kg, respectively.

Anticonvulsant activity (*in vivo*)

The anticonvulsant activity of produced derivatives (4a-f and 5a-f) was examined using the standard methods of subcutaneous pentylenetetrazol (scPTZ) caused convulsions models in either female or male albino mice as shown in (Tables 3 and 4, Figure 1). These tests looked for drugs that could stop tonic-clonic or generalized grand-mal seizures. All the synthetic medications were administered intravenously in a dose of 30, 100, or 300 mg/kg of body weight to the selected mouse groups, and observations were conducted over the course of two different periods (0.5 hr and 4 hr). The synthesized derivatives 4a, 4b, and 4d were discovered to display the most noticeable behavior in the scPTZ model and showed protection against seizures spaced throughout both time duration at a dosage level of 30 mg/kg body weight, indicating a promising fast start and a prolonged period of activity. At 30 mg/kg of body weight for 0.5 hr and 100 mg/kg for 4 hr of protection, compounds 4e and 5f showed moderate activity. Compounds 4f and 5d exhibited moderate action at 100 mg/kg of body weight for both time duration of protection. Compounds 5b and 5c required larger dosages i.e., 100 mg/kg and 300 mg/kg of body weight for 0.5 hr and 4 hr interval protection, respectively. Produced derivatives 4c and 5a demonstrated potential as anti-epileptic because they were protected at doses of 300 mg/kg of body weight at intervals of 0.5 hr and 4 hr, suggesting that these substances need to be administered at higher doses. However, compound 5e produced no protection at the same dose over the course of 4.0 hr, demonstrating clearly that there is no longer duration of effect at the levels being used. Compound 5e is neurotoxic whereas other compounds showed no neurotoxicity as none of the mice could balance on the rotarod device's rotating rod.

Neurotoxicity Screening

The rotarod apparatus was used to test the synthetic drugs' *in vivo* neurotoxicity in either sex of mice. A healthy mouse that has not experienced any neurological damage can maintain balance for at least 1 min on a rotating rod. Every effort in which the individual was unable to maintain equilibrium on a rotating rod revealed neurological damage. Tests were conducted on each synthetic derivative in comparison to the positive control (carbamazepine) at dosages of 30, 100, and 300 mg/kg body weight. No other substances examined showed any symptoms of neurotoxicity

Table 2: Studies on the acute toxicity of synthesized compounds (4a-f), (5a-f), and standard drugs.

Compound	Number of animal's dead/total number of animals tested (Dose in mg/kg)			
	30 mg	100 mg	200 mg	300 mg
4a	0/3	0/3	0/3	1/3
4b	0/3	0/3	0/3	2/3
4c	0/3	0/3	0/3	2/3
4d	0/3	0/3	0/3	1/3
4e	0/3	0/3	1/3	2/3
4f	0/3	0/3	0/3	2/3
5a	0/3	0/3	0/3	1/3
5b	0/3	0/3	0/3	3/3
5c	0/3	0/3	0/3	2/3
5d	0/3	0/3	0/3	1/3
5e	0/3	0/3	0/3	3/3
5f	0/3	0/3	1/3	3/3
Phenytoin	0/3	0/3	0/3	0/3
Carbamazepine	0/3	0/3	0/3	0/3

Table 3: Anti-convulsant activity synthesized derivatives 4(a-f) and 5(a-f).

Compounds	Intraperitoneal injection in mice[a]			
	scPTZ		Neurotoxicity	
	0.5 hr	4.0 hr	0.5 hr	4.0 hr
4a	30	30	-	-
4b	30	30	-	-
4c	300	300	-	-
4d	30	30	-	-
4e	30	100	-	-
4f	100	100	-	-
5a	300	300	-	-
5b	100	300	-	-
5c	100	300	-	-
5d	100	100	-	-
5e	-	300	300	300
5f	30	100	-	-
Carbamazepine	30	100s	100	100

The dosages administered were 30, 100, and 300 mg/kg, and obtained result here show the lowest doses at which bioactivity was observed in a middle or higher number of mice. We checked on the mice after 0.5 and 4 hr. The (-) indicates that there was no response at the maximum dosage (300 mg/kg).

because none of them could balance on the rotarod apparatus's rotating rod, except for compound 5e, which was neurotoxic at 300 mg/kg body weight.

In silico Studies

ADME Parameters

All synthesized compounds (4a–f) and (5a–f) underwent *in silico* studies to assess their physiochemical characteristics. Lipinski's rule states that there is a direct correlation between the anti-epileptic medication's absorption and their physicochemical characteristics, such as their log P, molecular weight, number of hydrogen bond donors, and number of hydrogen bond acceptors. Synthesized compounds demonstrated no violation of Lipinski's rule. The data was analyzed and it revealed that every compound is sufficiently lipophilic (>2) and able to pass the blood-brain barrier to have a sizable anticonvulsant effect. In all generated compounds,

Table 4: Anticonvulsant activity of synthesized compounds (4a-f) and (5a-f).

Compound Code	Onset of clonus		Onset of tonic	
	(0.5 hr)	(4.0 hr)	(0.5 hr)	(4.0 hr)
4a	63.2±1.24**	107.8±2.79**	63.2±1.24**	107.8±2.79**
4b	91.2±2.81**	110.4±1.83**	91.2±2.81**	110.4±1.83**
4c	143.2±3.92**	140±2.40**	143.2±3.92**	140±2.40**
4d	79.8±1.35**	100±1.79**	79.8±1.35**	100±1.79**
4e	-	96.4±2.36**	-	96.4±2.36**
4f	63.8±1.82**	105.8±2.31**	63.8±1.82**	105.8±2.31**
5a	62.4±1.72**	90.8±1.35**	62.4±1.72**	90.8±1.35**
5b	-	98±3.24**	-	98±3.24**
5c	64.6±1.80**	92±1.64**	64.6±1.80**	92±1.64**
5d	74±1.87**	105.6±2.90**	74±1.87**	105.6±2.90**
5e	96.2±2.13**	89.6±2.90**	96.2±2.13**	89.6±2.90**
5f	61.6±1.72**	89±1.30**	61.6±1.72**	89±1.30**

Table 5: ADME profile of synthesized compound (4a-f) and (5a-f).

Compound	% Absorption	Molecular Weight	TPSA	Hydrogen bond donor	Hydrogen bond acceptor	No. of rotatable bond	Log P	Violation	Drug likeness score
4a	86.064	392.46	63.17	1	5	5	5.98	1	-0.03
4b	85.705	406.49	63.17	1	5	5	6.38	1	-0.04
4c	79.978	498.59	83.40	2	6	7	7.61	1	0.57
4d	84.047	448.57	63.17	1	5	7	7.25	1	0.06
4e	81.093	468.56	63.17	1	5	6	7.67	1	0.36
4f	80.94	482.59	63.17	1	5	7	7.97	1	0.48
5a	86.297	330.39	63.67	1	5	5	4.42	0	-0.35
5b	86.007	344.42	63.17	1	5	5	4.82	0	-0.23
5c	80.27	436.51	83.40	2	6	7	6.06	0	0.48
5d	84.036	386.50	63.17	1	5	7	5.69	0	-0.22
5e	81.376	406.49	63.17	1	5	6	6.12	1	0.22
5f	81.267	420.52	63.17	1	5	7	6.41	1	0.32

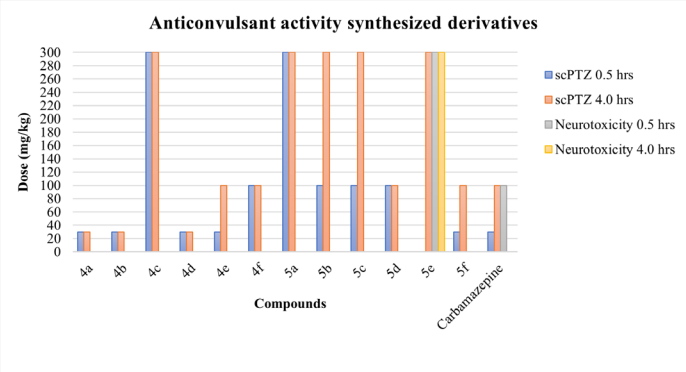


Figure 1: Anticonvulsant Activity of Synthesised commounds (4a-e and 5a-f).

the ABS% of the title compounds ranged from 79.978% to 86.297%. For optimal oral bioavailability, total hydrogen bond count (the sum of acceptors and donors) or low polar surface area are important indicators. All the functional variants adhere to Lipinski's criteria and have hydrogen bond acceptors between 5 to 6 and 1 to 2 hydrogen bond donor domains. To determine the Topological Polar Surface Area, molinspiration was utilized (TPSA). For molecule conformational flexibility and receptor interaction, the number of rotatable bonds is also essential. It is estimated that 10 rotatable bonds will be necessary to achieve oral bioavailability standards. All synthesized compounds have a high number of rotatable bonds i.e., 5 to 7, and they all show good conformational flexibility. Using the pkCSM descriptors algorithm's protocol, the synthesized derivatives' preliminary ADME profiles were evaluated as shown in Table 5.

Table 6: Binding energies of synthesized derivatives (4a-f) and (5a-f), and carbamazepine on 2COJ.

Sl. No.	Compound	Binding Energy	Number of Hydrogen		Amino acid	Number of Other interactions
			Conventional Hydrogen Bond	Carbon Hydrogen		
1.	4a	-6.66	2	0	ILE A:108, GLN A:142	10
2.	4b	-6.84	2	0	GLN A:142, LEU A:110	13
3.	4c	-6.72	0	2	LYS A:39, GLN A:77	10
4.	4d	-7.03	2	3	GLN A:142, ILE A:108, LEU A:110	14
5.	4e	-6.42	1	3	LEU A:110, GLN A:112	8
6.	4f	-6.05	1	0	GLN A:142	9
7.	5a	-5.53	3	2	GLY A:97, LYS A:99, PHE A:111	9
8.	5b	-5.31	3	0	LEU A:79, LEU A:81	7
9.	5c	-6.56	1	3	GLN A:142, LYS A:146	8
10.	5d	-6.29	2	0	GLN A:142, ILE A:108	13
11.	5e	-5.56	0	0	-	8
12.	5f	-5.84	3	1	GLN A:142, LEU A:110, ILE A:108	12
13.	Carbamazepine	6.17	1	1	GLY A:23	5

Molecular Docking study

AutoDock 4.2 software was used to perform ligand docking experiments of the compounds (4a-f) or (5a-f) into the Gamma-Aminobutyric Acid Aminotransferase ($GABA_A$) target. The crystal structures of the Gamma-Aminobutyric Acid Aminotransferases ($GABA_A$) enzyme were retrieved from the Proteins Data Bank (PDB ID: 2COJ), which is a validated target for AEDs. The protein preparation was carried out in three steps by using the "Discovery studio visualizer": pre-processing, review and change, and refining. The Protein Data Bank (PDB ID: 2COJ) showed the enzymes' crystal structure with the human gamma-aminobutyric acid receptor. The binding energy of carbamazepine (-6.17), while synthesized compounds (4a-f) or (5a-f) have binding energy between (-5.31 to -7.03) indicating that the molecule made significant contact with the GABAA-R active site. The docking position of the produced compounds showed that the NH of the amide group forms a hydrogen connection with the various amino acid side chains, while the nitrogen atom of the benzimidazole ring is linked to the active GABAA-R site via various amino acid side chains. (Figures 2 to 5) displays the 2D ligand interaction graphs for the most active drugs and carbamazepine (standard). The π-π interaction with the amino acid side chain and their distance are shown in Table 6. These interactions highlight the importance of nitrogen atoms for binding and, therefore, for inhibitory power. The benzimidazole scaffolds bind on the same ligand. The inhibitory effects of the benzimidazole scaffold against GABAA- R is significantly influenced by the p-p interaction and two hydrogen bonds. The interaction investigation offers a molecular understanding of the binding mechanism, which is essential for creating new benzimidazole-based ligands that interfere with $GABA_A$. The above research indicates that the synthesized compounds have potent anti-epileptic properties and work primarily by inhibiting inhibitory-aminobutyric acid (GABA) type A ($GABA_A$) receptors, just like conventional medicines (carbamazepine).

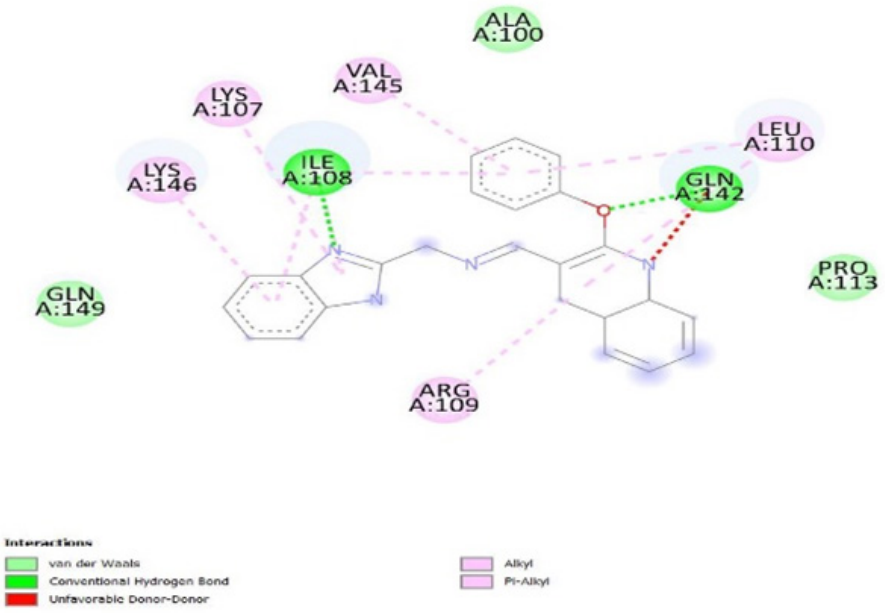


Figure 2: Compound 4a at the site of 2COJ is depicted in a 2D interaction picture.

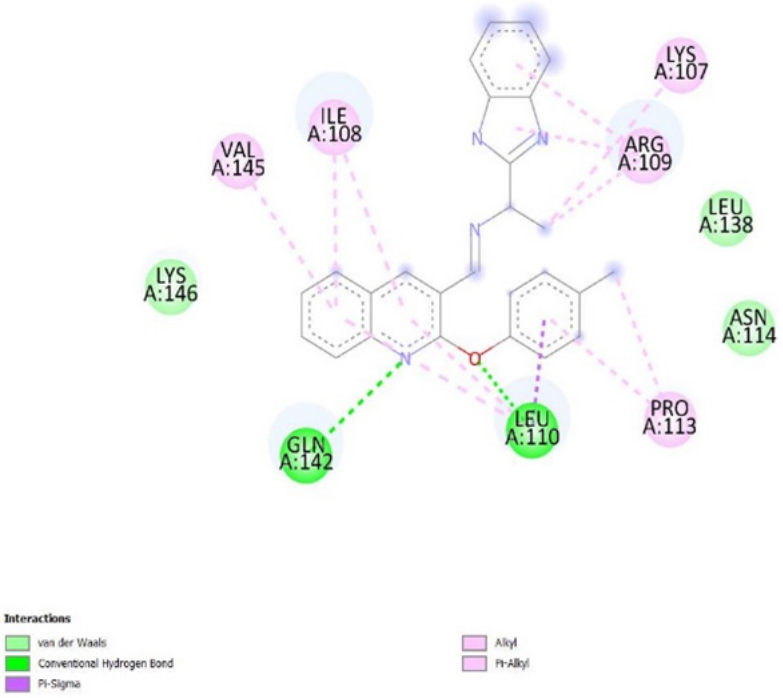


Figure 3: Compound 4b at the site of 2COJ is depicted in a 2D interaction picture.

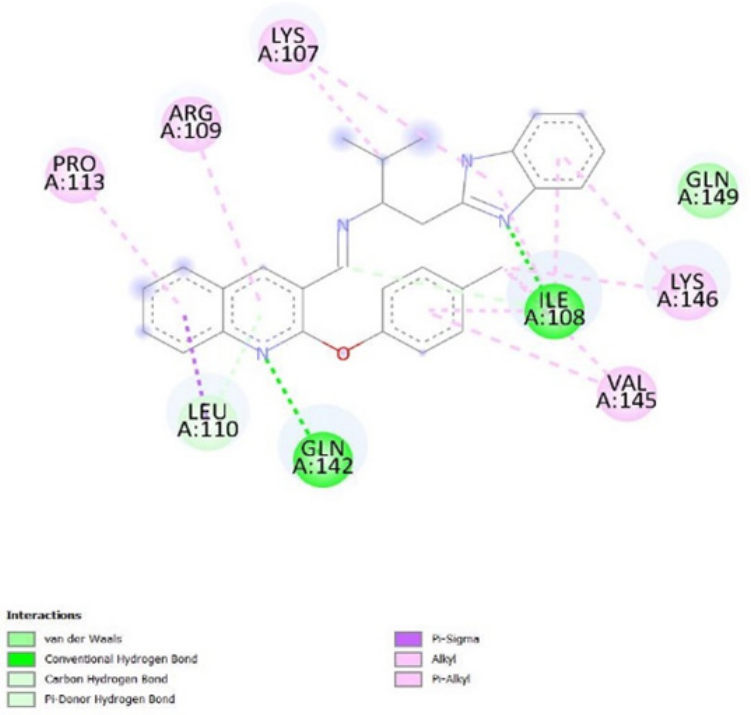


Figure 4: Compound 4d at the site of 2COJ is depicted in a 2D interaction picture.

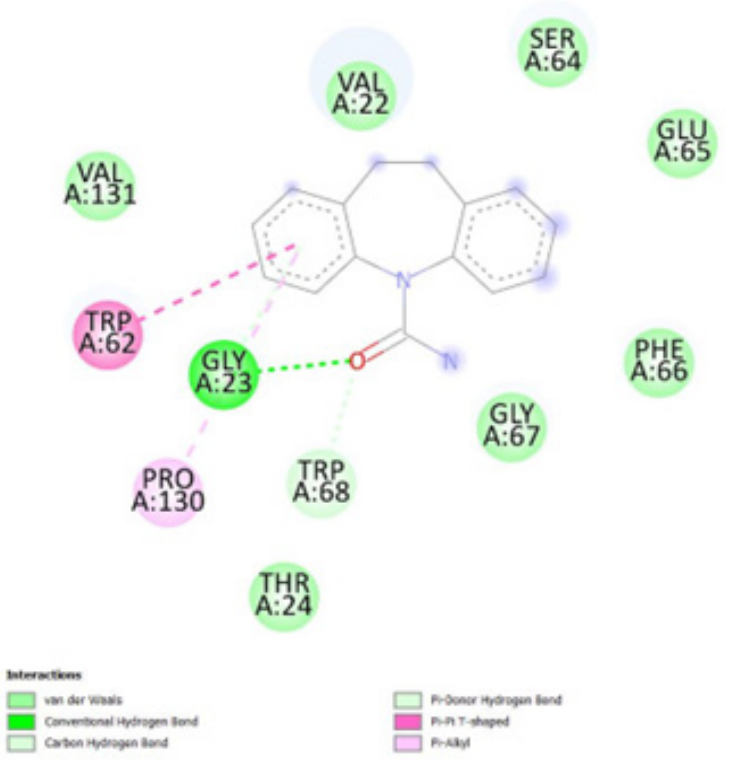


Figure 5: Carbamazepine at the site of 2COJ is depicted in a 2D interaction picture.

CONCLUSION

A novel series of quinoline-bearing benzimidazole were synthesized with the help of amino acids showing good anticonvulsant potential. It was evaluated by using scPTZ models. Among them, derivatives 4a, 4b, and 4d were shown to be most active among all synthesized derivatives at a dose of 30mg/kg after 0.5 hr as well as 4 hr with no neurotoxicity during preliminary anticonvulsant screening. The study also showed that, compared to conventional medications, the most potent compounds, 4a, 4b, and 4d had a better interaction with $GABA_A$ binding sites. Synthesized compounds are determined to have a decent drug-likeness score and do not break any of Lipinski's rule's parameters, according to an *in silico* analysis. The results above suggest that the synthesized compounds 4a, 4b, and 4d are effective anti-epileptic molecules with the potential to be developed and produced as AEDs after the appropriate research work.

ACKNOWLEDGEMENT

The authors are thankful to the Management of Noida Institute of Engineering and Technology (Pharmacy Institute), Greater Noida, India, for his continuous support and encouragement.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AEDs: Anti-epileptic Drugs; **GABA:** Gamma-aminobutyric acid; **NS:** Central Nervous System; **DNA:** Deoxyribonucleic acid; **V_{max}:** Maximum wavelength; **mg/mL:** Milligram per millilitre; **mL:** Millilitre; **Mol:** Mole; **mg/Kg:** Milligram per Kilogram; **R_f value:** Refractive value; **TLC:** Thin Layer Chromatography; **PTZ:** Pentylenetetrazole; **MSO:** Dimethyl sulfoxide **NMR:** Nuclear Magnetic Resonance; **^{1}HNMR:** Proton Nuclear Magnetic Resonance; **$CDCl_3$:** Chloroform; **IR:** Infrared; **DMF:** Dimethyl Formamide; **OECD:** Organisation for Economic Co-operation and Development; **m/z:** Mass to charge ratio; **LD_{50}:** Median lethal dose; **IUPAC:** International Union of Pure and Applied Chemistry.

SUMMARY

In the present work synthesized quinoline-bearing benzimidazole derivatives were synthesized with help of various amino acids and carbaldehyde by Vilsmeier-Haack reaction. The synthesized compounds were characterized by IR, ^{13}C NMR, ^{1}H NMR, and mass spectral techniques and evaluated for their anticonvulsant activity in swiss albino mice by the ScPTZ model. Among all synthesized compounds, 4a, 4b, and 4d were found to be most potent against anti-convulsion at a dose of 30 mg/kg after 0.5 hr as well as 4 hr with no neurotoxicity during screening and have a high docking score of -6.66, -6.84, and -7.03 as compared to Carbamazepine (-6.17). All the synthesized compounds were found to be active and stable biological activity. We provided a convenient synthetic process for the synthesis of novel quinoline-bearing benzimidazole derivatives and the results of the anticonvulsant screening are encouraging. Further investigations with suitable structural modifications of novel title compounds may result in therapeutically useful.

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Cite this article: Tyagi S, Salahuddin, Mazumder A, Kumar R, Datt V, Shabana K. Synthesis, Characterization, *in silico* and *in vivo* Evaluation of Amino Acid Derived Schiff Bases of Quinoline-Benzimidazole Hybrids as Antiepileptic Agents. Indian J of Pharmaceutical Education and Research. 2024;58(1s):s80-s92.