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Advances in Bioresearch

## ORIGINAL ARTICLE

# Molecular Docking, Synthesis and Evaluation of *in-vivo* Anticonvulsant potential of some novel Pyrido[2,3-d] Pyridazines

**Tanjima Tarique Laskar[1]*, Monica Arora[2], Meghna H Seta[3]**
[1]* School of Pharmaceutical Sciences, University of Science and Technology Meghalaya, India-793101
[2]Faculty of Health Sciences, Villa College, QI Campus, Rahdhebai Hingun, Male' 20373, Republic of Maldives
[3]Department of Pharmaceutical Sciences, Faculty of Health Sciences, Marwadi University, Rajkot-Morbi Highway, Rajkot, Gujarat, 360003, India.
Corresponding Author: Tanjima Tarique Laskar
Email- tanjimatarique.12@gmail.com
ORCID ID- https://orcid.org/0000-0002-6098-0114

**ABSTRACT**
*Ten substituted pyrido[2,3-d] pyridazines (5a-5j) was prepared using pyridine dicarboxylic acid and evaluated for in-vivo Anticonvulsant properties. The structure of the derivatives was confirmed using analytical methods such as IR, NMR ($^1H$ & $^{13}C$), Mass spectroscopy and Elemental analysis. In silico docking analysis was carried out using software Glide 5.9 of Schrodinger (executed in Maestro 9.4) which was used for extra-precision docking. The in-vivo anticonvulsant activity was performed using Pentylene tetrazole (PTZ) test in which 25 mg/kg dose of each synthesized compound was administered subcutaneously and compared with commercial drug Phenytoin (10 mg/kg). Compound **5b** exhibited excellent anticonvulsant potential in both docking study and in vivo experimental test and displayed the highest anticonvulsant potential at 25 mg/kg dose of the synthesized test compounds with 87% protection from mortality. It took 132.33 ± 12.53 sec ($P<0.01$ in comparison to control group) to cause a delay in development of myoclonic seizure and 141.38 ± 22.92 sec for onset of clonic seizures at a dose of 25mg/kg. In silico study suggested that the anticonvulsant action exerted by the compound **5b** might be due to higher binding affinity with GABA-A receptor and neuronal membranes hyperpolarization. This study highlights the therapeutic potential of compound **5b** as a potent anticonvulsant agent as evinced by their efficacy in subcutaneous-PTZ tests.*
***Keywords**: Pyridazines, Docking, synthetic procedure, in-vivo Anticonvulsant activity*

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## INTRODUCTION

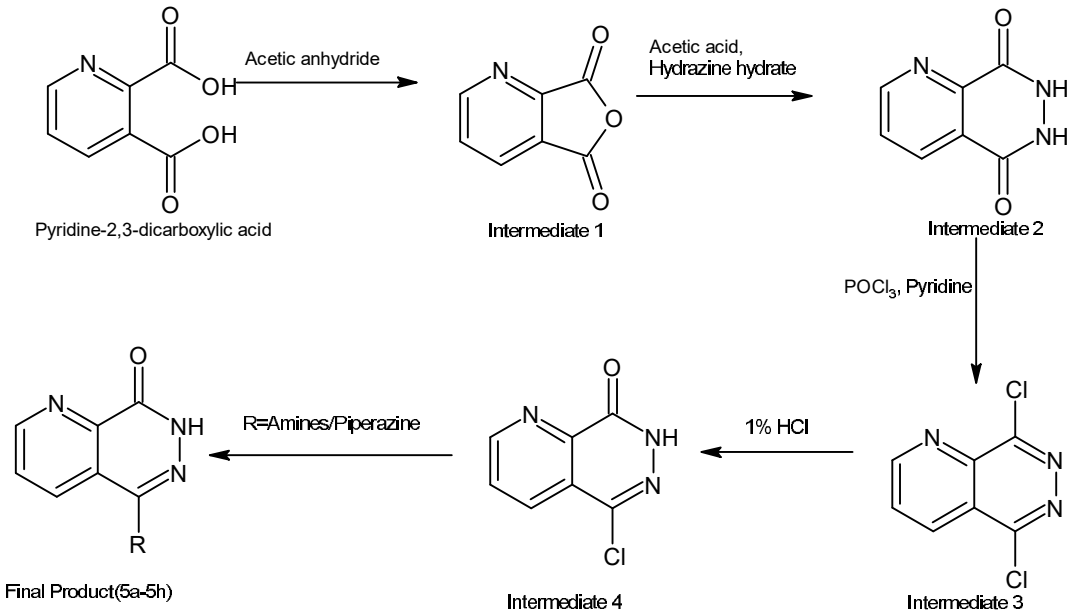
Heterocyclic compounds especially those containing pyridazine ring demonstrates significant therapeutic potential which renders them promising drug candidates in the field of medicinal chemistry[1,2]. Pyridazines in particular, are noteworthy molecules due to the presence of two adjacent functional nitrogen atoms in their core skeleton [3]. Pyridazine compounds exhibit diverse pharmacological effects encompassing anti-inflammatory, antibacterial, antihypertensive, antidiabetic, antioxidant, anticancer, antitubercular and antipsychotic properties [4,5]. Additionally, numerous studies have confirmed the efficacy of pyridazine derivatives in preventing epileptic attacks [6]. Several well-known compounds including Minaprine, Pipofezine (antidepressant), Imazodan (inotropic agent), Levosimendan, Pimobendan (heart failure), Zardaverine (Inhibitor of Phosphodiesterase III & IV isozymes), Zopolrestat (neuropathic pain & eye dysfunction) and Emorfazone (Pain killer) featuring a pyridazine ring moiety have been employed for management of numerous health implications and disorders exemplifying the broad utility of this heterocyclic scaffold [7,8]. One such category of pyridazine containing moiety is pyridopyridazines which are fused heterocyclic compounds, combining pyridazine with a pyridine ring similar to the isomeric structure of phthalazine presenting eight possible isomeric forms [9]. These compounds exhibit a wide spectrum of medicinal activities including antimicrobial activity, kinase

inhibitory activity, (Imatinib, Gefitinib and Sunitinib), gastric adenocarcinoma inhibitory activity (Vistusertib) & anticancer activity (Palbociclib) [10-15]. The growing demand for nitrogen-containing molecules has raised the importance of pyrido-pyridazines in the context of drug discovery and development. Notably, these biologically active compounds also excellent source of and anticonvulsant or antiepileptic activity which is defined as excessive neural activity and periods of altered consciousness, often accompanied by uncontrolled body movements. Seizures are typically categorized into two primary types based on electroencephalographic patterns: Partial (local or focal) seizures and Generalised seizures that fall into two categories: convulsive and non-convulsive [16-18]. Although the second and third generation antiepileptic medications are in use for many decades due to their potential in managing epileptic symptoms, but they do not address the underlying cause of the genesis of epilepsy. Furthermore, multiple-drug therapies may lead to development of toxicity due to drug-drug interactions and often cause unwanted side effects [19,20]. This investigation explores synthesis of some new series of pyridopyrimidine derivatives and conducts *in silico* and *in vivo* antiepileptic activity of the synthesized compounds via molecular docking study and PTZ tests with the aim to develop novel antiepileptic medications with enhanced tolerability, reduced drug interactions and simplified pharmacokinetics.

## MATERIAL AND METHODS

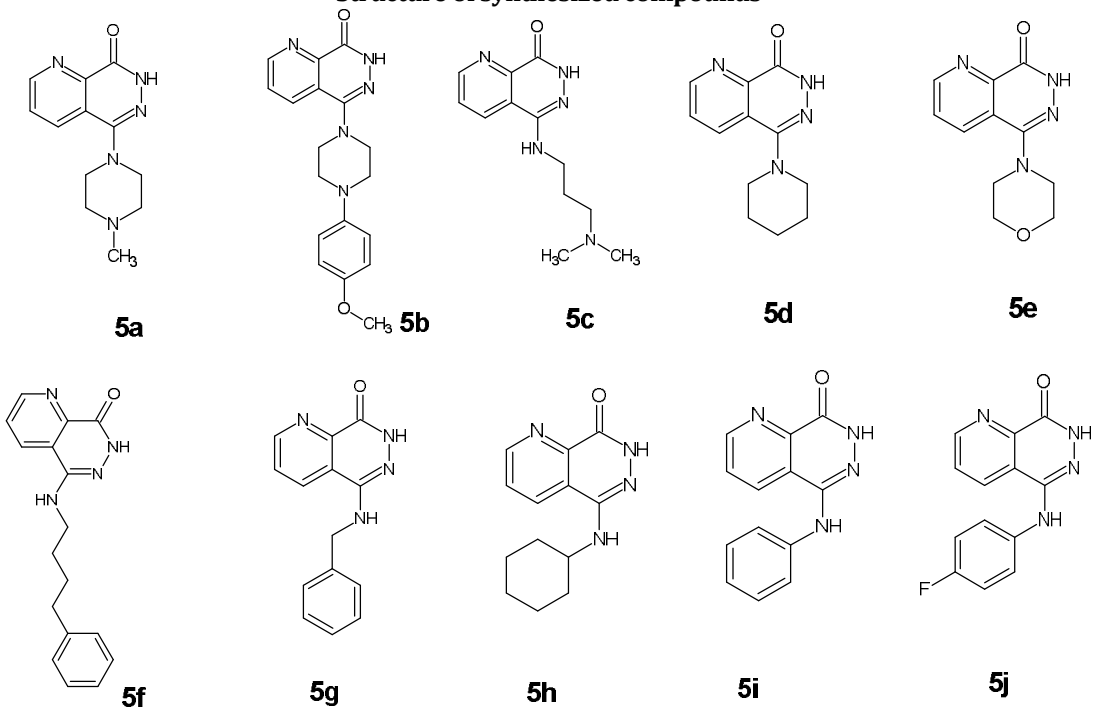
**Molecular Docking:** Schrodinger (Glide) software was employed for extra-precision (XP) docking of the designed pyridopyrimidines for assessing anticonvulsant potential. The target protein for molecular docking study was $GABA_A$ receptor (PDB ID: 4COF, 2.97 Å resolution, co-crystallized with Benzamidine**)** obtained from the Data Bank of Protein (RCSB). The target protein to be docked was prepared using Protein Preparation Wizard in software Maestro which treated formal charges, enclosed hydrogen atoms, eliminated water particles and bond ordering. To optimise the hydrogen bonding grid, a comprehensive sampling option was used. The force field OPLS_2005 along with the impref module (Impact 5.9) was used for minimization of energy of target receptor. The protonation of the compounds at pH 7.0 ± 2.0 was done using LigPrep 2.6 and Epik 2.4 and the energy was further reduced.

**Synthetic procedure:** The chemical process depicted in Scheme 1 was followed in the synthesis of the derivatives. Initially, 2,3-pyridine dicarboxylic acid (PDA) was subjected to dehydration using acetic anhydride and was refluxed at $110^0$C. The contents were stirred for 3-4 hours and the temperature was brought to 25 $^0$C following stirring. Dichloromethane was mixed to the contents at ice cold condition(0-$5^0$C) for precipitation of the contents. The white solid precipitated mass was allowed for filtration under vacuum condition and washed with Dichloromethane to produce the intermediate 1 (Furo[3,4-*b*] pyridine-5,7-dione). Acetic acid was mixed together with intermediate 1 with vigorous stirring and in a dropwise manner hydrazine hydrate was added slowly. After complete addition, the contents were refluxed at $95^0$C for 3hours to obtain intermediate 2(6,7-Dihydropyrido[2,3-*d*] pyridazine-5,8-dione). In the presence of pyridine, intermediate 2 was chlorinated with phosphorous oxychloride followed by stirring and refluxing for 5hrs. The contents were then mixed with ice and to that, sodium bicarbonate was added for neutralization of the contents in order to obtain the desired intermediate 3 (5,8-Dichloropyrido[2,3-*d*] pyridazine). The intermediate so formed was filtered under suction filtration and dried at $30^0$C for 3-4hrs followed by heating for 3hrs with dilute HCl (1%). Once the contents were cooled, intermediate 3 was precipitated and was filtered for separation. The separated contents were dried and crystallized with acetic acid to obtain the intermediate 4 (5-Chloropyrido[2,3-*d*] pyridazin-8(7*H*)-one). Intermediate 4 was neutralized with ammonium hydroxide for precipitating the contents. The precipitated contents were washed and filtered for separation and chlorine group was substituted with amines in the presence of polyethylene glycol-400 at 120-140 °C to obtain the desired final products. Progression of the reaction was tracked using thin-layer chromatography and column chromatography was employed to purify the final derivatives using various gradients of ethyl acetate and hexane.




**Scheme 1:** Schematic pathway for synthesis of desired compounds ( 5a-5j)

**Structure of synthesized compounds**




**Anticonvulsant activity:** *In-vivo* activity of synthesized compounds was investigated for pentylenetetrazol-induced seizures employing Swiss albino rats(n=6) of 150-200 grams weight. Under well-maintained and controlled experimental conditions (half day/night schedule at 25˚C), the Swiss albino rats were kept in a polypropylene cage with bedding made of rice husk (free of dust). The IAEC (reference number- AACP/IAEC/SEP2021/08; Registration No. 83/ReBi/SS/99/CPCSEA) had reviewed and approved all pharmaceutical procedures. The various pharmacological methods used to carry out anticonvulsant activity are as follows**:**

**PTZ (Pentylenetetrazol) model:** PTZ is a proconvulsant chemical that acts by antagonistically affecting $GABA_A$ activity and causes long-lasting convulsions in rats at a convulsive dose ($CD_{50}$). The Pentylene tetrazole (PTZ) test was used to screen compounds for their effectiveness in treating absence seizures or

petit mal epilepsy. In the experiment, six healthy rats from each of test and standard group were identified and labelled. All the derivatives to be screened were made into fine particles and suspended in a 5% (w/v) gum acacia solution. The animals in the test group received an intraperitoneal injection of 25 mg/kg dose of synthesised compounds prior to PTZ administration while the standard group received 10 mg/kg dose of Phenytoin. An intraperitoneal injection of 5% (w/v) gum acacia was injected to the control animals as well. After 30 minutes, PTZ (100 mg/kg) was subcutaneously injected into the animal's scruff and monitored for myoclonic jerks, clonic seizures and maximal tonic seizures. To assess the effectiveness of the medication, the onset of jerky motion and the length of the clonus phase were noted and the animals were kept under surveillance for 12hrs [21-23]. The formula for recording percentage protection is as follows:

$$\% \text{ Protection} = Mean\ of\ control - Mean\ of\ test / Mean\ of\ control \times 100$$

**RESULTS AND DISCUSSION**

**Molecular Docking:** The designed compounds, co-crystallized ligand Benzamidine and the standard drug Phenytoin were docked with $GABA_A$ receptor using Glide XP docking of Maestro GUI software, Schrodinger. The all the compounds showed moderate to good binding affinity except for which **5b** showed excellent binding affinity with the receptor with a docking score of -9.410 kcal/mol. The docking/binding affinity score and the 2D interactions determining the binding pattern of standard drug Phenytoin, co-crystallized drug and the test compounds of the receptor is given in Table 1.

**Table 1:** Binding affinity scores, hydrogen bonding and hydrophobic interactions with amino acid residues of target receptor

| Compound code | Binding affinity score of compounds with 4COF (Kcal/mol) | Hydrogen bonding interaction | Hydrophobic interaction |
|---|---|---|---|
| 5a | -6.923 | Ser A:172, Ala A: 240 | Glu A: 118 |
| 5b | -9.410 | Ala A: 240, Ser A:172 | Ile A: 116 |
| 5c | -6.445 | Asn A: 219 | Ala A:216, Leu A:279 |
| 5d | -7.467 | Ala A:240 | Gly A: 134, Ser A: 172, Ile A: 135, |
| 5e | -6.698 | Thr A: 704 | Phe A: 751, Ile A: 1280 |
| 5f | -8.545 | Cys A: 759 | Leu A: 707 |
| 5g | -8.134 | Leu A:758 | Val A: 793 |
| 5h | -7.667 | Ala A: 240 | Arg A: 175 |
| Co-crystallized ligand (Benzamidine) | -7.890 | Tyr A:157, Ser A:156 | Phe A:200, Tyr A: 205, Glu A:155 |
| Phenytoin | -7.843 | Gln A:65, Thr A:96 | Ile A: 130, Ala A:216 |

**Experimental section:** The chemicals were procured from Fisher Scientific, Finar Chemicals, Spectrochem, Sigma Aldrich and Merck. Open end capillary tubes and a digital device were employed for measurement of melting points. The progression of reaction was tracked using a TLC (Thin layer chromatography) plate made up of silica gel (thickness 0.25mm) employing various mobile phase liquids such as methanol, hexane, dichloromethane and ethyl acetate were used. UV light with wavelengths of 256 and 360 nm was used to screen and visualise the spots or bands on the silica gel plates. Using column chromatography, the synthesized derivatives were purified and FT-IR analysis of the compounds was conducted with a Shimadzu spectrometer. Bruker spectrometer (400 MHz) was employed for of NMR spectral study and mass spectra were obtained using an MS-2010A mass spectrophotometer (model Shimadzu). The Thermo Fisher FlashEA 1112 Analyser was used to analyse elemental analysis.

**Spectral and physical data**

(i) 5-(4-methylpiperazin-1-yl) pyrido[2,3-*d*] pyridazin-8(7*H*)-one(5a): Brownish solid; Percentage yield: 82%; Melting point:230.3°C; IR (KBr): 3160.8 (-NH str), 1662.5 (C=O str) cm-1; 1597.2(C-H str); $^1$H NMR (300 MHz, DMSO) δ (ppm): 12.28 (s, 1H, -NH), 9.11 (d, 1H, Ar-H), 8.63 (d, J=8.1Hz, 1H), 7.84 (q, J=12.0 Hz, 1H), 3.51 (s, 4H), 2.72(s, 4H,), 2.37(s, 3H); $^{13}$C NMR (400 MHz, DMSO) δ (ppm): 158.9(C-8), 154.2(C-5), 148.7(C-2), 142.8(C-9), 135.1(C-4), 126.4(C-3), 125.4(C-10), 69.77(C-3, C-5), 53.5(C-2), 48.7(C-6), 40.16(CH3); LC-MS(m/z): 246 [M+1]; Elemental analysis(C12H15N5O): C (57.96%) H (7.06%) N (28.65%) O (6.33%); Rf Value: 0.32.

(ii)5-[4-(4-methoxyphenyl)piperazin-1-yl]pyrido[2,3-*d*]pyridazin-8(7*H*)-one(5b): Brown solid; Percentage Yield: 73%; Melting point: 272°C; IR (KBr): 3160.8 (-NH str ), 1660.5 (C=O str ) cm- 1, 1265.5 (C-N str) cm-1 , 1246.8(C-O-C str); $^{1}$H NMR (300 MHz, DMSO) δ (ppm): 12.27 (s, 1H, -NH), 9.13 (d, 1H, Ar-H), 8.60 (dd, 1H, Ar-H), 7.85 (t, 1H, Ar-H), 6.97 (d, 2H, Ar-H ), 6.84 (d, 2H, Ar-H), 3.69 (s, 4H, piperazine-H), 3.57 (s, 4H, O-CH3), 3.19 (d, 4H, piperazine-H); 13C NMR (400 MHz, DMSO) δ(ppm): 154.3(C-5), 153.1(C-1) 148.9(C-2), 145.2(C-9), 135.1(C-4), 127.1(C-3), 126.0(C-10), 116.5(C-2, C-6), 114.9(C-3, C-5), 48.5(C-2, C-6), 49.31(C-2, C-6), 56.18(CH3); LC-MS(m/z): 338 [M+1]; Elemental analysis( C18H19N5O2): C (63.91%), H (6.79%), N (20.23%), O (8.98%); Rf Value: 0.71.

(iii) 5-{[3-(dimethylamino)propyl]amino}pyrido[2,3-*d*]pyridazin-8(7*H*)-one(5c): Yellow solid; Percentage Yield: 53%; Melting point: 215°C; IR (KBr): 3165.3(-NH str), 3405.2(-NH str), 1658.7(C=O str), 1585.1(C-O-C str), 1592.2( C-H str), 1095.6 (C-N str); $^{1}$H NMR(300 MHz, DMSO) δ (ppm): 11.75 (s, 1H, NH), 9.07 (q, J= 6.4 Hz, 1H), 8.56 (q, J=10 Hz, 1H), 7.87( q, J= 12.4, 1H), 6.96(t, J= 11.6 Hz, 1H), 3.49(m, 4H), 2.82(d, J=5.2 Hz, 2H), 2.66(d, J=1.6 Hz, 4H), 2.48(s, 2H); 13C NMR (400 MHz, DMSO) δ(ppm): 156.7(C=O), 155.1(C-2), 14.7(C-9), 145.9(C-5), 135.2(C-4), 125.7(C-3), 124.8(C-10), 72.43(C-3), 60.38(C-1), 24.34(C-2), 42.60(-CH3), 55.34(-CH3); LC-MS (m/z): 247[M+]; Elemental analysis (C12H17N5O): C (58.36%) H (6.52%) N (28.65%) O (6.47%); Rf Value: 0.12.

(iv) 5-(piperidin-1-yl)pyrido[2,3-*d*]pyridazin-8(7*H*)-one (5d): White solid; Percentage Yield: 47%; Melting point: 220°C; IR (KBr): 3020.1(-NH str), 2937.6(C-H str), 1656.7(C=O str), 1575.1(Ar C-H str), 1443.6(C-H bending); $^{1}$H NMR(300 MHz, DMSO) δ (ppm): 12.19 (s, 1H), 9.13 (d, J= 3 Hz, 1H), 8.56 (t, J=7.8 Hz, 1H), 7.86 (q, J= 12.6Hz, 1H), 3.38 (q, J= 20.4 Hz, 4H), 2.76 (m, 2H), 1.68 (d, J=25 Hz, 4H); 13C NMR (400 MHz, DMSO) δ(ppm): 159.8(C=O), 146.3(C-2), 142.1(C-9), 155.0(C-5), 136.0(C-4), 124.9(C-3), 125.9(C-10), 51.4 (C-2, C-6), 27.33 (C-3, C-5), 26.17 (C-4); LC-MS(m/z): 231 [M+1]; Elemental analysis(C12H14N4O):C (62.69%) H (6.03%) N (24.44%) O (6.84%); Rf Value: 0.80.

(v) 5-(morpholin-4-yl)pyrido[2,3-*d*]pyridazin-8(7*H*)-one(5e): White solid; Percentage Yield: 64%; Melting point: 225°C; IR (KBr): 3155.1(-NH str), 2936.3 (C-H str), 1693.3(C=O str), 1594.3(Ar C-H str), 1484-1353(-CH bending), 1122.1(C-O-C str); $^{1}$H NMR(300 MHz, DMSO) δ (ppm): 12.27 ( s, 1H), 9.11 ( d, J= 6.1 Hz, 1H), 8.63 (m, J=9.6 Hz, 1H), 7.86 (m, J= 16.5Hz, 1H), 3.80 ( t, J= 9 Hz, 4H), 3.50 (s, 4H); 13C NMR (400 MHz, DMSO) δ(ppm): 158.9(C=O), 148.3(C-2), 142.8(C-9), 154.1(C-5), 135.3(C-4), 126.7(C-3), 125.5(C-10), 66.14 (C-2, C-6 morpholine), 49.86 (C-3, C-5 morpholine); LC-MS(m/z): 232 [M+]; Elemental analysis(C11H12N4O2): C (56.62%), H (6.26%), N (23.12%), O (14.01%); Rf Value: 0.60.

(vi) 5-[(4-phenylbutyl)amino]pyrido[2,3-*d*]pyridazin-8(7*H*)-one(5f): Yellowish green solid; Percentage Yield: 74%; Melting point: 253°C; IR (KBr): 3152.1 (-NH str), 2896.3(C-H str), 1663.4(C=O), 1580.3(Ar-CH str), 1538.1(C=C str), 1477.1 (-CH bending); $^{1}$H NMR (300 MHz, DMSO) δ (ppm): 11.70 (s, 1H), 9.02 (q, J= 6.3 Hz, 1H), 8.59 (m, J=9.9 Hz, 1H), 7.89 (q, J= 12.6, 1H), 7.28 (m, J= 39.9 Hz, 5H), 6.72 (t, J=11.4 Hz, 1H), 2.51 (t, J=3.3Hz, 3H), 1.63 (t, J= 6.9Hz, 4H); 13C NMR (400 MHz, DMSO) δ(ppm): 159.6(C=O), 144.5(C-2), 143.1(C-9), 155.9(C-5), 137.0(C-4), 126.5(C-3), 124.7(C-10), 129.2 (C-2, C-4, C-5), 127.5 (C-3), 140.9 (C-1), 37.91(C-2, C-3), 28.68 (C-3, C-4); LC-MS(m/z): 295 [M+1]; Elemental analysis(C17H18N4O): C (68.40%), H (7.10%), N (19.08%), O (5.45%); Rf Value: 0.89.

(vii) 5-(benzylamino) pyrido[2,3-*d*] pyridazin-8(7*H*)-one (5g): White solid; Percentage Yield: 65%. Melting point: 198°C; IR (KBr): 3378.8, (-NH str), 1664.4(C=O str) 2928.2(C-H str), 1527.6(Ar-CH str), 1533.1(C=C str), 1459.3 (-CH bending); $^{1}$H NMR (300 MHz, DMSO) δ (ppm): 11.71 (s, 1H), 9.12 (d, J= 1.8 Hz, 1H), 8.58 (q, J=8.1 Hz, 1H), 7.91 (m, J=12.6Hz, 1H), 7.39 (m, J= 40.5 Hz 6H), 4.50 (d, J= 3.9 Hz, 2H); 13C NMR (400 MHz, DMSO) δ(ppm): 156.6(C=O), 143.5(C-2), 141.1(C-9), 155.9(C-5), 135.2(C-4), 125.6(C-3), 124.7(C-10), 126.2 (C-2, C-4, C-5), 125.5 (C-3), 140.2 (C-1), 34.81(C-2, C-3), 28.66 (C-3, C-4); LC-MS(m/z): 253 [M+1]; Elemental analysis(C14H12N4O): C (65.70%), H (5.71%), N (22.26%), O (6.32%); Rf Value: 0.88.

(viii) 5-(cyclohexylamino)pyrido[2,3-*d*]pyridazin-8(7*H*)-one(5h): Brown solid; Percentage Yield: 65%; Melting point: 213°C; IR (KBr): 3166.5(-NH str), 1697.8 (C=O str), 1586.3 (Ar C-H str), 1528.2 (C-H bending), 1010.2(C-N str); $^{1}$H NMR (300 MHz, DMSO) δ (ppm): 13.08 (s, 1H), 9.20 (q, J= 6.0 Hz, 1H), 8.62 (s, 1H), 8.63 (q, J= 8.0Hz, 4H), 7.95 (q, J=12.4 Hz, 3H), 3.79(m, 4H), 2.31(s, 6H); 13C NMR (400 MHz, DMSO) δ(ppm): 159.6(C=O), 144.5(C-2), 143.9(C-9), 155.8(C-5), 135.1(C-4),

125.2(C-3), 123.9(C-10), 49.02(C-1), 46.02(C-2), 34.20(C-6), 32.32(C-3), 29.05(C-5), 25.43(C-4); LC-MS(m/z): 245 [M+1]; Elemental analysis(C13H16N4O): C (65.20%), H (5.87%), N (21.42%), O (6.51%); Rf Value: 0.65

(ix) 5-anilinopyrido[2,3-*d*]pyridazin-8(7*H*)-one (5i): Black solid; Percentage Yield: 55%; Melting point: 253°C; IR (KBr): 3356.5(-NH str), 1667.8 (C=O str), 1571.3 (Ar C-H str), 1431.2 (C-H bending), 1310.2(C-N str),; $^{1}$H NMR (300 MHz, DMSO) δ (ppm): 13.09 (s, 1H), 9.23 (m, J= 13.8 Hz, 1H), 8.93 (s, 1H), 8.67 (q, J= 9.0Hz, 1H), 7.96 (t, J=13.5 Hz, 2H), 7.87(d, J=7.8 Hz, 1H), 7.33(t, J=16.2 Hz, 1H), 6.96(s, 2H), 3.33(s, 1H); 13C NMR (400 MHz, DMSO) δ(ppm): 156.6(C=O), 143.5(C-2), 142.9(C-9), 154.8(C-5), 136.2(C-4), 126.1(C-3), 125.2(C-10), 139.9(C-1), 127.5(C-2), 126.5(C-6), 122.8(C-3), 126.8(C-5), 118.7(C-4); LC-MS(m/z): 238 [M+]; Elemental analysis(C13H10N4O): C (66.40%), H (3.45%), N (23.56%), O (6.59%); Rf Value: 0.73

(x) 5-(4-fluoroanilino)pyrido[2,3-*d*]pyridazin-8(7*H*)-one (5j): Purple solid; Percentage Yield: 40%; Melting point: 258°C; IR (KBr): 3375.5(-NH str), 1686.8 (C=O str), 1586.1 (Ar C-H str), 1444.3 (C-H bending), 1317.2(C-N str), 1012.8(C-F str); $^{1}$H NMR (300 MHz, DMSO) δ (ppm): 13.09 (s, 1H), 9.21 (m, J= 13.6 Hz, 1H), 8.91 (s, 1H), 8.63 (q, J= 9.0Hz, 1H), 7.96 (t, J=13.5 Hz, 2H), 7.86(d, J=7.8 Hz, 1H), 7.36(t, J=16.2 Hz, 1H), 6.97(s, 2H), 3.32(s, 1H); 13C NMR (400 MHz, DMSO) δ(ppm): 159.6(C=O), 143.3(C-2), 142.4(C-9), 155.9(C-5), 135.2(C-4), 125.2(C-3), 124.2(C-10), 138.9(C-1), 127.5(C-2), 127.0(C-6), 120.29(C-3), 124.2(C-5), 115.1(C-4); LC-MS(m/z): 257 [M+1]; Elemental analysis(C13H19FN4O): C (61.04%), H (4.05%), F(8.45%), N (21.80%), O (4.69%); Rf Value: 0.85

**Anticonvulsant activity:**

**Pentylenetetrazole (PTZ) method:** The group of control animals receiving PTZ had displayed the myoclonic jerks within a time interval of 11.68 ± 6.39 sec while the time taken for exhibiting generalized clonic seizure was 17.1 ± 0.11 sec (P value <0.001 as compared to group of control mice). It was observed that all the animals suffered from convulsive hyperexcitability with a mortality rate of 100% after few minutes of administering chemo-convulsant agent whereas the animals which was administered with diazepam had displayed 100% protection from convulsions. Out of all the synthesized derivatives compound **5b** had shown the highest anticonvulsant potential activity with 87% protection from mortality and it took 132.33 ± 12.53 sec (P<0.01 in comparison to control group) to cause a delay in development of myoclonic seizure and 141.38 ± 22.92 sec for onset of clonic seizures. This compound had also exerted more strong anticonvulsant effect at higher doses (50 mg/kg) which might be a result of higher binding affinity with $GABA_A$ receptor and neuronal membranes hyperpolarization with a decreased rate of hyperexcitability. Table 2 demonstrates the duration of various convulsive phases caused by PTZ and the percentage convulsion and protection from seizures.

**Table 2:** PTZ induced seizure protection screening of synthesized derivatives(5a-5j) in animals

| Code of synthesized compounds | Administered doses (mg/kg) | Time taken(sec) for dormancy to onset of Tonic seizures | Time taken(sec) for dormancy to onset of Clonic seizures | Convulsion (%) | Seizure protection (%) |
|---|---|---|---|---|---|
| 5a | 25 | 162 ± 18.15 | 412.5 ± 12.50 | 13 | 69 |
| 5b | 50 | 328.00 ± 47.15*** | 623.13 ± 45.22*** | 31 | 87 |
| | 25 | 132.33 ± 12.53** | 141.38 ± 22.92ns | | |
| 5c | 25 | 82 ± 18.01 | 201.4 ± 0.01 | 64 | 36 |
| 5d | 25 | 103.34 ± 10.57 | 321.33 ± 22.50 | 50 | 50 |
| 5e | 25 | 63.90 ± 31.59 | 112.9 ± 7.18 | 72 | 28 |
| 5f | 25 | 75.3 ± 0.07 | 89.1 ± 0.11 | 84 | 16 |
| 5g | 25 | 69.6 ± 0.11 | 119.3 ± 0.67 | 70 | 30 |
| 5h | 25 | 36.7 ±0.28 | 31.2 ± 0.17 | 89 | 11 |
| PTZ (Control) | 100 | 11.68 ± 6.39 | 17.1 ± 0.11 | 100 | 0 |
| Phenytoin | 10 | No Seizure | No Seizure | 0 | 100 |

Data is generated using one-way ANOVA and Dunnett's test and represented by Mean ± SEM; ****P < 0.001; **P < 0.01; P < 0.05;

ns = not significant (in comparison to group of control animals treated with 100mg PTZ)

**CONCLUSION**

The strategic synthetic pathway allowed efficient synthesis of a diverse set of novel pyridopyrimidine compounds which were confirmed by characterization studies using spectral techniques. Molecular docking studies provided valuable insights of binding pattern within the receptor which facilitated the comprehensive understanding of the potential mechanisms underlying their antiepileptic properties. The evaluation of epileptic seizures employing PTZ model offered a prospective potential of the compound's

efficacy with compound **5b** demonstrating praiseworthy anticonvulsant effect both in docking study and PTZ seizure model. This study enhances the robustness of our findings and showcases the potential of the compound as potent anticonvulsant agents, addressing the complex challenges associated with epilepsy treatment.

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**CONFLICT OF INTEREST**
The authors declare no conflict of interests.

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