Phenyl-138.9(C-1), 127.6(C-2), 127.0(C-6), 120.39(C-3), 124.2(C-5), 115.1(C-4). LCMS m/z calcd. for $C_{13}H_9FN_4O$ Calcd: (m/z): 257.08$(M+1)^+$ Found 257.0$(M+1)^+$. Anal. Calcd for $C_{13}H_9FN_4O$ C(60.94%) H(3.54%) F(7.41%) N(21.87%) found C(60.93%) H(3.54%) N(21.82%).

Pharmacological activity

Anticonvulsant activity: The protection against the convulsions by synthesized compounds were studied *in-vivo* against MES-induced seizures and pentylenetetrazol induced seizures. Swiss albino rats weighing 150-200 g (n=6) of female sex were used in the experimentation. The animals were housed under a well-maintained and controlled conditions of light/dark schedule (12 hr) and temperature 25 ± 1˚C in a polypropylene cage with a dust free rice husk as a bedding. Animals had free access to food and water ad libitum. Before subjecting to the study, the animals were given a week of time to acclimatized with the laboratory condition. All the procedures for the pharmacological activities were priorly reviewed and approved by IAEC (Institutional Animal Ethical Committee) with reference no. AACP/IAEC/SEP2021/08(Approved by CPCSEA Regd No. 83/ReBi/SS/99/CPCSEA).

Anticonvulsant activity (MES method)

The anticonvulsant activity of the test drugs (synthesized pyridazine compounds) against grand mal type of epilepsy was determined by using MES (Maximal Electro shock) method (Model: Techno Electro Convulsometer). Test compounds and phenytoin was injected intraperitonially (i.p) at dose level 25 mg/kg body weight to each animal of different groups. A freshly prepared solution of solution of 5% gum acacia was used as a vehicle. Control animals were treated with vehicle only. The convulsions were induced 30min after drug treatment by application of ear clip electrode passing an alternating current of 50 mA for 0.2 sec. The abolition of the Hind Limb Tonic Extensor (HTLE) spasm was recorded as a measure of anticonvulsant activity. The results are tabulated in Table 2.

Anticonvulsant activity (PTZ model)

Pentylenetetrazol (PTZ) 80 mg/kg of body weight was administered subcutaneously into the scruff of the neck the animals. Diazepam was used as positive control at dose of 4 mg/kg body weight to each animal of different group of test drugs containing 6 animals each. test drug at a dosage of 25 mg/kg body weight was administered i.p, suspended in 5% gum acacia as pentylenetetrazol and diazepam respectively. Control animals were treated with vehicle only. PTZ was administered after 0.5 hr of administration of Diazepam i.p, in the standard group and test compounds in the test group of animals. The onset and severity of the myoclonic jerks and generalized seizure were recorded and tabulated in Table 2. The death of animals during the procedure was also recorded.

Anti-inflammatory activity by denaturation bovine serum albumin assay

The anti-inflammatory activity of the synthesized target compounds was determined by denaturation of the bovine serum albumin technique as per Mizushima and Kobayashi with slight modification. The sample to be analyzed were taken in 5ml total volume which contain 2% aqueous solution of bovine serum albumin and the pH of reaction mixture was adjusted to 7.4 using phosphate buffer saline. The test samples were further incubated for 30 mins at 370 C, and then heated to 510 C for 20 mins and cooled to room temperature and the solution was measured at 660 nm using UV-visible spectrometer. Percentage of inhibition of denaturation was calculated with reference to standard solutions of Ibuprofen and control samples with BSA and were tabulated in Table 3. The percentage inhibition of denaturation was calculated by using following formula,

%Inhibition=(Absorbance of control-Absorbance of test)/(Absorbance of control)*100

Molecular docking

ChemDraw ultra 12.0 software was used to prepare the ligand chemical structure, and were saved in mol format. The structures were later optimized by Chem3D Pro by Molecular mechanical MM2 algorithm, the 3D structures of the proteins were downloaded from RCSB Protein Data Bank (RCSB PDB). The binding interaction of all the compouds

determined with the protein targets NMDAR is an ionotropic glutamate receptor (PDB ID: 5TP9), gated by the endogenous coagonists glutamate and glycine, Gamma-aminobutyric acid receptor complexed with co-crystalized Benzamidine (PDB ID: 5cOF), and Voltage-gated sodium channel (PDB ID: 6AGF) co-crystallized with (2~{R})-1-(2-azanylethoxy)oxidanyl)phosphoryl) oxy-3-hexadecanoyloxy-propan-2-yl))~{Z})-octadec-9-enoate).
PyRx(AutoDock Vina 4.0 and AutoDock Vina) was used to predict binding energy of compounds by Empirical Fee Energy Scoring function and Lamarckian Genetic Algorithm. Macromolecules were prepared for docking by removing water molecules and with addition of the polar groups. The cocrystal molecule was isolated and hydrogen molecule were addedd to stabilise the molecule. active site was identified by cocrystal ligand and the grid box was placed to fit the binding site. The protein ligand interaction of compounds with respective ligand was visualized by Discovery Studio software.

CONCLUSION

The successful synthesis of novel 5-substituted amino pyridazines and their screening by *in silico* and *in vivo* anticonvulsant activities of compound 5a and 5b exhibited 100% in comparison with Phenytoin and compound 5g exhibiting a considerable potent anticonvulsant activity as well as the considerable anti-inflammatory activity by compound 5c showed that these derivatives may be used as a lead compound for developing newer anticonvulsant drugs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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