ANTICONVULSANT ACTIVITY OF SOME SCHIFF BASES SYNTHESIZED FROM 2- AMINOPYRIDINE

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Summary

A series of new schiff bases of 2-aminopyridine were synthesized through the condensation reaction between 2-aminopyridine with different aldehydes / ketones and cyclic ketones. These schiff bases were characterized by elemental analysis, FT-IR, ^{1}H NMR spectroscopy. The compounds were evaluated for anticonvulsant properties against seizures induced by maximal electroshock (MES), and chemically induced seizures in mice. The acute adverse effects profiles were assessed with respect to impairments of motor co-ordination by rotorod test in mice. These schiff bases shows better anticonvulsant potency against MES and Sc.-PTZ induced seizures while found moderately active against Sc.-STY induced seizure screen. The compound I_6, II_2 and VII shows better ED_{50} values (6.16, 3.07, and 9.62) with PI >10, against MES induced seizures than that of reference drug (Phenytoin and Phenobarbital). Compound I_5, I_6 and II_1 in Sc.-PTZ shows promising ED_{50} while in Sc.-STY induced seizures only I_2 shows good ED_{50}.

Keywords: 2-Aminopyridine, Anticonvulsant Agents, Maximal Electro Shock, Pentylenetetrazole, Strychnine.

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Introduction

Epilepsy, one of the oldest conditions known to mankind, and is one of the most common neurological disorder. According to the World Health Organisation (WHO) around 60 million people worldwide affected by this condition. At least 50 per 100,000 of the general population found with epilepsy at any one time in their life span. The majority (60-70%) of these cases occur without clear etiology of disease. The epilepsy is characterised by recurrent, transient seizures, caused by sudden excessive firing of neurons in the central nervous system. Despite advances in epilepsy treatment over the past 15 years, currently available drugs are only effective in 60-80% of epileptic patients [1] This efficacy is limited due to resistance to drug treatment. Thus the search for the new anticonvulsant drugs continued to be an active area of investigation in medicinal and pharmaceutical chemistry.

2-Aminopyridine derivatives are well known class of compounds due to their wide applicability in the synthesis of various pharmaceuticals (Antihistaminic, anti-inflammatory etc.)[2], and still continued to be the objects of considerable important in medicinal and pharmaceutical research. Various 2-substituted pyridine derivatives with significant anticonvulsant properties [3-4] had been reported earlier. 2-Aminopyridine derivatives also been reported for lipid absorption inhibitors [5]. Substituted benzoyl pyridines [6] are known to prevent convulsions induced by electroshock in mice. Previously few aminopyridine derivatives are reported as sodium channel modulators [7] with number of therapeutic applications, particularly in the treatment of convulsions, depression, and in pain. As sodium-channel plays major role in treatment of convulsions, various heterocyclic and pyridine derivatives were synthesized which may leads to development of effective anticonvulsants of future generation.

Schiff bases are the condensation product of aldehydes/ ketones with amines [8]. The schiff bases derived from various pyridine derivatives were reported to possess anticonvulsants [9-10],

cardiotonic [11] cytotoxic and antiproliferative [12], and antihypertensive [13-14], antifungal [15] and anticancer [16] activities. Some schiff bases from 2 and 3-aminopyridine [17] with cinnamaldehyde had been reported for iron carbonyl complexes but not evaluated for anticonvulsant activity. As part of our program directed toward the search for novel anticonvulsant agents, it was envisaged that the schiff bases from 2-Aminopyridine with various aryl aldehydes and ketones and cyclic ketones may have some interesting biological activities.

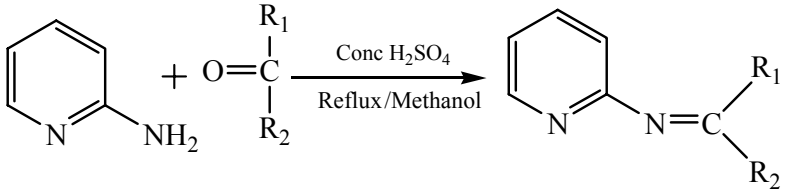
Materials and Methods

The melting points were determined by using BARNSTEAD / ELECTROTHERMAL STUART-SMP10, open capillary melting point apparatus and are uncorrected. Spectroscopic data were recorded on the following instruments SHIMADZU, RF-1501-UV Spectro-fluorophotometer. IR spectra were recorded on KBr disks using SHIMADZU, Infrared Spectrophotometer, FTIR-8400S. The NMR spectra's were measured at 25 ^{0}C in DMSO-d_6/$CDCl_3$ by JEOL AL300, FT-NMR Spectrophotometer (300MHz). Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Elemental analyses (C, H, and N) were undertaken with Perkin-Elmer model 240C analyzer. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminium plates, visualized in UV light. The log P values were determined using chem. Draw Ultra version 8.0software [18]. All the chemicals and solvents used for this work were obtained from Merck (Germany), S.D. Fine (Mumbai), Himedia (Mumbai) and Sigma-Aldrich chemical company (U.S.A.). The chemicals purchased were of analytical reagent grade or were purified by standard methods prior to use [19]. All the compounds had its IR, and 1H NMR spectra consistent with their assigned structure.

General method for synthesis of schiff bases from 2- amino pyridine (I_1- II_3):

Equimolar quantities 4.7 g (0.05mol.) of 2-Aminopyridine was taken in a round bottom flask which was dissolved in 40ml methanol and stirred at room temperature for 15 minutes to give a clear solution. The (0.05mol) of aryl aldehyde was added and few drops of concentrated sulphuric acid (catalyst) to this reaction mixture and was left for stirring for 8-14 hrs at temperature 90^0C on magnetic stirrer. The progress of reaction was monitored by thin layer chromatography (TLC), on completion of reaction the solvent was evaporated to dryness and to this residue 10ml ethyl acetate was added to precipitate the compound. The recrystallization of the synthesized compound was done in absolute ethanol and ethyl acetate at different proportions depending on the nature of the compound.

Figure-1: ***Preparation of schiff bases from aldehydes / ketones (I_1-IV_2)***



2-Aminopyridine Aldehyde / ketone I_1 - IV_2 (Schiff bases)

(I_1) *N-benzylidenepyridin-2-amine*: Recrystallization was done in ethyl acetate and absolute ethanol 95:5 v/v solution; yield 74.4%; mp 110-108 ^{0}C; TLC *Rf* 0.67in chloroform-methanol, (9/1, v:v); ^{1}H NMR(DMSO-d_6); 6.63-6.71(m, 4H, Py-H), 6.89 (s, 1H, -CH=N-), 7.60-7.90 (m, 5H, Ar-H), IR (ν, cm^{-1}) 2929 (CH), 1631(C=N), 1520, 1476, 1433 (C=C), 1261 (C-N) 1132, 806, 711. Analysis (% C, H, N) calculated/ found: 79.10/79.24, 5.53/5.51, 15.37/15.31.

(I_2) *2-((pyridin-2-ylimino) methyl) phenol*: Recrystallization was done in ethyl acetate and absolute ethanol 95:5 v/v solution;

yield 53.4%; mp 202-200 ^{0}C; TLC *Rf* 0.52 in chloroform-methanol, (9/1, v:v); ^{1}H NMR(DMSO-d_6); 5.01 (s, 1H, Ar-OH), 6.63-6.72(m, 4H, Ar-H), 6.94 (s, 1H, -CH=N-), 7.61-7.91 (m, 4H, Py-H), IR (ν, cm^{-1}) 3444-3425 (OH), 2992 (CH), 1602(C=N), 1550, 1467, 1433 (C=C), 1359, 1315 (C-N),1122, 871, 619. Analysis (% C, H, N) calculated/ found: 72.71/72.58, 5.08/5.11, 14.13/14.39.

(I_3) *N-(2-nitrobenzylidene) pyridin-2-amine*: Recrystallization was done in ethyl acetate and absolute ethanol 99:1 v/v solution; yield 58.6%; mp 171-169 ^{0}C; TLC *Rf* 0.71 in chloroform-methanol, (9/1, v:v); ^{1}H NMR(DMSO-d_6); 6.67-6.72(m, 4H, Ar-H), 6.95 (s, 1H, -CH=N-), 7.62-7.91 (m, 4H, Py-H), IR (ν, cm^{-1}) 2995, 2899 (CH), 1603(C=N), 1498, 1435, (C=C), 1384, 1352 (C-N), 1120, 1035, 972. Analysis (% C, H, N) calculated/ found: 63.43/63.68, 4.00/4.12, 18.49/18.26.

(I_4) *N-(4-nitrobenzylidene) pyridine 2- amine*: Recrystallization was done in ethyl acetate and absolute ethanol 99:5 v/v solution; yield 61.2%; mp 139-137 ^{0}C; TLC *Rf* 0.44 in chloroform-methanol, (9/1, v:v); ^{1}H NMR($CDCl_3$); 7.25-7.83 (m, 4H, Ar-H), 8.15-8.53 (m, 4H, Py-H), 9.29 (s, 1H, -CH=N-), IR (ν, cm^{-1}) 3074, 2926 (CH), 1589(C=N), 1518, 1431, (C=C), 1342, (C-N), 1105, 692, 621. Analysis (% C, H, N) calculated/ found: 63.43/63.21, 3.99/3.91 18.49/18.35.

(I_5) *N-(2-chlorobenzylidene) pyridin-2-amine*: Recrystallization was done in ethyl acetate solution; yield 44.5%; mp 113-111 ^{0}C; TLC *Rf* 0.52 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 6.87-7.32 (m, 4H, Ar-H), 7.95 (s, 1H, -CH=N-), 7.64-7.81 (m, 4H, Py-H), IR (ν, cm^{-1}) 2926, 2862 (CH), 1600(C=N), 1408, (C=C), 1362 (C-N), 1126, 1047, 912. Analysis (% C, H, N) calculated/ found: 66.52/66.78, 4.19/4.10, 12.93/12.78.

(I_6) *2-methoxy-4-((pyridin-2-ylimino)methyl)phenol*:
Recrystallization was done in ethyl acetate and absolute ethanol 95:5 v/v solution, yield 65.5%; mp 207-205 ^{0}C; TLC *Rf* 0.71 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 3.38 (s, 3H, -OCH_3-), 4.88 (s, 1H, Ar-OH)

6.68-6.80 (m, 4H, Py-H),7.69-7.74 (m, 3H, Ar-H), 7.26 (s, 1H, -CH=N-), IR (ν, cm^{-1}) 3416-3379 (OH), 2978 (CH), 1624(C=N), 1545, 1481, (C=C), 1383, 1325 (C-N),1126, 995, 769. Analysis (% C, H, N) calculated/ found: 68.41/68.78, 5.30/5.07, 12.27/12.30.

(I_7) *N-(3,4-dimethylbenzylidene)pyridin-2-amine*: Recrystallization was done in ethyl acetate and chloroform 90:10 v/v solution, yield 44.2%; mp 206-204 ^{0}C; TLC *Rf* 0.41 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 2.50 (s, 6H, -OCH_3-), 6.64-6.73 (m, 4H, Py-H),7.62-7.67 (m, 3H, Ar-H), 7.89 (s, 1H, -CH=N-), IR (ν, cm^{-1}) 2982 (CH), 1633(C=N), 1522, 1481, (C=C), 1390, 1321 (C-N),1124, 999, 769. Analysis (% C, H, N) calculated/ found: 80.17/80.07, 6.70/6.51, 13.39/13.70.

(I_8) *N-(3,4,5-trimethoxybenzylidene)pyridin-2-amine*:
Recrystallization was done in hexane and ethyl acetate 80:20 v/v solution, yield 48.6%; mp 58-56 ^{0}C; TLC *Rf* 0.68 in benzene-chloroform, (50/50, v:v); ^{1}H NMR ($CDCl_3$); 3.94 (s, 9H,-OCH_3-), 7.04 (s, 2H, Ar-H),7.61-7.78 (m, 4H, Py-H), 9.87 (s,1H,-CH=N-), IR (ν, cm^{-1}) 2995, 2901 (CH), 1604(C=N), 1489, 1437, (C=C), 1350, 1319, (C-O), 1242, (C-N), 1037, 972, 709. Analysis (% C, H, N) calculated/ found: 66.16/66.35, 5.92/5.81, 10.29/10.43.
(II_1) *Phenyl-N-pyridin-2-yl formimidate*: Recrystallization was done in ethyl acetate and absolute ethanol 95:5 v/v solution, yield 56.6%; mp 210-108 ^{0}C; TLC *Rf* 0.74 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 6.62-6.71 (m, 4H, Py-H), 6.92 (s, 1H, -CH=N-), 7.60-7.90 (m, 5H, Ar-H), IR (ν, cm^{-1}) 3057, 2926 (CH), 1605 (C=N), 1520, 1446, (C=C),1334 (C-O), 1211 (C-N) 991, 750, 684. Analysis (% C, H, N) calculated/ found: 72.71/72.51, 5.08/5.41, 14.13/14.40.

(II_2) *N-((1H-indol-3-yl)methylene)pyridin-2-amine*: Recrystallization was done in absolute ethanol solution, yield 56.3%; mp 176-174 ^{0}C; TLC *Rf* 0.70 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 7.07-7.19 (m, 4H, Py-H), 8.41 (s, 1H, -CH=N-), 7.35-7.44 (m, 4H, Ar-H), 9.93 (s, 1H, Indole NH) (D_2O exchangeable, IR (ν, cm^{-1}) 3093-2922 (CH), 1631 (C=N), 1577, 1450, (C=C), 1444 (C-N) 1126, 1035, 856, 754. Analysis (% C, H, N) calculated/ found: 76.00/76.25, 5.01/5.12, 18.99/18.66.

(II_3) *N-(3,7-dimethylocta-2,6-dienylidene)pyridin-2-amine*:
Recrystallization was done in distilled water, yield 38.6%; mp 240-238 ^{0}C; TLC *Rf* 0.44 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 1.3 (s, 9H, CH_3), 2.10 (m, 4H, CH_2), 4.11-4.18 (m, 2H, =CH), 6.74-6.88 (m, 4H, Py-H), 9.16 (s, 1H, -CH=N-), IR (ν, cm^{-1}) 2999, 2899 (CH), 1604 (C=N), 1490, 1476, (C=C), 1116 (C-N) 1041, 902, 819. Analysis (% C, H, N) calculated/ found: 78.90/79.54, 8.83/8.69, 12.27/12.41.

General method for Synthesis of Schiff bases from 2-Aminopyridine (III_1- VII): In a 20ml round bottom flask hot methanolic solution of (4.7 g, 0.05mol.) of 2-Amino pyridine was taken and to this 20ml hot methanolic solution (0.05mol) of respective aryl ketone / cyclic ketones added drop-wise. Few drops of glacial acetic acid were added to this reaction mixture to make the resulting reaction mixture slight acidic. The resulting solution was left for stirring for 6-12 hrs at temperature 60-80^{0}C on magnetic stirrer. The progress of reaction was monitored by thin layer chromatography (TLC). At the completion of reaction the 2/3rd solvent was evaporated and resulting solution was kept for crystallization. The recrystallization was done accordingly in appropriate solvent system.

(III_1) *N-(1-phenylethylidene) pyridin-2-amine*: Recrystallization was done in ethyl acetate and chloroform 95:5 v/v solution, yield 46.5%; mp 215-210 ^{0}C; TLC *Rf* 0.77 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 1.02 (s, 3H, CH_3) 6.63-6.73(m, 4H, Py-H), 7.60-7.91 (m, 5H, Ar-H), IR (ν, cm^{-1}) 2922 (CH), 1624 (C=N), 1549, 1479, (C=C), 1119 (C-N) 1000, 768, 619. Analysis (% C, H, N) calculated/ found: 79.56/79.31, 6.16/6.02, 14.27/14.48.

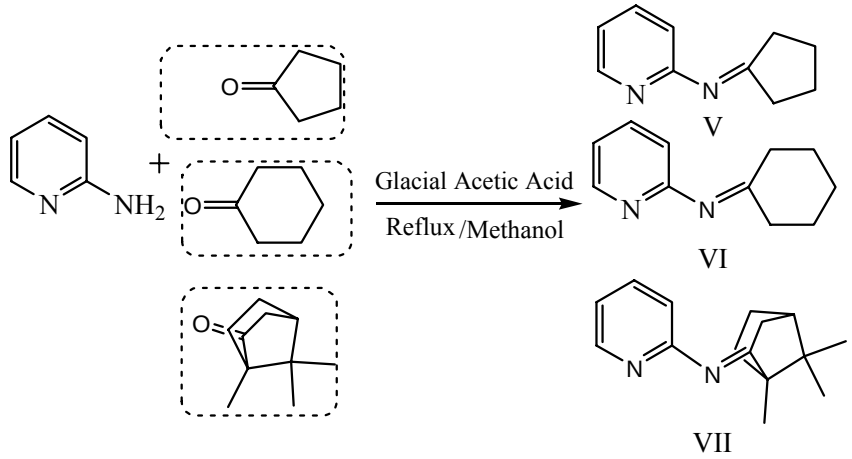
(III_2) *N-(1-(4-aminophenyl)ethylidene)pyridin-2-amine*:
Recrystallization was done in ethyl acetate and chloroform 95:5 v/v solution, yield 46.5%; mp 215-210 ^{0}C; TLC *Rf* 0.77 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 0.89 (s, 3H, CH_3) 6.63-6.73 (m, 4H, Py-H), 7.63-7.84 (m, 4H, Ar-H), 6.43 (s, 2H, NH_2)

(D_2O exchangeable), IR (ν, cm^{-1}) 3444 (NH), 2989 (CH), 1604 (C=N), 1554, 1468, (C=C), 1130 (C-N) 1045, 776,. Analysis (% C, H, N) calculated/ found: 73.91/73.84, 6.20/6.41, 19.89/19.68.

(IV$_1$) *N-(diphenylmethylene) pyridine-2-amine*: Recrystallization was done in ethyl acetate and absolute ethanol 95:5 v/v solution, yield 62.6%; mp 174-172 ^{0}C; TLC *Rf* 0.76 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 6.63-6.71 (m, 4H, Py-H), 7.10-7.60 (m, 10H, Ar-H), IR (ν, cm^{-1}) 2970 (CH), 1608 (C=N), 1520, 1464, 1445 (C=C), 1221 (C-N) 1112, 852,. Analysis (% C, H, N) calculated/ found: 83.69/83.54, 5.46/5.61, 10.84/10.50.

(IV$_2$) *1,2-diphenyl-2-(pyridin-2-ylimino)ethanol*: Recrystallization was done in ethyl acetate and chloroform 90:10 v/v solution, yield 48.9%; mp 142-140 ^{0}C; TLC *Rf* 0.48 in chloroform-methanol, (9/1, v:v); ^{1}H NMR (DMSO-d_6); 1.05 (d, 1H, CH), 3.24 (d, 1H, OH), 7.11-7.96 (m, 4H, Py-H), 7.55-8.69 (m, 10H, Ar-H), IR (ν, cm^{-1}) 3421 (OH), 2991 (CH), 1604 (C=N), 1545, 1489, (C=C), 1218 (C-N) 1147, 905,. Analysis (% C, H, N) calculated/ found: 79.14/79.55, 5.59/5.74, 9.72/9.51.

Figure-2*: *Preparation of schiff bases from cyclic ketones (V-VII)



2-Aminopyridine Cyclic ketones V, VI, VII (Schiff bases)

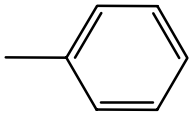
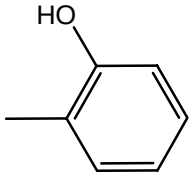
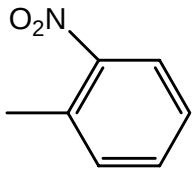
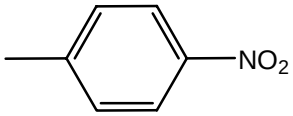
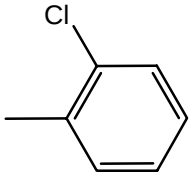
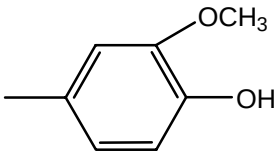
(V) *N-cyclohexylidene pyridin-2-amine*: Recrystallization was done in hexane and ethyl acetate 98:2 v/v solution, yield 61.6%; mp 221-219 ^{0}C; TLC *Rf* 0.62 in benzene-chloroform, (50/50, v:v); ^{1}H NMR(DMSO-d_6);1.25(s, 10H, CH_2), 6.93-7.86(m, 4H, Py-H), IR (ν, cm^{-1}) 2993 (CH), 1602(C=N), 1552, 1467, (C=C), 1230 (C-N) 873, 682. Analysis (% C, H, N) calculated/ found: 75.82/75.88, 8.10/8.52, 16.08/16.41.

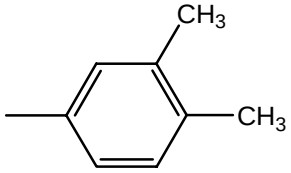
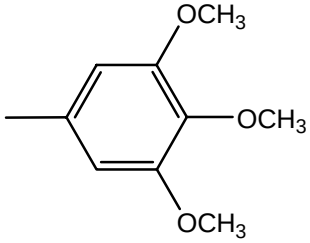
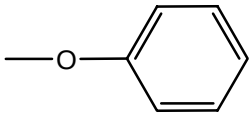
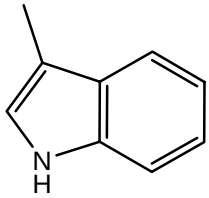
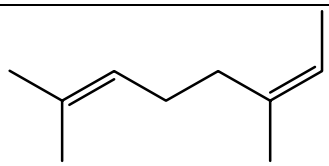
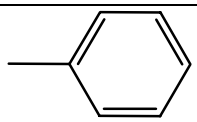
(VI) N-cyclopentylidene pyridin-2-amine: Recrystallization was done in hexane and ethyl acetate 95:5 v/v solution, yield 59.3%; mp 198-196^{0}C; TLC *Rf* 0.57 in benzene-chloroform, (50/50, v:v); ^{1}H NMR (DMSO-d_6); 1.65 (s, 8H, CH_2), 6.63-7.76 (m, 4H, Py-H), IR (ν, cm^{-1}) 2978, 2899 (CH), 1597(C=N), 1485, (C=C), 1134 (C-N) 873, 621. Analysis (% C, H, N) calculated/ found: 75.17/75.06, 7.55/7.98, 17.48/17.86.

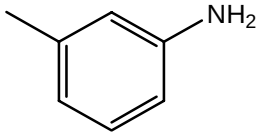
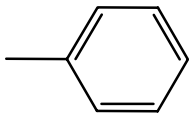
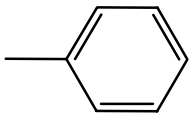
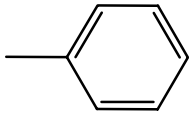
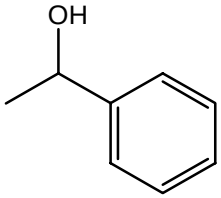
(VII) *N-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)pyridin-2-amine*: Recrystallization was done in ethyl acetate and absolute ethanol 95:5 v/v solution, yield 62%; mp 207-205^{0}C; TLC *Rf* 0.64 in chloroform-methanol, (9/1, v:v); ^{1}H NMR ($CDCl_3$); 1.25 (s, 9H, CH_3), 1.58 (s, 6H, CH_2), 1.58 (s, 1H, -CH-), 6.63-7.06 (m, 4H, Py-H), IR (ν, cm^{-1}) 2928, (CH), 1666, 1624(C=N), 1541,1477 (C=C), 1126 (C-N). Analysis (% C, H, N) calculated/ found: 78.90/78.58, 8.33/8.37, 12.27/12.65.

Chemistry: The different schiff base synthesis accomplished from 2-Aminopyridine with several aromatic aldehyde, ketones, and cyclic ketones (Fig. 1&2). The structures of the schiff bases were confirmed by different spectral analysis (^{1}H NMR, FTIR and elemental analysis). The purities of synthesized compounds were determined by its melting point and R_f (TLC) and ^{1}H NMR spectra. ^{1}H NMR, and IR spectral data of the schiff bases confirm the synthesis of proposed structure of the schiff bases (Table: 1&2).

Table-1: Physical constant's of the synthesized schiff bases:

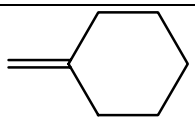
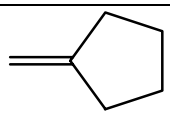
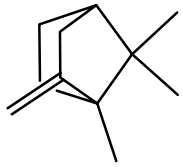
Comp.	R1	R2	Mol.* formula	Mol.* weight	Log P
I_1	H		$C_{12}H_{10}N_2$	182.0	3.28
I_2	H		$C_{12}H_{10}N_2O$	198.2	2.89
I_3	H		$C_{12}H_9N_3O_2$	227.2	2.31
I_4	H		$C_{12}H_9N_3O_2$	227.2	2.31
I_5	H		$C_{12}H_9N_2Cl$	216.7	3.38
I_6	H		$C_{13}H_{12}N_2O_2$	228.2	2.76

Comp.	R1	R2	Mol.* formula	Mol.* weight	Log P
I_7	H		$C_{14}H_{14}N_2$	210.3	4.25
I_8	H		$C_{15}H_{16}N_2O_3$	272.1	2.90
II_1	H		$C_{12}H_{10}N_2O$	198.2	3.01
II_2	H		$C_{14}H_{11}N_3$	221.3	2.82
II_3	H		$C_{15}H_{20}N_2$	228.3	3.68
III_1	CH3		$C_{13}H_{12}N_2$	196.2	2.85

Comp.	R1	R2	Mol.* formula	Mol.* weight	Log P
III_2	CH3		$C_{13}H_{13}N_3$	211.3	2.04
IV_1			$C_{18}H_{14}N_2$	258.3	4.74
IV_2			$C_{19}H_{16}N_2O$	288.3	4.03

*Mol. (Molecular)

Table-2: Physical constants of the synthesized schiff bases from cyclic ketones:

Compound	R	Mol. formula	Mol. weight	Log P
V		$C_{11}H_{14}N_2$	174.2	2.93
VI		$C_{10}H_{12}N_2$	160.2	2.51
VII		$C_{15}H_{20}N_2$	228.3	4.42

Pharmacology: The preliminary anticonvulsant screening of the synthesized compounds were achieved by three major convulsant tests: maximal electro shock (MES), subcutaneous pentylenetetrazole (Sc.-PTZ) and subcutaneous strychnine (Sc.-STY) and neurotoxicity screen (rotorod method in mice).

The pharmacological experiments were carried out on mice of either sex (Swiss-albino, 20–25 g), and male albino rats (Sprague–Dawley, 100–150 g). The animals were obtained from Central Animal House, Institute of Medical science, Banaras Hindu University (Regd. No. 542/02/ab/CPCSEA). The animals were randomly housed in polypropylene cages with free access to standard pellets (Hindustan Unilever Limited India) and water ad libitum. The experimental temperature was 25±1 ^{0}C, relative humidity around 45-55% with natural light–dark cycle. The experiments were conducted according to the norms of committee for the purpose of control the supervision of the experiments in animals (CPCSEA), New Delhi India. Prior permission was taken from institutional animal ethical committee (IAEC) to carry out the experiments. After adaptation to laboratory conditions (7 days), the animals were randomly assigned to experimental groups (six animals), and each animal was used only once. The experiments were conducted between 9:00 am to 4:00pm. The schiff bases were dissolved in water and polyethylene glycol (PEG-200) According to their solubility.

In Phase-I screening (Table: 3) each compound was administered at three dose levels 30, 100, and 300 (mg/kg) i.p., in different groups to evaluate its anticonvulsant potential. The anticonvulsant activity and neurotoxicity were assessed at 30 min and 4 hrs intervals after administration. Pharmacologically active compounds were further quantified in phase-II screening (Table: 4). In the determination of the ED_{50} and TD_{50} values, groups of 10 mice were given a range of intraperitoneal doses of the schiff bases until at least three points were established in the range of 10 to 90% seizure protection or minimal observed neurotoxicity. ED_{50} and TD_{50} values at 95% confidence intervals were calculated by using Probit analysis program [20]. The time topeak effect of all compounds was determined at the interval of 0.5 hr to 4-hr range.

Anticonvulsant Screening: The anticonvulsant evaluation of the synthesized compounds were under taken by the following the Anticonvulsant drug development protocol [21-23].

Maximal Electro Shock (MES) Method: In the MES test, seizures were elicited by 60 Hz alternating current of 50 mA intensity in mice. The current was applied via ear electrodes for 0.2 seconds. Abolition of the hind-leg tonic-extensor component of the seizure indicated protection against the spread of MES-induced seizures. The anticonvulsant activities of synthesized compounds were determined as its median effective doses ED_{50}, (dose in required to protect 50% of the animals tested against MES-induced seizures). The ED_{50} was calculated according to log-probit method [20]. In the MES test one can readily preselect compounds that are effective in suppression of tonic–clonic seizures and, to a certain extent, of partial seizures with or without secondary generalization in humans [24].

Chemically Induced Seizures: The seizures were induced by subcutaneous injection of a convulsant dose of pentylenetetrazole (CD_{97}) 85mg/kg and strychnine 2mg/kg in mice. Elevation of the pentylenetetrazole induced seizure threshold was indicated by the absence of clonic spasms for at least 5 seconds duration over 30 minutes with an accompanying loss of righting reflex, following administration of the test compound. The mice were placed separately into polypropylene cages (25·15·10, cm) and observed for 30 minutes for the occurrence of seizures. The number of animals convulsing out of the total number of mice tested was noted for each treatment condition.

Neurotoxicity Screening: The rotorod test was utilised to measure the minimal motor impairment in mice. The mice were trained to stay on revolving rod of diameter 3.2 cm, and rotating at the speed of 6 revolutions per min. The trained mice were given test compounds intraperitoneally in dose 30, 100, and 300 (mg/kg) body-weights. The neurotoxicity was measured by the inability of the animal to maintain equilibrium on that revolving rod for at least one minute in each of the three trails.

The adverse effects of synthesized compounds were expressed as its TD_{50} (the doses at which synthesized compounds shows impaired motor coordination in 50% of the animals tested). To evaluate TD_{50} value, at least four groups of animals (each group consisted of six mice) were injected with various doses of the different drug, and were challenged with the rotorod test. Impairment of motor performance, ataxia and loss of skeletal muscular strength are the most visible signs of acute neurotoxicity produced by drugs in preclinical studies [25-26].

Protective index: Protective index (PI) was calculated by dividing a given TD_{50} value evaluated in the rotorod test, by the respective ED_{50} values determined separately in the MES test and various chemically induced seizures. The PI is considered an index of the margin of safety and tolerability between anticonvulsant doses and doses of various synthesized compounds exerting acute adverse effects (e.g. sedation, ataxia, impairment of motor coordination or other neurotoxic manifestations) in preclinical studies [26].

Table-3: The anticonvulsant activity and minimal motor impairment evaluation after intraperitoneal (I.P.) injection in mice and oral administration in rats

Compounds	Intraperitoneal injection in mice[a]								Oral administration to rats[b]					
	MES screen		Sc PTZ screen		Sc STY screen		Toxicity screen		MES screen (time)hr					
	0.5	4	0.5	4	0.5	4	0.5	4	Dose (mg/kg)	0.25	0.5	1	2	4
I_1	-	-	100	300	300	300	100	100	50	0	1	1	1	1
I_2	100	100	100	300	30	300	300	-	50	1	2	3	3	2
I_3	300	-	300	-	-	-	100	100	50	-	-	1	-	-
I_4	100	100	30	100	300	300	300	-	12.5	2	2	3	3	2
I_5	30	100	30	30	-	-	-	-	12.5	2	3	4	4	2
I_6	30	30	30	300	100	-	-	-	12.5	3	4	4	4	2
I_7	-	-	300	-	100	-	-	-	50	1	-	1	-	-
I_8	-	300	300	300	300	-	100	300	50	-	-	-	-	-
II_1	30	100	30	100	100	300	300	-	12.5	2	4	4	4	3
II_2	30	30	300	-	100	300	-	-	12.5	2	4	4	3	3
II_3	300	-	-	-	100	100	100	300	50	-	-	-	-	-
III_1	-	-	300	-	30	300	-	300	50	-	-	-	-	-

Compounds	Intraperitoneal injection in mice[a]								Oral administration to rats[b]					
	MES screen		Sc PTZ screen		Sc STY screen		Toxicity screen		MES screen (time)hr					
	0.5	4	0.5	4	0.5	4	0.5	4	Dose (mg/kg)	0.25	0.5	1	2	4
III_2	300	300	100	300	100	300	300	300	50	-	1	-	-	-
IV_1	30	300	100	100	30	30	100	100	12.5	1	3	4	4	3
IV_2	30	100	300	300	300	-	-	-	12.5	2	3	3	4	3
V	30	100	100	300	100	100	-	-	12.5	1	4	4	4	4
VII	30	30	-	-	100	100	-	-	12.5	2	4	4	4	3
VI	-	-	-	-	30	300	100	100	50	-	-	1	-	-
Phenytoin[c]	30	30	-	-	-	-	100	100	..	..	..	..	..	..
Phenobarbital[c]	100	30	30	30	-	-	100	300	..	..	..	..	..	..

*a The compounds were administered at the dose of 30, 100 and 300 (mg/kg) in mice. The figures in the table indicate minimum dose needed to elicit the pharmacological effects in 50% or more in mice. The mice were examined 0.5h and 4h after injection of respective dose.
The (-) indicates an absence of anticonvulsant activity and neurotoxicity.
*b The figure in the screen indicates the number of rats out of 4 which were protected. The line (-) means no activity and (..) designation indicates that the compound was not screened.*c The data was taken from references [32].

Result and Discussion

Chemistry: The IR and ^1H NMR spectra's of synthesized compounds confirms the synthesis of schiff bases. The chemical schift at δ (8–6) has been ascribed for the aromatic protons. The singlet at δ (11.60–12.10) due to aldehyde (-CH=O) and resonance due to NH-group in (2-Aminopyridine) around δ (8-9) characteristics of starting material were absent in all schiff bases. The presence of (O-CH_3) resonating spectral peak at δ (3.38 & 3.94) in compounds (I_6 & I_8), the NH proton resonance in the compound (III_2 & II_2) at δ (6.43-9.93), and characteristics azomethine (–CH=N–) resonances between δ (6.89–9.87) ppm in the spectra of synthesized schiff bases strongly recommends the synthesis of proposed structures. In a study of somewhat similar type the azomethine (–CH=N–) resonance of schiff bases have been reported around δ (7–9) region, [27-28].

The IR spectra of schiff bases are found to be almost very similar to each other. Very strong and sharp bands at 1600– 1640 cm^{-1} are assigned to the ν(C=N) of azomethine of the schiff base [28-29]. Infrared spectra (FT-IR) of starting material (aldehyde/ketone) / (amine) display the characteristic bands associated with the (1700-1750) cm^{-1} (C=O) bonds and a sharp peak due to (N–H) stretching at (3320–3395) cm^{-1} these both peaks were absent in synthesised compounds strongly recommends the formation of schiff bases. The IR spectra of schiff bases display characteristic aromatic C=C stretching at 1510–1470 cm^{-1} in all schiff bases. In addition to this a very broad characteristic (O–H) vibration was observed in compounds (I_2 & I_6) at 3425–3444 cm^{-1}. A strong band observed around 1270–1260 cm^{-1} in the free schiff bases has been assigned to phenolic C–O stretching while 1120-1150 cm^{-1} is characteristic (C-N) stretch and band around 650-760 is due to aromatic (C-H) stretching. All these data are well in accordance with those of reported schiff bases [27-31] strongly supports the synthesis of proposed structures.

Anticonvulsant activity: The compounds were screened for anticonvulsant activity by using the procedures described previously. For the initial evaluation (phase-I) of anticonvulsant activity (Table: 3) three tests were performed for each compound, maximal electroshock (MES)-induced convulsions, subcutaneous pentylenetetrazole (Sc.-PTZ)-induced convulsions, subcutaneous strychnine (Sc.-STY) induced convulsions. Some selected schiff bases were examined for oral toxicity studies in the rat. In mice MES screen and rotorod neurotoxicity test were also performed to know the potential of motor impairment in co-ordination of movement of the tested compounds (Table: 3) as common side effect of most of marketed anticonvulsant drugs. As a result of preliminary screening, compounds I_2, I_4, I_5, I_6, II_1, II_2, III_1, III_2, IV_1, IV_2, V, VI, and VII were found potentially active for the consideration of their quantification of anticonvulsant activity and neurotoxicity evaluation (phase-II) in mice. The median effective dose (ED_{50}) and the median neurotoxic dose (TD_{50}) were established by this quantification study (Table: 4).

Experimental results were compared with standard antiepileptic drugs (Phenytoin, and Phenobarbital) [32]. Most of schiff bases showed a high degree of protection against MES-induced seizures, and very few were found effective against Sc.-PTZ induced seizures only II_2, IV_4, and VII. Compound II_2 shows the best protection against MES with ED_{50} of 3.07 (mg/kg) and PI of >100 amongst all synthesized schiff bases. Compound I_6 & VII with ED_{50} 6.16 & 9.62 (mg/kg), with PI >10 also have better potential to subside seizures than that of Phenytoin ED_{50} 9.5 (mg/kg) with PI 3.2 in the MES test. Compounds I_5 and IV_2 with ED_{50} values 15.82 and 16.92 (mg/kg) can also be evaluated for next stage of anticonvulsant screening.

Most of the synthesized compounds showed protection against MES seizures after 0.5 hr of drug administration. Compounds II_1, II_2, I_5, VI_6, IV_1, IV_2 and VII, were active at 30 (mg/kg), while I_2, I_4, at the dose 100(mg/kg) after 0.5 hr of administration of dose.

Compounds I_2, I_6, and VII at 30 (mg/kg) and compounds I_2, I_4, I_5, II_1, IV_2, and V, at 100 (mg/kg) dose shows better protection even after 4 hrs of drug administration. These synthesized schiff bases exhibited their ability to diminish the magnitude of tonic-clonic seizures with prolonged duration of action.

Table-4: Quantitative anticonvulsant data of some compounds in mice (Test drug administered i.p.)

Comp.	Time to peak effect	ED_{50} (mg/kg)			Rotorod toxicity TD_{50}	Protection index (PI) = TD_{50}/ ED_{50}		
		MES	Sc. PTZ	Sc. STY		MES	Sc. PTZ	Sc. STY
I_2	1	-	46.94	10.39	68.61	-	1.46	6.60
I_4	1	-	20.49	-	118.00	-	5.75	-
I_5	1	15.82	4.35	--	233.26	14.74	53.62	-
I_6	0.5	6.16	8.55	34.93	197.39	32.04	23.08	5.65
II_1	0.5	21.62	11.82	34.93	201.64	9.32	17.05	5.77
II_2	0.5	3.07	-	61.97	>500	>100	-	>10
III_1	0.5	-	-	28.96	201.64	-	-	6.97
III_2	0.5	-	55.49	46.94	201.64	-	3.63	4.29
IV_1	1	21.62	32.28	-	54.77	2.53	1.69	-
IV_2	2	16.92		-	157.00	9.27	-	-

V	0.5	20.36	32.27	52.23	>500	>10	>10	>10
VI	1	-	-	36.24	68.61	-	-	1.89
VII	0.5	9.62	-	34.93	>500	>10	-	>10
phenytoin[a]	2.0	9.5 (8.1–10.4)	-	-	65.5 (52.5–72.1)	6.9	<0.22	-
Phenobarbital[a]	1.0/0.5	21.8 (15.0–25.5)	13.2(5.87–15.9)	-	69.0 (62.8–72.9)	3.2	5.2	-

[a*] Data from reference [30].

In the MES screening of the compounds II_1, II_2, I_5, VI_6, IV_1, IV_2 and VII, at dose 100 (mg/kg) exhibits their prolonged duration of action. Compounds II_1, I_5, I_6, V, and VII showed rapid and longer duration of anticonvulsant action in this screen. Compounds I_4 and II_1 shows good ED_{50} (20.49, and 11.82) can be comparable to marketed anticonvulsants in Sc.-PTZ induced seizures. Only compound I_5 and I_4, II_1, and IV_1 were active after 4 hrs of drug administration in Sc.-PTZ screen at respective doses of 30, and 100(mg/kg). In case of strychnine induced seizures (Sc.-STY) only four compounds (I_2, III_1, IV_1, and VI,) shows protection at 30 (mg/kg) dose, amongst I_2 shows the best ED_{50} 10.39 (mg/kg with PI, 6.6). Compound I_7, II_1, II_2, II_3, III_2, V, and VII shows protection at 100(mg/ kg) dose after 0.5hr, while compound IV_1 was found active at 30(mg/kg) even after 4 hrs of drug administration. Four compounds (II_3, IV_1, V, and VII,) show's longer duration of action and were active even 4hrs after drug administration at 100(mg/kg).

Neurotoxicity: Neurotoxicity screening revealed that some compounds showing neurotoxicity at dose 100 and 300(mg/kg), 0.5 hr after the drug administration. The neurotoxicity level of standard drugs, (Phenytoin TD_{50} is 65.5mg/kg and of Phenobarbital is 69.0mg/kg) after 0.5 hrs. Compounds (I_2, IV_1, and VI) showed neurotoxicity somewhat comparable to reference drugs, while four compounds (I_1, I_3, IV_1, and VI,) showed prolonged neurotoxicity after (4hrs) and cannot be recommended for further screening. All compounds were screened for oral activity in rat at dose ranging from 12.5-50 (mg/kg) body weights to study their time to peak effect and toxicity profiles (Table: 3). From the above study it was clear that the time to elicit the peak anticonvulsant effect by different synthesized schiff bases varies from compound to compound, and there is no apparent toxicity in rats at oral dose 12.5-50 (mg/kg) body weights.

The inhibition of MES induced seizures by synthesized schiff bases (I_5, I_6, II_1, II_2, IV_1, IV_2, and VII) predicts their activity against generalized tonic-clonic and cortical focal seizures. The mechanism may involve here probably the blockage of neuronal voltage dependent Na^+ channels [33]. Pentylenetetrazole is a most frequently used substance as well as an acute experimental model used in the preliminary screening of potential anticonvulsant drugs. Compounds protecting against tonic-clonic seizures induced by PTZ are useful to control myoclonic and absence seizures in humans. The mechanism by which PTZ is believed to exert its action is by acting as an antagonist at GABA receptor complex [34]. Only compound I_2 exhibits good protection against Sc.-STY induced seizures and hence it is believed that they might acts through inhibitory glycine receptors [35]. Here it would be hasty to attribute the effect of schiff bases to the GABAergic system. The precise mechanisms of possible anticonvulsant effect of schiff bases are not yet clear.

Conclusion

In this study synthesized schiff bases mimicking the effects of anti epileptic drug by reducing tonic convulsion and mortality. Most of aryl-aldehyde schiff bases (I_4, I_5, I_6, and II_1, etc.) were found useful in suppressing generalized tonic-clonic seizures, while the schiff bases synthesized from aryl ketones and cyclic ketones (III_2, IV_1, and V) were found effective against absence seizures. Above discussion concluding that the synthesized schiff bases shows more selectivity towards MES & Sc.-PTZ seizure screen than that of Sc.-STY screen. The present research will guide our future development of potent and selective anticonvulsant drugs. However further studies on other species of animals with drug-induced epilepsy models is recommended, and comparison with other antiepileptic drugs in different species need to perform to fill the future need of model drug.

Statistical Analysis

Both ED_{50} and TD_{50} values of synthesized compounds from numerous seizure models and rotorod test were calculated by computer program log-probit analysis [20].

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