Synthesis, Preliminary Anticonvulsant and Toxicity Screening of (Z)-4-[(substitutedmethylene)amino]-5-phenyl-4H-1,2,4-triazole-3-thiols

Anand Kumar Srivastava[1*], Zeashan Hussain[2], Savita Upadhyay[3]

[1]*Research Scholar Dr. A. P. J. Abdul Kalam Technical University, Uttar Pradesh, Lucknow, Email: anand.srivastava275@gmail.com*

[2]*Professor and Principal, Faculty of Pharmacy, Mahatma Gandhi Institute of Pharmacy, Lucknow*

[3]*Professor and Principal, Faculty of Pharmacy, T.S. Mishra School of Pharmacy, Lucknow*

Corresponding Author

Anand Kumar Srivastava

Research Scholar Dr. A. P. J. Abdul Kalam Technical University, Uttar Pradesh, Lucknow, Email: anand.srivastava275@gmail.com

ABSTRACT

Keeping in view the structural requirements suggested in the pharmacophore model for anticonvulsant activity, a novel series of 20 Schiff bases (Z)-4-[(substitutedmethylene)amino]-5-phenyl-4H-1,2,4-triazole-3-thiols (AKS – 1 – AKS – 20) were synthesized by condensation of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol and different aromatic aldehyde with aromatic hydrophobic aryl ring, nitrogen atom as electron donor, and phenyl as distal aryl ring. Synthesized compounds were characterized by FTIR, 1H NMR, mass spectroscopy, and elemental analysis. Preliminary in vivo anticonvulsant screening (phase I) was performed by most adopted seizure model, maximal electroshock seizure (MES). Based on anticonvulsant screening results, three compounds, 4-(2-chloro-4-methylbenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 1), 4-(3-chloro-4-methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 3) and 4-(2-chloro-3-hydroxy-4-methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 7), were found to be most active; they exhibited activity comparable to standard drugs phenytoin (PHY). These active compounds were subjected to phase II and phase III screening, where they displayed much higher protective index (PI) in comparison to the standard drugs. In phase IV screening, the bioavailability of active compounds was assessed on oral administration. Further, preliminary safety profiles of AKS – 1, AKS – 3 and AKS – 7 were evaluated by the neurotoxicity testing and liver enzyme estimation.

Keywords: Schiff bases, 1,2,4-triazole, Anticonvulsant screening, Maximal electroshock seizure, Protective index, Bioavailability, Neurotoxicity

How to cite this article: Srivastava AK, Hussain Z, Upadhyay S. Synthesis, preliminary anticonvulsant and toxicity screening of (Z)-4-[(substitutedmethylene)amino]-5-phenyl-4H-1,2,4-triazole-3-thiols. Int J Drug Deliv Technol. 2026;16(73s): 1497-1511. DOI: 10.25258/ijddt.16.73s.159

INTRODUCTION

Recurrent convulsions are the hallmark of epilepsy, a persistent neurological condition brought on by aberrant activation of cerebral nerve cells in the central nervous system. Epidemiological research indicates that the condition affects about 60 million people globally. In India, around 10 million people suffer from epilepsy. Compared to the urban population (0.6%), the prevalence of epilepsy is higher in rural areas (1.9%). These numbers are increased by almost 2.4 million additional cases annually. It was believed that an imbalance between excitatory and inhibitory neurotransmitters causes convulsions.

For scientists and medical professionals, treating epilepsy is always extremely difficult. About 30% of epileptic patients are resistant to existing treatments and many of the available antiepileptic therapies, despite the fact that several new drugs have been developed in recent decades to treat epilepsy. Only 60–70% of individuals may achieve good seizure control with the current antiepileptic medications.

Vigabatrin, lamotrigine, gabapentin, topiramate, felbamate, rufinamide, and levetiracetam are some of the new anticonvulsants that have recently entered clinical settings. Up to one-third of epilepsy patients developed resistance to the best medication treatment,

despite the release of these new medications in the last ten years. Some negative side effects, including headache, nausea, hepatotoxicity, gastrointestinal problems, and hirsutism, outweigh the therapeutic effectiveness of these well-known, proven medications in lowering seizures.

As a result, medicinal and pharmaceutical chemistry researchers are still actively searching for novel anticonvulsant medications.

Numerous anticonvulsants fall under the Schiff bases group. Conjugation, typically an aromatic ring connected to the imine double bond, is a common structural characteristic that raises the iminium form's reduction potential. Partial seizures can be prevented with iminium form. Carbamazepine, oxcarbazepine, and eslicarbazepine acetate are a few well-known anticonvulsant medications that have an imine group in their structure. An important component of a drug's broader molecular architecture is the imine group, which is a carbon-nitrogen double bond (C=N).

The development of new 1,2,4-triazole compounds for their anticonvulsant properties has been the main focus of research. Compared to current medications, some of these substances might be more potent or have fewer adverse effects. It has been demonstrated that compounds containing a 1,2,4-triazole-3-thione core exhibit anticonvulsant properties. The benzodiazepines triazolam and alprazolam, as well as experimental substances like loreclezole, are examples of anticonvulsant medications that contain the 1,2,4-triazole ring. Numerous different 1,2,4-triazole compounds are being investigated for their possible anticonvulsant qualities; some have demonstrated promise by interacting with voltage-gated sodium channels or exhibiting extra antioxidant benefits.

Given these facts, it was deemed interesting to synthesize some newer derivatives of 1,2,4-triazole containing Schiff bases as anticonvulsant drugs as part of our ongoing research in the field. A good tool for creating prototypic molecules and explaining likely interactions is a pharmacophore model combined with physicochemical determination. The aromatic hydrophobic aryl ring, nitrogen atom as an electron donor, and phenyl as a distal aryl ring are structural characteristics shared by the named compounds in terms of interaction at the binding site.

In the present study, therefore, we hereby describe the synthesis and preliminary anticonvulsant evaluation of some new (Z)-4-((substitutedmethylene)amino)-5-phenyl-4H-1,2,4-triazole-3-thiols.

EXPERIMENTAL PROTOCOLS

Measurement

The chemicals and reagent were procured from S. D. fine Chemicals Mumbai and were used as such. Melting range was determined by Thiele tube and are uncorrected. Reaction progress was monitored by performing thin layer chromatography on silica gel G plates, using iodine vapours and UV chamber as visualizing agent. After physical characterization, compounds were subjected to spectral analysis. IR spectra were recorded on Shimadzu 8400S and Perkin Elmer Spectrum RX1 FTIR spectrophotometers and values are expressed in cm-1. Mass spectra were recorded on JEOL-AccuTOF JMS-T100LC spectrometer and NMR spectra were recorded on Bruker DRX-300 spectrometer and chemical shifts are reported in parts per million (δ values) from TMS (δ 0 ppm for 1HNMR) as an internal standard. Elemental analysis was performed on Elemental Vario EL III analyzer from Central Drug Research Institute, Lucknow.

General method for synthesis of (Z)-4-((substitutedmethylene)amino)-5-phenyl-4H-1,2,4-triazole-3-thiols (AKS-1-AKS-20)

Step I: Synthesis of benzoic acid hydrazide: A mixture of ethyl benzoate (0.01 mol, 1.5 grams), hydrazine hydrate (0.01 mol, 0.5 mL) and 30 mL ethanol was refluxed for 4 hours and the excess of ethanol was distilled off and the contents were poured into ice cold water and the precipitated hydrazides were filtered, dried and recrystallized from ethanol. TLC was carried out on a precoated silica plate.

Step II: Synthesis of potassium – benzoic acid hydrazide dithiocarbamate: A mixture of benzoic acid hydrazide (0.01 mol, 1.34 grams), KOH (0.015 mol, 0.84 gram) and 1.5 ml CS2 in absolute alcohol was stirred for 21 hours and product was isolated from diethyl ether. TLC was carried out on a precoated silica plate.

Step III: Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol: Potassium salt (0.01 mol, 2.5 grams) was taken in hydrazine hydrate and heated up to the evolution of H2S gas nearly 5 hours in oil bath. The reaction mixture was poured into crushed ice and treated with glacial acetic acid. The product was filtered and purified by KOH treatment and crystallized from ethanol. TLC was carried out on a precoated silica plate.

Step IV: Synthesis of (Z)-4-((substitutedmethylene)amino)-5-phenyl-4H-1,2,4-triazole-3-thiols: Equimolar quantities of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (0.01 mol, 1.92 grams) and respective aromatic aldehyde (0.01 mol) were

dissolved in ethanol. 4 to 5 drops of concentrated sulphuric acid was added and refluxed/stirred the reaction mixture for 2 - 10 hours (In microwave assisted synthesis the reaction mixture was subjected to microwave irradiation at 160 W intermittently at 30 second interval for 2 to 6 minutes) to give clear solution. The resulting solution was cooled to room temperature and the precipitate were separated by filtration, recrystallized from hot ethanol, washed with cold ethanol three times, and dried in vacuum desiccator containing anhydrous calcium chloride. TLC was carried out on precoated silica plates using solvent system, benzene-acetone (9:1) or ethyl acetate-hexane (1:19). In some cases, the reaction mixture was poured in cold water and allowed to stand overnight. Oily product obtained was separated by separating funnel (Figure 1).

4-(2-chloro-4-methylbenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 1)

Light yellow crystals, yield C.M.=77% , MWI=87%, mp: 144-150 ºC, logP=9.3, IR (KBr Cm^{-1}): 1486.4 (aromatic C=Cstr), 1599.4 (imine HC=N), 2853.7 (C-H alkane), 2555.6 (S-H thiol), 762.2 (C-Clstr), 1446 (C-Nstr), 696 (C-Sstr), ^{1}H NMR (300 MHz,CDCl3 δ ppm) 3.310 (s, 1H), 4.853 (t, 3H), 6.844 (s, 1H), 6.873-6.878 (d, 1H), 6.898-6.906 (d, 1H), 6.929 (s, 1H), 6.955 (s, 1H), 7.204-7.242 (q, 2H), 7.980 (s, 1H), DART-MS (M^++1 = 329.43), Anal. Calcd. For $C_{16}H_{13}ClN_4S$: C, 58.44%; H, 3.98%; N, 17.04%, Found: C, 58.90%; H, 4.08%; N, 17.23%.

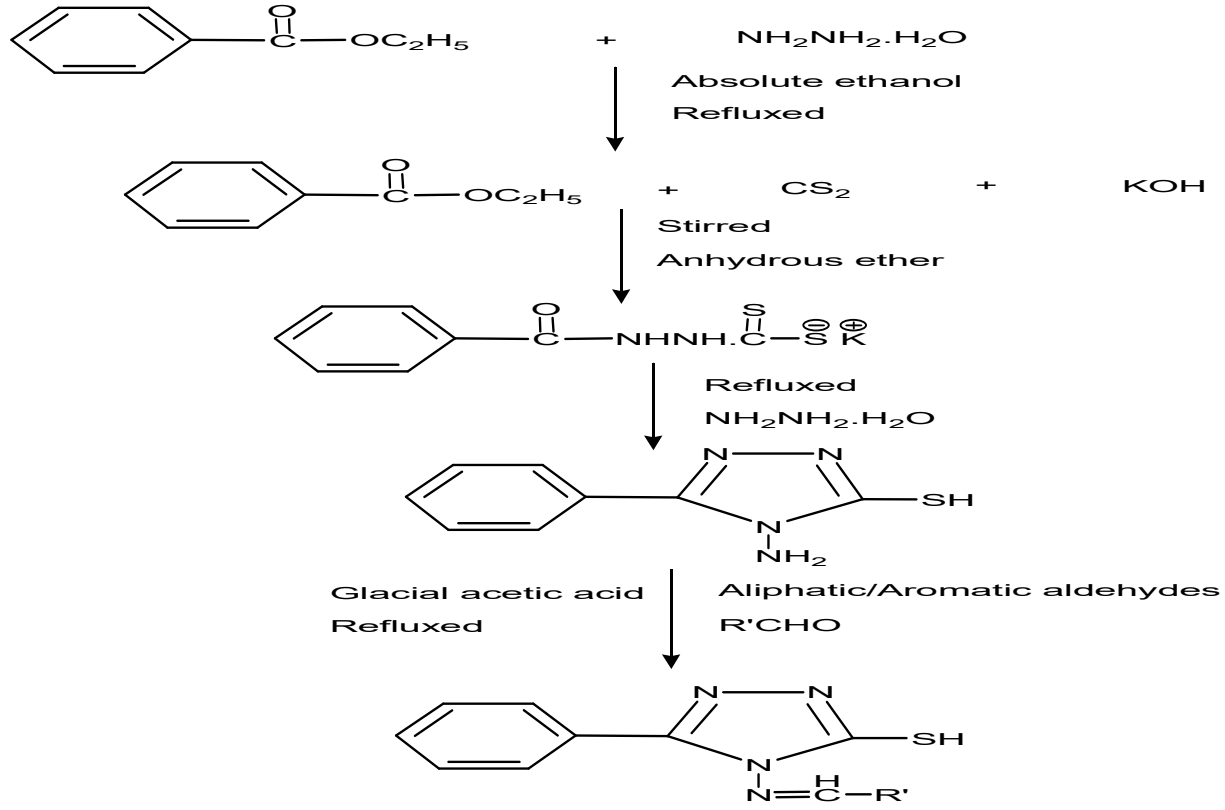

Fig. 1: Synthesis of (Z)-4-[(substitutedmethylene)amino]-5-phenyl-4H-1,2,4-triazole-3-thiols (AKS-1 - AKS-20)

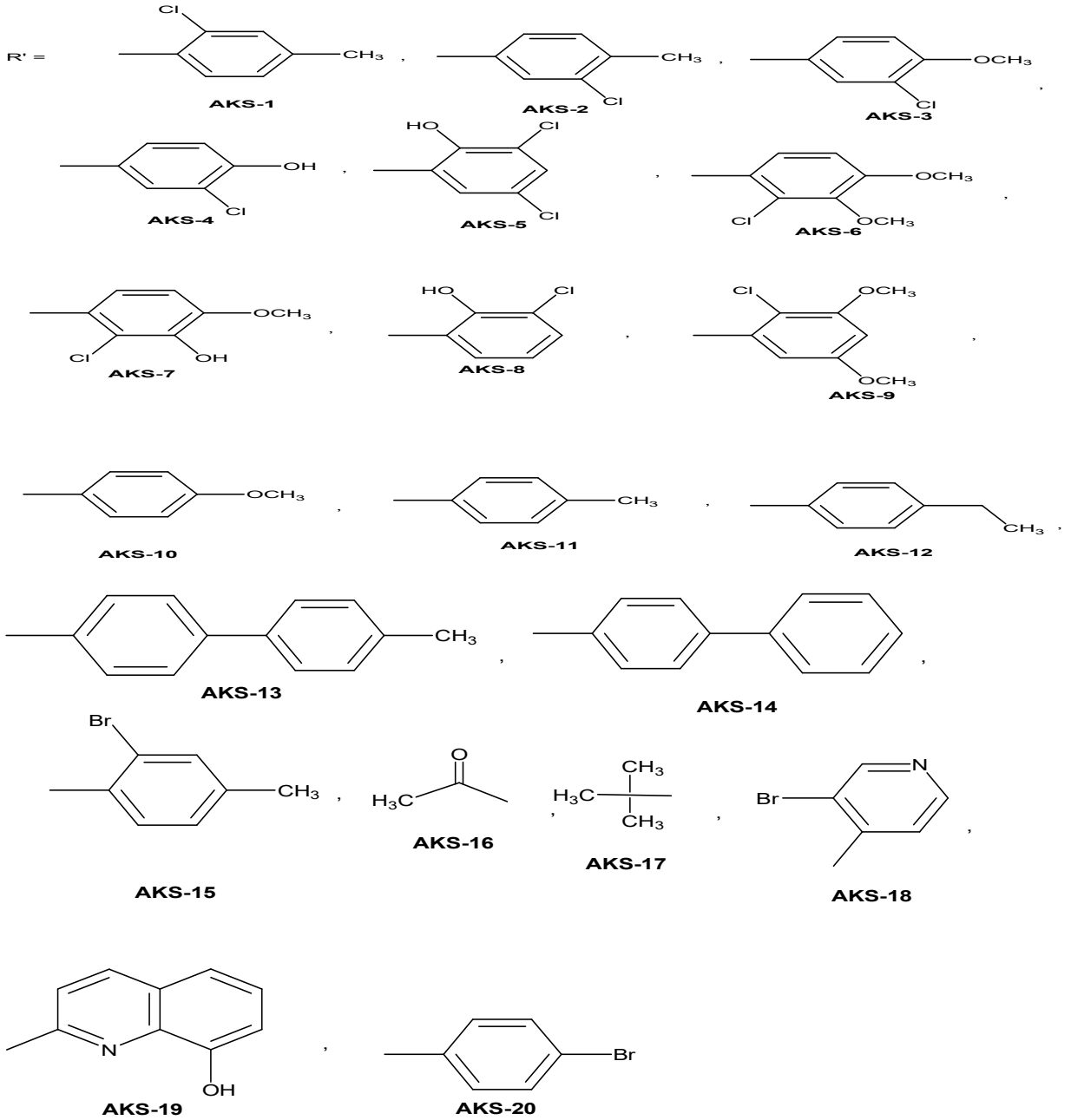

Fig. 2: Various R' attached in compounds (AKS–1 – AKS–20)

4-(3-chloro-4-methylbenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 2)

Red crystal, yield CM=60%, MWI=76%, mp: 252-256 ºC, logP=8.46, IR (KBr Cm^{-1}): 1486.4 (aromatic C=Cstr), 1599.4 (imine HC=N), 2853.7 (C-H alkane), 2550.3 (S-H thiol), 763.2 (C-Clstr), 1446 (C-Nstr), 696 (C-Sstr),^{1}H NMR (300 MHz,CDCl3 δ ppm) 6.978-7.029 (t, 3H), 7.360-7.411 (t, 1H), 7.621-7.647 (d, 1H), 7.941-7.973 (d, 1H), 8.486-8.527 (q, 1H), 8.901 (s, 1H), 9.040-9.048 (d, 1H), 9.941 (s, 1H), DART-MS (M^{+}+1 = 329.12), Anal. Calcd. For $C_{16}H_{13}ClN_4S$: C, 58.44%; H, 3.98%; N, 17.04%, Found: C, 58.90%; H, 4.18%; N, 17.25%.

4-(3-chloro-4-methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 3)

Yellowish green crystal, yield CM=60%, MWI=76%, mp: 170-176 ºC, logP=6.77, IR (KBr Cm^{-1}): 1574.1 (aromatic C=Cstr), 1615.9 (imine HC=N), 2853.7 (C-H alkane), 762.2 (C-Clstr), 1446 (C-Nstr), 696 (C-Sstr), 3114 (Ar – Hstr),^{1}H NMR (300 MHz,CDCl3 δ ppm) 5.600 (s, 1H), 6.777-6.846 (q, 3H), 7.015-7.156 (t, 2H), 7.311-7.337 (d, 1H), 7.606-7.670 (d, 1H), DART-MS (M^{+}+1 = 345.83), Anal. Calcd. For $C_{16}H_{13}ClN_4OS$: C,

55.73%; H, 3.80%; N, 16.25%, Found: C, 55.33%; H, 3.51%; N, 16.52%.

4-(3-chloro-4-hydroxybenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 4)

Orange crystal, yield CM=63%, MWI=72%, mp: 50-54 ºC, logP=4.16, IR (KBr Cm^{-1}): 1574.1 (aromatic C=Cstr), 1615.9 (imine HC=N), 1274.2 (C-O alcohol), 3422.4 (O-H alcohol), 762.2 (C-Clstr), 1446 (C-Nstr), 696 (C-Sstr), 3114 (Ar – Hstr), ^{1}H NMR (300 MHz,CDCl3 δ ppm) 6.978-7.029 (t, 3H), 7.360-7.411 (t, 1H), 7.621-7.647 (d, 1H), 7.941-7.973 (d, 1H), 8.486-8.527 (q, 1H), 8.901 (s, 1H), 9.040-9.048 (d, 1H), 9.941 (s, 1H), DART-MS (M^++1 = 331.52), Anal. Calcd. For $C_{15}H_{11}ClN_4OS$: C, 54.46%; H = 3.35%; N, 16.96%, Found: C, 54.89%; H, 3.20%; N, 17.07%.

4-(2-hydroxy-3,5-dichlorobenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 5)

Yellow Melting Solid, yield CM=79%, MWI= 91%, mp: at r. t., logP=1.91, IR (KBr Cm^{-1}): 1506.5 (aromatic C=Cstr), 1628.2 (imine HC=N), 1243.6 (C-O alcohol), 3348.1 (O-H alcohol), 2853.7 (C-H alkane), 2555.6 (S-H thiol), 762.2 (C-Clstr), 1446 (C-Nstr), 696 (C-Sstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm) 3.320 (s, 1H), 4.866 (s, 3H), 6.590-6.641 (t,1H), 6.926-6.954 (d, 2H), 7.310-7.361 (t, 1H), 7.529-7.688 (t, 1H), 8.007-8.035 (d, 1H), DART-MS (M^++1 = 366.13), Anal. Calcd. For $C_{15}H_{10}Cl_2N_4OS$: C, 49.33%;H, 2.76%; N, 15.34%, Found: C, 49.99%; H, 3.70%; N, 15.03%.

4-(2-chloro-3,4-dimethoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 6)

Reddish black oily liquid, yield CM=61%, MWI= 72%, BP: 226 ºC, logP=2.10, IR (KBr Cm^{-1}): 1493.5 (aromatic C=Cstr), 1628.2 (imine HC=N), 1279.7), 2545.7 (S-H thiol), 772.2 (C-Clstr), 1447 (C-Nstr), 696 (C-Sstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm) 3.311 (s, 1H), 4.769-4.870 (d, 2H), 4.994 (s, 1H), 6.798-6.850 (q, 2H), 7.268-7.340 (q, 7H), DART-MS (M^++1 = 375.91), Anal. Calcd. For $C_{15}H_{11}ClN_4OS$: C, 54.47%; H, 4.03%;N, 14.95%, Found: C, 53.37%; H, 4.33%; N, 14.05%.

4-(2-chloro-3-hydroxy-4-methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 7)

Reddish black oily liquid, yield CM=66%, MWI= 78%, BP: 202 ºC, logP=1.65, IR (KBr Cm^{-1}): 1584.0 (aromatic C=Cstr), 1632.8 (imine HC=N), 1280.0 (C-O alcohol), 3571.2 (O-H alcohol), 2963.6 (C-H Alkane), 762.2 (C-Clstr), 1446 (C-Nstr), 696 (C-Sstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm) 3.310-3.354 (q, 1H), 4.865 (s, 8H), 6.740-6.789 (t, 2H), 7.491-7.548 (q, 1H), 7-650-7.680 (q, 1H), DART-MS (M^++1 = 361.82), Anal. Calcd. For $C_{16}H_{13}ClN_4O_2S$: C, 54.46%; H, 3.35%; N, 16.94%, Found: C, 54.90%; H, 4.18%; N = 16.25%.

4-(2-hydroxy-3-chlorobenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 8)

Reddish black oily liquid, yield CM=83%, MWI= 90%, BP: 224 ºC, logP=2.11, IR (KBr Cm^{-1}): 1584.8 (aromatic C=Cstr), 1663.0 (imine HC=N), 1278.6 (C-O alcohol), 3630.9 (O-H alcohol), 3061.1 (C-H Alkane), 2535.8 (S-H thiol), 1446 (C-Nstr), 694 (C-Sstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm) 3.308 (s, 1H), 3.441 (s, 2H), 4.848 (s, 3H), 5.641 (s, 2H), 8.393 (s, 1H), DART-MS (M^++1 = 331.13), Anal. Calcd. For $C_{16}H_{13}ClN_4O_2S$: C, 54.47%; H, 4.03%; 14.95%, Found: C, 55.03%; H, 4.51%; N = 14.52%.

4-(2-chloro-3,5-dimethoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 9)

Reddish Brown Solid, yield CM=66%, MWI= 78%, mp: 234-238 ºC, logP=5.70, IR (KBr Cm^{-1}): 1583.1 (aromatic C=Cstr), 1629.9 (imine HC=N), 2555.6 (S-H thiol), 762.2 (C-Clstr), 1446 (C-Nstr), 696 (C-Sstr)., ^{1}H NMR (300 MHz, CDCl3 δ ppm) 3.310-3.354 (q, 1H), 4.865 (s, 8H), 6.740-6.779 (t, 2H), 7.481-7.548 (q, 1H), 7-650-7.680 (q, 1H), DART-MS (M^++1 = 375.92), Anal. Calcd. For $C_{17}H_{15}ClN_4O_2S$: C, 61.92%; H, 4.55%; N, 18.05%, Found: C, 61.89%; H, 4.20%; N, 18.27%.

4-(4-methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 10)

Black Melting Solid, yield CM=73%, MWI= 80%, mp: at r. t., logP=5.70, IR (KBr Cm^{-1}): 1490.87 (aromatic C=Cstr), 1608.52 (imine HC=N), 2853.7 (C-H alkane), 2555.6 (S-H thiol), 1446 (C-Nstr), 698 (C-Sstr)., ^{1}H NMR (300 MHz, CDCl3 δ ppm)2.820-2.853 (d, 2H), 6.914-6.979 (q, 2H), 7.136-7.167 (d, 2H), 7.268-7.324 (t, 1H), 7.399-7.424 (d, 1H), 8.203-8.233 (d, 1H), 8.308 (s, 1H), 10.374-10.431 (s, 1H), DART-MS (M^++1 = 311.13), Anal. Calcd. For $C_{16}H_{14}CN_4OS$: C, 65.28%; H, 4.79%; N, 19.03%, Found: C, 65.99%; H, 3.70%; N, 19.43%.

4-(4-methylbenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 11)

Yellow Solid, yield CM=62%, MWI= 71%, mp: 168 - 172ºC, logP=5.23, IR (KBr Cm^{-1}): 1571.6 (aromatic C=Cstr), 1617.3 (imine HC=N), 2963.6 (C-H Alkane), 1446 (C-Nstr), 696 (C-Sstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm)3.310-3.354 (q, 1H), 4.865 (s, 8H), 6.740-6.779 (t, 2H), 7.481-7.548 (q, 1H), 7-650-7.680 (q, 1H), DART-MS (M^++1 = 295.38), Anal. Calcd. For $C_{16}H_{14}CN_4OS$: C, 66.21%; H, 5.23%; N, 18.17%, Found: C, 66.37%; H, 5.33%; N, 18.05%.

4-(4-ethylbenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 12)

Orange Solid, yield CM=73%, MWI= 80%, mp: 212 - 214ºC, logP=3.23, IR (KBr Cm^{-1}): 1506.5 (aromatic

C=Cstr), 1628.2 (imine HC=N), 1243.6 (C-O alcohol), 3348.1 (O-H alcohol), 2853.7 (C-H alkane), 2555.6 (S-H thiol), 1446 (C-Nstr), 696 (C-Sstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm)3.320 (s, 1H), 4.866 (s, 3H), 6.590-6.641 (t,1H), 6.926-6.954 (d, 2H), 7.310-7.361 (t, 1H), 7.529-7.688 (t, 1H), 8.007-8.035 (d, 1H), DART-MS (M^++1 = 309.13), Anal. Calcd. For $C_{17}H_{16}N_4S$: C, 66.26%; H, 4.23%; N, 13.84%, Found: C, 66.90%; H, 4.08%; N, 13.23%.

4-[N-{4'-(methyl)–[1,1'-biphenyl]-4-ylmethylideneamino}]-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 13)

Black Oily Liquid, yield CM=77%, MWI= 93%, BP: 212 – 214 ºC, logP=4.91, IR (KBr Cm^{-1}): 1506.5 (aromatic C=Cstr), 1628.2 (imine HC=N), 1243.6 (C-O alcohol), 3348.1 (O-H alcohol), 2853.7 (C-H alkane), 2555.6 (S-H thiol), 1446 (C-Nstr), 696 (C-Sstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm)3.310-3.354 (q, 1H), 4.865 (s, 8H), 6.740-6.779 (t, 2H), 7.481-7.548 (q, 1H), 7-650-7.680 (q, 1H), DART-MS (M^++1 = 371.38), Anal. Calcd. For $C_{22}H_{18}N_4S$: C, 71.32%; H, 4.91%; N, 15.13%, Found: C, 71.90%; H, 4.38%; N, 15.83%.

4-[N-{[1,1'-biphenyl]-4-ylmethylideneamino}]-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 14)

Light Yellow Solid, yield CM=73%, MWI= 80%, mp: 138 – 142 ºC, logP=4.53, IR (KBr Cm^{-1}): 1574.1 (aromatic C=Cstr), 1615.9 (imine HC=N), 1274.2 (C-O alcohol), 3422.4 (O-H alcohol), 1446 (C-Nstr), 696 (C-Sstr), 3114 (Ar – Hstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm)2.820-2.853 (d, 2H), 6.914-6.979 (q, 2H), 7.136-7.167 (d, 2H), 7.268-7.324 (t, 1H), 7.399-7.424 (d, 1H), 8.203-8.233 (d, 1H), 8.308 (s, 1H), 10.374-10.431 (s, 1H), DART-MS (M^++1 = 357.56), Anal. Calcd. For $C_{22}H_{18}N_4S$: C, 70.77%; H, 4.52%; N, 15.72%, Found: C, 70.90%; H, 4.88%; N, 15.87%.

4-(2-bromo-4-methylbenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 15)

Yellow Green Oily Liquid, yield CM=79%, MWI= 91%, BP: 232 ºC, logP=2.67, IR (KBr Cm^{-1}): 1506.5 (aromatic C=Cstr), 1628.2 (imine HC=N), 1243.6 (C-O alcohol), 3348.1 (O-H alcohol), 2853.7 (C-H alkane), 2555.6 (S-H thiol), 549.2 (C-Brstr), 1446 (C-Nstr), 696 (C-Sstr)., ^{1}H NMR (300 MHz, CDCl3 δ ppm)3.310-3.354 (q, 1H), 4.865 (s, 8H), 6.740-6.779 (t, 2H), 7.481-7.548 (q, 1H), 7-650-7.680 (q, 1H)]., DART-MS (M^++1 = 389.38), Anal. Calcd. For $C_{17}H_{16}BrN_4S$: C, 53.64%; H, 4.09%; N, 22.75%, Found: C, 54.09%; H, 4.20%; N = 22.07%.

1-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-ylimino)propan-2-one (AKS – 16)

Reddish Black Oily Liquid, yield CM=77%, MWI= 85%, BP: 244 ºC, logP=1.15, IR (KBr Cm^{-1}): 1584.8 (aromatic C=Cstr), 1663.0 (imine HC=N), 1678.6 (C-O alcohol), 3061.1 (C-H Alkane), 2535.8 (S-H thiol), 762.2 (C-Clstr), 1446 (C-Nstr), 694 (C-Sstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm)3.308 (s, 1H), 3.441 (s, 2H), 4.848 (s, 3H), 5.641 (s, 2H), 8.393 (s, 1H), DART-MS (M^++1 = 247.33), Anal. Calcd. For $C_{11}H_{10}N_4OS$: C, 59.97%; H, 6.19%; N, 21.52%, Found: C, 59.99%; H, 6.60%; N, 21.03%.

4-(2,2-dimethylpropylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 17)

Green Crystals, yield CM=85%, MWI= 91%, mp: 222-226 ºC, logP=4.36, IR (KBr Cm^{-1}): 1506.5 (aromatic C=Cstr), 1628.2 (imine HC=N), 1243.6 (C-O alcohol), 3348.1 (O-H alcohol), 2853.7 (C-H alkane), 2555.6 (S-H thiol), 549.2 (C-Brstr), 1446 (C-Nstr), 696 (C-Sstr)., ^{1}H NMR (300 MHz, CDCl3 δ ppm3.310-3.354 (q, 1H), 4.865 (s, 8H), 6.740-6.779 (t, 2H), 7.481-7.548 (q, 1H), 7-650-7.680 (q, 1H), DART-MS (M^++1 = 261.38), Anal. Calcd. For $C_{13}H_{16}N_4S$: C, 62.91%; H, 6.33%; N, 19.56%, Found: C, 62.37%; H =6.03%; N = 19.05%.

4-[(3-bromopyridin-4-yl)methyleneamino]-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 18)

Yellow Crystals, yield CM=71%, MWI= 89%, mp: 268-272 ºC, logP=4.13, IR (KBr Cm^{-1}): 1506.5 (aromatic C=Cstr), 1628.2 (imine HC=N), 1243.6 (C-O alcohol), 3348.1 (O-H alcohol), 2853.7 (C-H alkane), 2555.6 (S-H thiol), 549.2 (C-Brstr), 1446 (C-Nstr), 696 (C-Sstr)., ^{1}H NMR (300 MHz, CDCl3 δ ppm 3.320 (s, 1H), 4.866 (s, 3H), 6.590-6.641 (t,1H), 6.926-6.954 (d, 2H), 7.310-7.361 (t, 1H), 7.529-7.688 (t, 1H), 8.007-8.035 (d, 1H), DART-MS (M^++1 = 361.33), Anal. Calcd. For $C_{14}H_{10}BrN_5S$: C, 46.68%; H, 2.80%; N, 19.44%, Found: C, 47.90%; H, 2.08%; N, 19.23%.

4-[N-(quinolin-8-ol-2-ylmethylidene)amino]-5-phenyl-4H-1, 2, 4-triazole-3-thiol (AKS – 19)

Orange Crystals, yield CM=81%, MWI= 91%, mp: 202-204 ºC, logP=1.73, IR (KBr Cm^{-1}): 1571.6 (aromatic C=Cstr), 1617.3 (imine HC=N), 2963.6 (C-H Alkane), 1446 (C-Nstr), 696 (C-Sstr), 3348.1 (O-H alcohol), ^{1}H NMR (300 MHz, CDCl3 δ ppm 3.310-3.354 (q, 1H), 4.865 (s, 8H), 6.740-6.779 (t, 2H), 7.481-7.548 (q, 1H), 7-650-7.680 (q, 1H), DART-MS (M^++1 = 348.46), Anal. Calcd. For $C_{18}H_{13}N_5OS$: C, 62.33%; H=3.77%; N, 20.13%, Found: C, 62.90%; H, 3.18%; N, 19.25%.

4-(4-bromobenzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol (AKS – 20)

Black Crystals, yield CM=76%, MWI= 89%, mp: 226-230 ºC, logP=2.33, IR (KBr Cm^{-1}): 1490.6 (aromatic C=Cstr), 1618.2 (imine HC=N), 3063.6 (C-H Alkane), 1456 (C-Nstr), 676 (C-Sstr),603.3 (C-Brstr), ^{1}H NMR (300 MHz, CDCl3 δ ppm 9.310-9.454 (s, 1H), 14.865

(s, 1H), 7.740-7.779 (d, 2H), 7.281-7.542 (m, 4H), 7.150-7.480 (t, 1H), DART-MS (M^++1 = 360.66), Anal. Calcd. For $C_{15}H_{11}BrN_4S$: C, 50.15%; H = 3.09%; N, 15.60%, Found: C, 50.90%; H, 3.08%; N, 15.25%.

BIOLOGICAL SCREENING

First Phase Screening:

Animal and Environmental Condition

Swiss albino mice weighing (20 ± 25gm) of either sex will be procured from the Laboratory Animal Facility, Central Drug Research Institute, Lucknow. They will be kept in an animal house in a well cross – ventilated room at 25 ± 2ºC, and with relative humidity 44 – 56%, light and dark cycles of 10 and 14 hours respectively for one week before and during the experiments. Animals will be provided with a standard rodent pellet diet (Bharat Ansh Scientific Industries) and food will be withdrawn 18 – 24 hours before the experiments, water will be allowed ad libitum. All studies will be performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 1957/PO/Re/S/17CPCSEA).

Anticonvulsant activity

The albino mice were divided in to three groups (n = 6). Control group (Received 30%v/v PEG aqueous solution). Synthesized compounds (AKS-1 to AKS-20) i.e. treated groups (Received 30, 100 and 300 mg/kg in 30% v/v PEG400 aqueous solution doses i.p. route). Standard group (Received Phenytoin 30mg/kg in 30% v/v PEG400 aqueous solution).

Maximal Electroshock Seizure (MES)

All the novel synthesized compounds (AKS 1 – AKS - 20) preliminary screen for anticonvulsant activity by maximal electroshock seizures method. In mice, seizures were elicited with a 60 Hz alternating current of 150 mA intensity; apply current on the upper eye lid for 0.2 second. After intraperitoneal administration of test compounds (AKS -1 – AKS - 20) at doses of 30, 100 and 300 mg/kg in 30% v/v PEG400 aqueous solution, the pharmacological response was evaluated at two-time intervals 0.5 and 4 hours. Different phases of seizures (a) tonic flexion (b) tonic extensor (c) clonic convulsion (d) stupor (e) recovery/death were observed in all groups of animals. The animals of the control and standard groups undergo the same procedure mentioned above. The observation of the after electroshock showed reduced time or abolished extensor phase are represented in Table 1 and Table 2.

Neurotoxicity Studies

Motor impairment confirmation done in albino mice by using the Rota rod apparatus (Medicraft Pharma. Pvt. Ltd). Prior to conducting the experiment every experimental animal was trained to stay on accelerating rota rod at 10 rpm. At 30 minutes after the administration of synthesized novel compounds (AKS – 1 – AKS - 20) the animals were screened for neurotoxicity on a knurled rotating rod and mice failing to hold the rod for one minute at least in each of three trials were potentially experiencing neurotoxicity.

3.1.2.3. Statistical Analysis

All the values are represented in the form of mean ± standard error mean (SEM) and these all values analyzed by ANOVA then multiple comparison tests apply by Dennett's test. All the statistical analysis were analyzed and collect the data by using graph pad prism 5 version software.

Table 1: Anticonvulsant activity of synthesized compounds (AKS -1 – AKS -20) using MES model

Compound No.	Doses (mg/kg)	Time (sec) in various phases of convulsion after 0.5 hrs				Recovery/ Death	Neurotoxicity screen
		Flexion (Mean±SEM)	Extensor (Mean±SEM)	Clonus (Mean±SEM)	Stupor (Mean±SEM)		
AKS - 1	30	4.43 ± 0.34***	7.67 ± 0.44***	15.66 ± 0.21***	116.13 ± 0.63***	Recovery	Absent
	100	3.59 ± 0.28**	9.28 ± 0.07***	10.48 ± 0.92***	49.50 ± 1.43***	Recovery	Absent
	300	5.09 ± 0.30***	11.3 ± 0.47***	35.97 ± 0.94***	119.48 ± 0.68***	Recovery	Absent
AKS - 2	30	5.14 ± 0.80^{ns}	16.35 ± 0.73^{ns}	18.67 ± 0.98***	120.05 ± 1.73**	Recovery	Absent
	100	3.46 ± 0.39**	23.26 ± 0.63***	33.05 ± 0.47**	55.02 ± 0.31**	Recovery	Absent
	300	5.35 ± 0.59**	25.20 ± 0.50***	37.81 ± 0.88*	131.62 ± 0.50**	Recovery	Absent
AKS - 3	30	4.04 ± 0.22**	6.47 ± 0.36***	9.06 ± 0.24***	47.02 ± 0.28***	Recovery	Absent
	100	2.44 ± 0.17***	4.47 ± 0.09***	8.40 ± 0.05***	46.22 ± 0.24***	Recovery	Absent
	300	4.19 ± 0.20***	18.21 ± 0.84***	34.01 ± 0.51***	113.87 ± 0.18***	Recovery	Absent
AKS - 4	30	8.41 ± 0.41^{ns}	23.75 ± 0.31**	42.87 ± 1.00**	173.48 ± 0.85**	Recovery	Absent
	100	9.23 ± 0.29**	11.14 ± 0.20^{ns}	20.74 ± 0.50***	61.39 ± 0.47***	Recovery	Absent
	300	4.85 ± 0.72**	22.71 ± 0.40***	34.51 ± 0.82***	113.99 ± 0.43**	Recovery	Absent
AKS - 5	30	3.46 ± 0.34***	4.87 ± 0.17***	8.78 ± 0.18***	43.07 ± 0.62***	Recovery	Absent
	100	2.84 ± 0.35***	3.09 ± 0.18***	8.32 ± 0.43***	45.06 ± 1.19***	Recovery	Absent
	300	3.17 ± 0.30***	7.9 ± 0.18***	18.84 ± 0.15***	114.74 ± 0.72***	Recovery	Absent
AKS - 6	30	8.28 ± 0.44^{ns}	22.73 ± 0.67**	41.03 ± 0.85**	193.48 ± 0.96**	Recovery	Absent
	100	5.35 ± 0.33**	12.41 ± 0.58***	44.81 ± 0.55**	52.40 ± 0.39**	Recovery	Absent
	300	10.75 ± 0.22**	20.82 ± 0.25	35.39 ± 0.50**	114.54 ± 0.40**	Recovery	Absent
AKS - 7	30	2.83 ± 0.36***	3.73 ± 0.18***	7.79 ± 0.32***	40.15 ± 0.37***	Recovery	Absent
	100	3.05 ± 0.17***	5.13 ± 0.24***	9.01 ± 0.19***	45.48 ± 0.50***	Recovery	Absent
	300	4.07 ± 0.23**	20.435 ± 0.29**	35.13 ± 0.42**	119.34 ± 0.45**	Recovery	Absent
AKS - 8	30	6.42 ± 0.21^{ns}	22.18 ± 0.27^{ns}	39.07 ± 0.09**	191.48 ± 0.47*	Recovery	Absent
	100	5.98 ± 0.40**	10.13 ± 0.32***	42.74 ± 0.69*	165.63 ± 0.72**	Recovery	Absent
	300	10.27 ± 0.32*	21.66 ± 0.28*	35.76 ± 0.37**	120.18 ± 0.63***	Recovery	Absent
AKS - 9	30	8.27 ± 0.44*	23.58 ± 0.28^{ns}	42.35 ± 0.81*	193.01 ± 0.53*	Recovery	Absent
	100	8.37 ± 0.35**	23.73 ± 0.28^{ns}	40.75 ± 1.04*	190.67 ± 0.70*	Recovery	Absent
	300	9.46 ± 0.28**	21.70 ± 0.67*	37.24 ± 0.51*	198.54 ± 0.75**	Recovery	Absent
AKS - 10	30	10.86 ± 0.31^{ns}	27.12 ± 0.91^{ns}	41.29 ± 0.71*	196.19 ± 1.64*	Recovery	Absent
	100	9.26 ± 0.49*	26.07 ± 0.56*	40.31 ± 0.77*	193.45 ± 1.17*	Recovery	Absent
	300	11.70 ± 0.38*	23.27 ± 0.56*	37.21 ± 0.44*	189.64 ± 0.39*	Recovery	Absent
AKS - 11	30	9.63 ± 0.40**	20.74 ± 0.50*	37.95 ± 0.34**	168.31 ± 0.56**	Recovery	Absent
	100	8.84 ± 0.20**	21.16 ± 0.51***	35.38 ± 0.45**	154.85 ± 0.77**	Recovery	Absent
	300	7.43 ± 0.48**	22.58 ± 0.33**	39.20 ± 0.43**	188.63 ± 0.64***	Recovery	Absent
AKS - 12	30	8.30 ± 0.18*	23.18 ± 0.59^{ns}	39.66 ± 0.30*	171.57 ± 0.53*	Recovery	Absent
	100	7.85 ± 0.43**	21.98 ± 0.74**	37.52 ± 0.78**	180.11 ± 0.65**	Recovery	Absent
	300	7.86 ± 0.39**	23.19 ± 0.64*	41.61 ± 0.82*	189.83 ± 0.91*	Recovery	Absent
AKS - 13	30	9.50 ± 0.46**	23.67 ± 0.31**	55.08 ± 0.67^{ns}	195.22 ± 0.76**	Recovery	Absent
	100	9.16 ± 0.39**	23.10 ± 0.32**	43.38 ± 0.45**	193.88 ± 0.61**	Recovery	Absent
	300	8.26 ± 0.44**	23.02 ± 0.39**	39.34 ± 0.50**	193.57 ± 0.56**	Recovery	Absent
AKS - 14	30	10.99 ± 0.21**	24.89 ± 0.20^{ns}	45.39 ± 0.45	198.69 ± 0.73**	Recovery	Absent
	100	9.59 ± 0.22**	24.62 ± 0.45^{ns}	44.32 ± 0.33**	195.01 ± 0.50**	Recovery	Absent
	300	8.85 ± 0.19**	22.79 ± 0.55**	40.31 ± 0.54***	193.61 ± 0.25**	Recovery	Absent
AKS - 15	30	6.40 ± 0.20***	24.12 ± 0.17***	26.39 ± 0.77***	137.34 ± 0.92**	Recovery	Absent
	100	5.01 ± 0.31***	7.72 ± 0.52***	17.63 ± 0.24***	54.59 ± 0.50***	Recovery	Absent
	300	6.59 ± 0.44***	11.47 ± 0.85***	36.13 ± 0.91**	127.21 ± 0.88***	Recovery	Absent
AKS - 16	30	7.15 ± 0.22*	24.37 ± 0.58^{ns}	25.55 ± 0.42**	126.01 ± 0.39**	Recovery	Absent
	100	8.66 ± 0.22*	22.97 ± 0.30*	43.56 ± 0.84*	194.25 ± 0.24*	Recovery	Absent
	300	7.25 ± 0.46**	24.83 ± 0.35*	39.49 ± 0.56**	129.89 ± 0.87**	Recovery	Absent
AKS - 17	30	6.08 ± 0.41**	22.81 ± 0.81*	19.61 ± 0.37***	123.59 ± 0.90^{ns}	Recovery	Absent
	100	4.47 ± 0.34**	8.77 ± 0.55***	12.99 ± 0.34***	52.20 ± 0.22***	Recovery	Absent
	300	6.18 ± 0.25***	10.54 ± 0.75***	40.57 ± 0.77*	128.96 ± 0.97***	Recovery	Absent
AKS - 18	30	6.67 ± 0.15**	23.97 ± 0.84**	19.91 ± 0.42***	124.56 ± 0.83**	Recovery	Absent
	100	5.62 ± 0.17**	22.04 ± 0.45**	22.02 ± 0.53**	120.99 ± 0.56**	Recovery	Absent
	300	6.49 ± 0.42**	24.37 ± 0.61***	41.77 ± 0.99**	129.61 ± 0.36**	Recovery	Absent
AKS - 19	30	5.52 ± 0.27***	21.01 ± 0.51**	17.17 ± 0.36***	118.19 ± 0.95^{ns}	Recovery	Absent
	100	3.13 ± 0.22***	5.87 ± 0.25***	9.45 ± 0.18***	48.96 ± 0.42***	Recovery	Absent
	300	5.76 ± 0.19***	9.80 ± 0.21***	39.25 ± 0.37***	125.91 ± 0.71***	Recovery	Absent
AKS - 20	30	6.35 ± 0.49**	21.23 ± 0.31**	18.31 ± 0.31**	119.24 ± 0.81**	Recovery	Absent
	100	6.35 ± 0.49**	21.88 ± 0.58**	22.67 ± 0.61*	121.52 ± 0.22**	Recovery	Absent
	300	6.85 ± 0.53**	23.63 ± 0.41**	40.47 ± 0.56**	126.60 ± 0.68**	Recovery	Absent
Control	30% v/v PEG400	8.74 ± 0.23	17.12 ± 0.12	23.5 ± 0.38	110.80 ± 0.66	Recovery	Absent
Phenytoin	30	2.47 ± 0.04***	3.50 ± 0.08***	7.69 ± 0.17***	42.32 ± 0.19***	Recovery	Absent

n = 6 in each group, ***p < 0.001 highly significant, **p < 0.01 significant, *p < 0.05 moderately significant compared against the control group (30% v/v PEG400) and standard drug (Phenytoin 30 mg/kg).

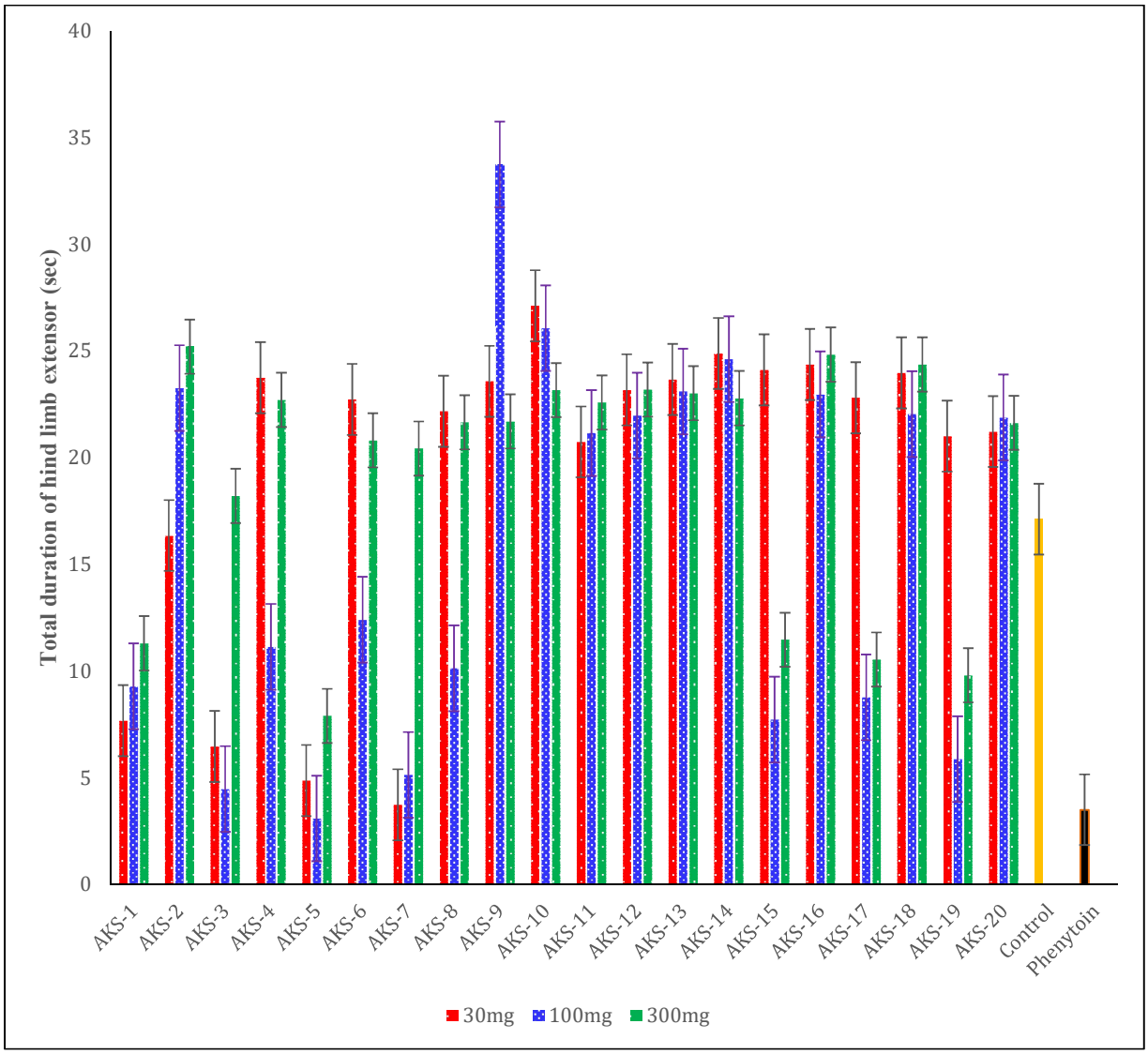

Fig. 3: Graphical representation of anticonvulsant evaluation after 0.5 hours administration of compounds (AKS-1 – AKS-20) using MES model.

Table 2: Anticonvulsant activity of synthesized compounds (AKS – 1 - AKS - 20) using MES model

Compound No.	Doses (mg/kg)	Time (sec) in various phases of convulsion after 4 hrs				Recovery/ Death	Neurotoxicity screen
		Flexion (Mean±SEM)	Extensor (Mean±SEM)	Clonus (Mean±SEM)	Stupor (Mean±SEM)		
AKS - 1	30	3.25 ± 0.26**	7.87 ± 0.44***	13.66 ±0.21***	110.97 ± 0.96***	Recovery	Absent
	100	3.47 ± 0.25***	8.91 ± 0.20***	9.83 ± 0.55***	46.66 ± 0.60***	Recovery	Absent
	300	4.94 ± 0.20*	10.29 ± 0.30***	35.47 ± 0.48***	119.48 ± 0.68***	Recovery	Absent
AKS - 2	30	5.47 ± 0.41**	16.95 ± 0.57ns	18.32 ± 0.71*	018.38 ± 0.47**	Recovery	Absent
	100	3.37 ± 0.31***	22.78 ± 0.89**	32.35 ± 0.54***	44.84 ± 0.32***	Recovery	Absent
	300	6.01 ± 0.58**	24.60 ± 0.48**	36.33 ± 0.92**	120.62 ± 0.60**	Recovery	Absent
AKS - 3	30	3.80 ± 0.22**	5.87 ± 0.28***	8.63 ± 0.33***	46.26 ± 0.54***	Recovery	Absent
	100	2.30 ± 0.45***	4.10 ± 0.20***	8.06 ± 0.32***	46.22 ± 0.24***	Recovery	Absent
	300	3.73 ± 0.32**	14.89 ± 0.80***	33.01 ± 0.59***	113.87 ± 0.18***	Recovery	Absent
AKS - 4	30	8.27 ± 0.44**	23.93 ± 0.28*	39.60 ± 0.20***	193.34 ± 0.84**	Recovery	Absent
	100	8.54 ± 0.31**	10.23 ± 0.19**	21.26 ± 0.36*	61.39 ± 0.47**	Recovery	Absent
	300	4.18 ± 0.32*	22.16 ± 0.51**	33.88 ± 0.50***	113.99 ± 0.5**	Recovery	Absent
AKS - 5	30	3.32 ± 0.29***	4.29 ± 0.23***	8.61 ± 0.17***	43.48 ± 0.27***	Recovery	Absent
	100	2.69 ± 0.25***	3.01 ± 0.19***	7.97 ± 0.39***	43.9 ± 0.62***	Recovery	Absent
	300	3.02 ± 0.33**	7.3 ± 0.24***	18.49 ± 0.28***	114.74 ± 0.72***	Recovery	Absent
AKS - 6	30	7.81 ± 0.21**	23.11 ± 0.44***	41.38 ± 0.78**	193.16 ± 0.73**	Recovery	Absent
	100	5.07 ± 0.30*	11.51 ± 0.30***	44.16 ± 0.53**	62.40 ± 0.49**	Recovery	Absent
	300	12.85 ± 0.25**	20.43 ± 0.29***	35.27 ± 0.41**	114.54 ± 0.30**	Recovery	Absent
AKS - 7	30	2.68 ± 0.36***	3.62 ± 0.14***	6.99 ± 0.39***	38.79 ± 0.76***	Recovery	Absent
	100	2.94 ± 0.16***	4.85 ± 0.20***	8.48 ± 0.29***	45.48 ± 0.50***	Recovery	Absent
	300	3.82 ± 0.19**	19.62 ± 0.47***	34.56 ± 0.32***	119.34 ± 0.45***	Recovery	Absent
AKS - 8	30	7.71 ± 0.21*	21.73 ± 0.35*	39.59 ± 0.60**	191.83 ± 0.42*	Recovery	Absent
	100	5.53 ± 0.31**	9.63 ± 0.33***	42.31 ± 0.69**	165.65 ± 0.72*	Recovery	Absent
	300	9.45 ± 0.32*	21.65 ± 0.17*	35.76 ± 0.37**	120.18 ± 0.63**	Recovery	Absent
AKS - 9	30	7.61 ± 0.32***	10.84 ± 0.18***	38.74 ± 0.68**	192.48 ± 0.64**	Recovery	Absent
	100	8.90 ± 0.47**	24.03 ± 0.25***	42.12 ± 0.66**	190.66 ± 0.70ns	Recovery	Absent
	300	7.71 ± 0.28***	22.54 ± 0.84***	37.82 ± 0.61**	189.20 ± 0.36***	Recovery	Absent
AKS - 10	30	6.69 ± 0.82***	10.21 ± 0.44***	41.08 ± 0.67**	193.47 ± 0.63**	Recovery	Absent
	100	8.93 ± 0.47***	25.56 ± 0.44**	40.95 ± 0.76***	192.45 ± 0.63***	Recovery	Absent
	300	7.36 ± 0.22***	22.96 ± 0.73***	38.03 ± 0.63***	188.89 ± 0.69***	Recovery	Absent
AKS - 11	30	8.27 ± 0.17***	12.35 ± 0.83**	39.09 ± 0.64***	169.16 ± 0.88**	Recovery	Absent
	100	7.78 ± 0.55***	21.6 ± 0.56***	37.66 ± 0.81**	164.85 ± 0.77**	Recovery	Absent
	300	8.41 ± 0.45***	22.93 ± 0.46***	41.62 ± 0.91***	188.63 ± 0.64***	Recovery	Absent
AKS - 12	30	7.74 ± 0.37**	33.58 ± 0.28ns	38.19 ± 0.91***	170.35 ± 0.68***	Recovery	Absent
	100	7.52 ± 0.50**	17.95 ± 0.79**	37.71 ± 0.79***	170.11 ± 0.55***	Recovery	Absent
	300	7.35 ± 0.36**	21.67 ± 0.62**	40.14 ± 0.74**	188.16 ± 0.85**	Recovery	Absent
AKS - 13	30	8.87 ± 0.18***	11.05 ± 0.30***	44.29 ± 0.27***	166.84 ± 0.40***	Recovery	Absent
	100	9.74 ± 0.44**	23.46 ± 0.36***	43.70 ± 0.47***	193.88 ± 0.61**	Recovery	Absent
	300	8.84 ± 0.40***	23.68 ± 0.28***	38.95 ± 0.89*	193.57 ± 0.56***	Recovery	Absent
AKS - 14	30	10.76 ± 0.17**	17.15 ± 0.41**	44.88 ± 0.53**	196.68 ± 0.95**	Recovery	Absent
	100	9.42 ± 0.32**	24.45 ± 0.43*	44.45 ± 0.28**	195.01 ± 0.50**	Recovery	Absent
	300	8.99 ± 0.19**	23.13 ± 0.47**	39.79 ± 0.68**	193.61 ± 0.25**	Recovery	Absent
AKS - 15	30	6.07 ± 0.17***	22.60 ± 0.86**	25.09 ± 0.70***	127.00 ± 0.91**	Recovery	Absent
	100	4.74 ± 0.27***	7.06 ± 0.48***	17.30 ± 0.26***	51.59 ± 0.50***	Recovery	Absent
	300	6.25 ± 0.26***	11.47 ± 0.42***	35.11 ± 0.36***	128.77 ± 0.55***	Recovery	Absent
AKS - 16	30	6.77 ± 0.29**	23.41 ± 0.39*	25.00 ± 0.30**	125.20 ± 0.34**	Recovery	Absent
	100	8.20 ± 0.35*	22.71 ± 0.04*	42.37 ± 0.06**	194.25 ± 0.24**	Recovery	Absent
	300	6.44 ± 0.29**	24.38 ± 0.40ns	35.71 ± 0.41**	132.50 ± 0.60**	Recovery	Absent

AKS - 17	30	5.82 ± 0.35**	21.27 ± 0.94***	19.08 ± 0.62*	121.91 ± 0.81***	Recovery	Absent
	100	4.21 ± 0.32ns	8.22 ± 0.35***	12.26 ± 0.42***	52.20 ± 0.22***	Recovery	Absent
	300	5.92 ± 0.15***	10.6 ± 0.61***	38.64 ± 0.49***	128.53 ± 0.95***	Recovery	Absent
AKS - 18	30	6.26 ± 0.40**	21.68 ± 0.83**	18.30 ± 0.37*	122.49 ± 0.96***	Recovery	Absent
	100	5.56 ± 0.13***	20.93 ± 0.40***	21.11 ± 0.11***	120.99 ± 0.56***	Recovery	Absent
	300	5.63 ± 0.12***	22.91 ± 0.81***	42.43 ± 0.98**	126.61 ± 0.36**	Recovery	Absent
AKS - 19	30	5.14 ± 0.29*	18.73 ± 0.43***	16.56 ± 0.31*	107.19 ± 0.45***	Recovery	Absent
	100	2.95 ± 0.23***	5.71 ± 0.25***	9.00 ± 0.17***	48.96 ± 0.42***	Recovery	Absent
	300	5.63 ± 0.12***	22.91 ± 0.81***	40.43 ± 0.98**	129.61 ± 0.36**	Recovery	Absent
AKS - 20	30	6.20 ± 0.15**	20.99 ± 0.30**	17.76 ± 0.27**	118.17 ± 0.34**	Recovery	Absent
	100	6.62 ± 0.17**	20.28 ± 0.60**	21.81 ± 0.40*	131.52 ± 0.22**	Recovery	Absent
	300	6.85 ± 0.53*	23.63 ± 0.41**	40.47 ± 0.56***	126.60 ± 0.68**	Recovery	Absent
Control	30% v/v PEG400	4.53 ± 0.10	15.67 ± 0.18	20.56 ± 0.31	109.08 ± 0.61	Recovery	Absent
Phenytoin	30	1.57 ± 0.11***	3.19 ± 0.13***	7.20 ± 0.20***	37.63 ± 0.30***	Recovery	Absent

N = 6 in each group, ***p < 0.001 highly significant, **p < 0.01 significant, *p < 0.05 moderately significant compared against the control group (30% v/v PEG400) and standard drug (Phenytoin 30 mg/kg).

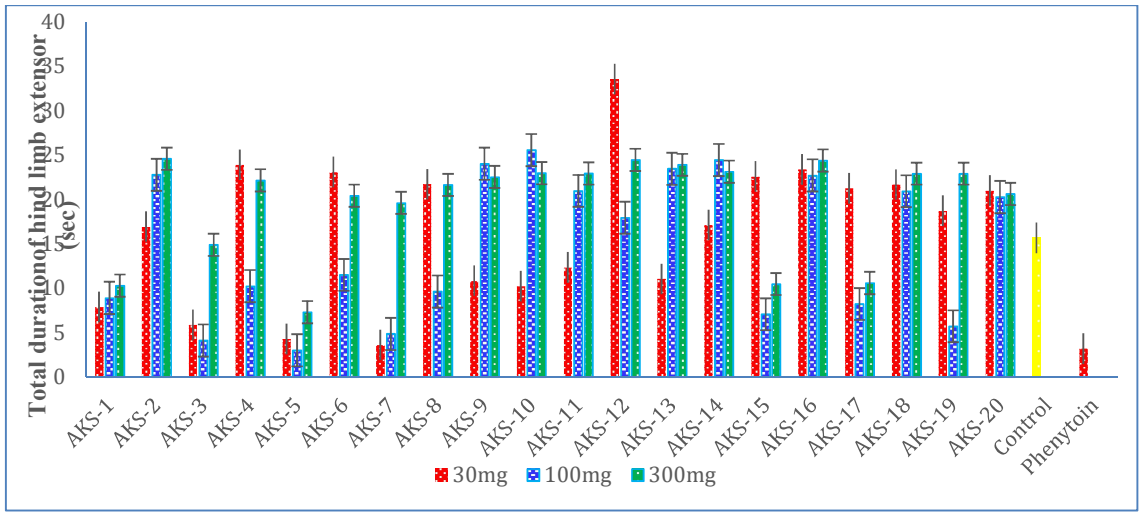

Fig. 4: Graphical representation of anticonvulsant evaluation after 4 hours administration of compounds (AKS-1 – AKS-20) using MES model.

Second Phase Screening:

In phase II anticonvulsant screening, the most active compounds AKS – 1, AKS – 3 and AKS – 7 exhibited, in the MES screen, ED_{50} of 27.5 mg/kg, 29.4 mg/kg and 30.9 mg/kg, respectively, TD_{50} of 388.9 mg/kg, 277.9 mg/kg and 257.9.9 mg/kg, respectively, and protective index (PI) of 13.9, 9.45 and 8.34, respectively, which is higher as compared to phenytoin.

Table 3: Phase II quantitative anticonvulsant evaluation of selected active compounds

Compounds	**ED_{50}**	**TD_{50}**	**PI**
	MES		
AKS -1	27.5 ± 1.44	388.9 ± 18.04	1.14
AKS -3	29.4 ± 0.89	277.9 ± 21.24	9.45
AKS -7	30.9 ± 1.40	257.9 ± 23.44	8.34
Phenytoin	9.5 ± 0.77	65.5 ± 12.07	6.9

Number of animal used = 8; solvent used: PEG (0.1 mL, i.p.); ED_{50} = Median effective dose; TD_{50} = Median toxic dose; PI = Protective index (TD_{50}/ED_{50})

Third Phase Screening

In phase III screening, the toxicity profile of compounds AKS – 1, AKS - 3 and AKS - 7 was determined. Mice were injected i.p. with the test compounds at different doses in order to determine the HD_{50} for the hypnotic activity of the compounds based on loss of the righting reflex. Groups of 10 animals were used for each dose. Solutions were prepared immediately before the test. Logarithmic dose-response curves for test compounds were fitted to calculate the HD_{50} using a linear regression analysis.

Data are reported as means ± SE. For LD_{50}, the selected compounds were administered intraperitoneally to mice at various doses in the multiple of TD_{50} and the toxicity per- suaded by them was portrayed by diminished motor activity, relaxation of muscles, failure of righting reflex, and decline level of respiration. At higher doses, animals also showed hypnosis, analgesia, and anesthesia. The median hypnotic dose (HD_{50}) of compound AKS - 1 was found to be 644.3 mg/kg, which is nearly twice the TD_{50} of the compound. It also showed the 24 h median lethal dose (LD_{50}) of 866.8 mg/kg.

Compound AKS - 3 displayed the HD_{50} value 714.3 mg/kg with LD_{50} 690.3 mg/kg. Compound AKS - 7 displayed the HD_{50} value 670.4 mg/kg with LD_{50} 650.4 mg/kg. All three compounds showed high safety profile as the HD_{50}/ED_{50} values of AKS – 1, AKS - 3 and AKS - 7 were found to be 23.4, 24.3 and 21.7 against MES induced seizures. These values are higher than that showed by phenytoin. They displayed a considerable safety profile in MES induced seizure also indicative of the efficiency of both the compounds as broad spectrum anticonvulsants.

Table 4: Phase III quantitative toxicity profile of selected compounds

Compounds	HD_{50}[a]	LD_{50}[b]	HD_{50}/ED_{50} MES
AKS – 1	644.3 (610.8-690.6)[c]	866.8 (825.2-903.6)	23.4
AKS - 3	714.3 (666.8-767.2)	690.3 (606.1-667.2)	24.3
AKS - 7	670.4 (662.8-765.2)	650.4 (642.3-635.2)	21.7
Phenytoin	183.4 (168.3-95.2)	225.4 (203.3-250.2)	19.3

[a]Median hypnotic dose in mg/kg; [b]median lethal dose in mg/kg; mortality was determined 24 hours after i.p. injection.
[C]95% confidence interval in parenthesis.

Fourth Phase Screening

In phase IV anticonvulsant screening, the selected compounds AKS – 1, AKS - 3 and AKS - 7 were further evaluated for ED_{50} and TD_{50} values after oral administration in mice to assess their bioavailability. The result indicated that, on oral administration, the bioavailability of test compounds decreased since the ED_{50} values were found to be higher than the ED_{50} values in phase II screening on i.p. administration. Phenytoin is a probable cause of acetaminophen hepatotoxicity. Selected active compounds AKS – 1, AKS - 3 and AKS - 7 were investigated for their hepato- toxic side effects by means of liver function tests. Compounds were administered chronically to mice for two weeks and the biochemical parameters were estimated. The values of alkaline phosphatase, serum glutamate oxaloacetatetransaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), albumin, and bilirubin suggested that none of the compounds had shown any considerable increase or decrease.

Further histopathological study of compounds AKS -1, AKS - 3 and AKS - 7 confirmed that there is no liver toxicity by showing normal hepatic parenchyma with portal triad, central vein, and hepatocytes in comparison to control.

Table 5: Phase IV quantitative anticonvulsant evaluation of selected active compounds after oral administration

Compounds	TPE[a] (h)	ED_{50}[b](mg /kg)	TD_{50}[c](mg/ kg)	PI[d]
AKS – 1	2	39.7 (34.1-44.6)[e]	466.3 (420.2-503.3)	11.6
AKS - 3	2	48.6 (41.2-54.8)	690.2 (642.3-721.4)	14.2
AKS - 7	2	49.4 (42.3-55.2)	649.4 (641.3-615.2)	13.1
Phenytoin	2	9.17 (7.4-12.4)	86.7 (79.4-99.2)	9.5

[a]Time peak pharmacodynamic activity. [b]ED_{50} median effective dose eliciting anticonvulsant protection in 50% animals; [c]TD_{50} median toxic dose eliciting minimal neurological toxicity in 50% animals; [d]PI (Protective index) was determined by TD_{50}/ED_{50}; [e]95% confidence interval in parenthesis

Table 6: Enzyme estimation of the selected compounds

Compounds	SGOT[a] level (IU/mL)	SGPT[b] level (IU/mL)	Alkaline Phosphatase (IU/mL)	Albumin (mg/dL)	Bilirubin (mg/dL)
Control	88.1± 2.16	27.67± 1.50	16.60± 0.47	1.69 ± 0.04	1.58 ± 0.04
AKS – 1	86.6 ± 2.25	26.81 ± 1.66	15.98 ± 0.90	1.69 ± 0.08	1.50 ± 0.08
AKS - 3	85.6 ± 1.80	24.96 ± 1.60	12.56 ± 0.40	1.57 ± 0.05	1.57 ± 0.04
AKS - 7	84.5 ± 1.70	25.97 ± 1.50	13.56 ± 0.41	1.60 ± 0.06	1.55 ± 0.05
Phenytoin	86.4 ± 4.22	27.91 ± 2.22	13.99 ± 0.73	1.53 ± 0.03	1.56 ± 0.02

Number of animal tested (n = 6), [a]serum glutamate oxaloacetate transaminase,

[b]serum glutamate transaminase, P> 0.05 versus control. The mean levels were calculated using ANOVA followed by Dunnett's test.

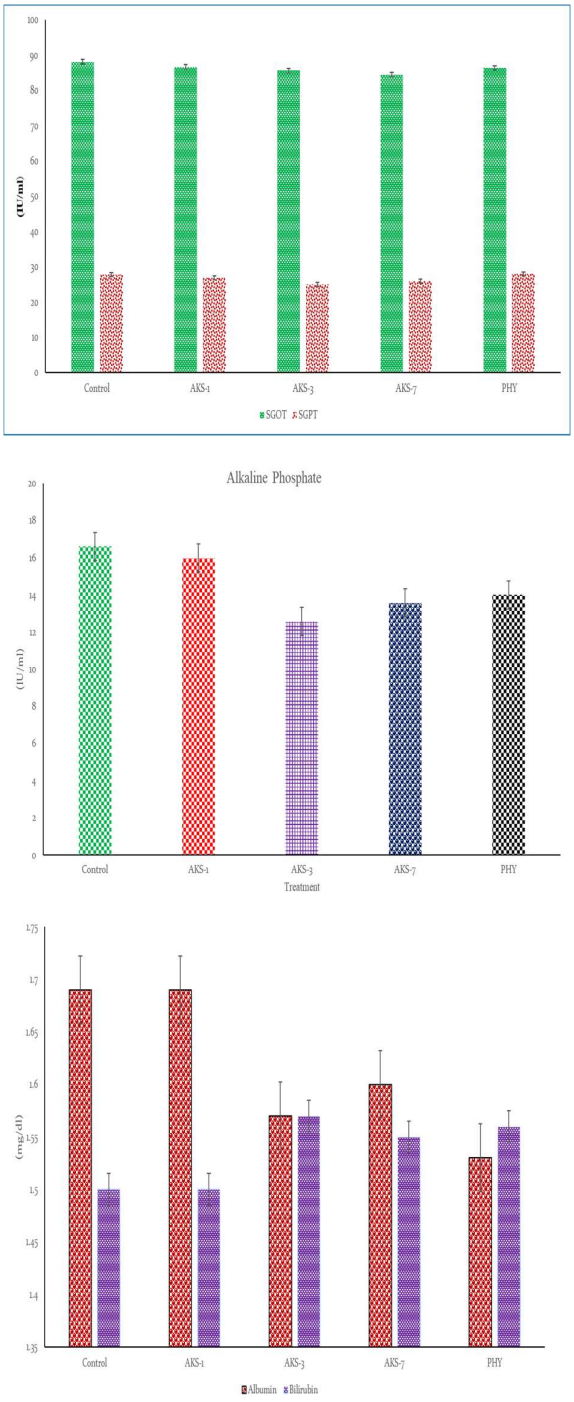

Fig. 5: Hepatic enzymes estimations after treatment with different test compounds. Number of animals tested (n = 6). The mean levels were calculated using ANOVA followed by Dunnett's test.

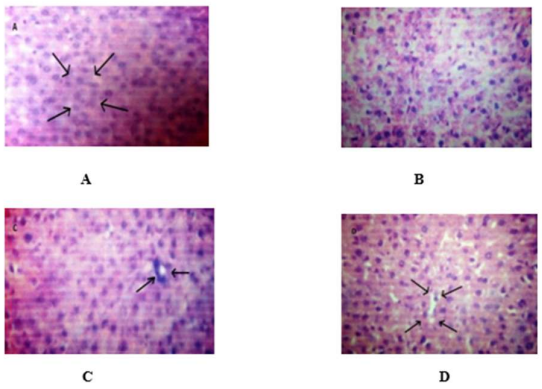

Fig. 6: High power photomicrograph of portal triad area of liver tissue from animals

A. Normal liver section shows prominent central vein, normal hepatocytes & sinusoids.

B. Liver sections of rats treated AKS – 1 showing inflammatory collection around central vein & focal necrosis with sinusoidal dilation.

C. Liver section treated with AKS – 3 showing regeneration of hepatocytes around central vein toward near normal liver architecture.

D. Liver section treated with AKS – 7 showing normal liver architecture

CONCLUSION

In the present study, a series of (Z)-4-((substitutedmethylene)amino)-5-phenyl-4H-1,2,4-triazole-3-thiols were synthesized successfully and all compounds were tested for anticonvulsant activity (phases I–IV) using MES screen. Compounds AKS-1, AKS-3 and AKS-7 represent valuable leads in the exploration of agents controlling both treatment of seizures and intoxication during epilepsy. Hence, we may conclude that a series of (Z)-4-((substitutedmethylene)amino)-5-phenyl-4H-1,2,4-triazole-3-thiols may be promising for the development of potential anticonvulsant agents after further optimization.

ACKNOWLEDGEMENT

It is my pleasure to express a deep sense of gratitude and offer my most sincere and humble thanks to Mahatma Gandhi Institute of Pharmacy, Lucknow for giving facilities for research work.

REFERENCES

1. Singh, S., Sharma, S. Synthesis, Characterization and Evaluation of 2-Imino Benzothiazole Derivatives as Anticonvulsant Agents. *International Journal of Pharmaceutical Sciences and Research*. 2014; 5: 213-217.

2. International Union of Pure and Applied Chemistry. "Schiff base". *Compendium of Chemical Terminology*, Internet ed.; http://en.wikipedia.org/wiki/Compendium of Chemical Terminology (accessed May 2007).

3. Verma, M., Pandeya, S. N., Singh, K. N., Stables, J. P. Anticonvulsant Activity of Schiff Bases of Isatin Derivatives. *Acta Pharm*. 2004; 54; 49–56.

4. Sharma, P. P., Pandeya, S. N., Roy, R. K., Chaudhary, A. Synthesis and Anticonvulsant Activity of Some Novel Isatin Schiff's Bases. *International Journal of ChemTech Research*. 2009; 1(3): 758-763.

5. Paneersalvam, P., Raj, T., Ishar, M. P. S., Singh, B., Sharma, V., Rather, B. A. Anticonvulsant Activity of Schiff Bases of 3-Amino-6,8-dibromo-2-phenyl-quinazolin-4(3H)-ones. *Indian J Pharm Sci*. 2010; 72(3): 375–378.

6. Ghadage, R. V., Shirote, P. J. Synthesis and anticonvulsant activity of Schiff's bases of 3-{[2-({(E)-[(substituted) phenyl] methylidene} amino) ethyl] amino}quinoxalin-2(1H)-one. *Bangladesh J Pharmacol*. 2011; 6: 92-99.

7. Swathi, K., Sarangapani, M. Synthesis and antiepileptic activity of Schiff's bases of dialkylamino alkoxy isatin derivatives. *Adv Exp Med Biol*. 2015; 822: 119-28.

8. Kulkarni, A. A., Wankhede, S. B., Dhawale, N. D.,Yadav, P. B., Deore, V. V., Gonjari, I. D. Synthesis, characterization and biological behaviour of some Schiff's and Mannich base derivatives of Lamotrigine. *Arabian Journal of Chemistry*. 2017; 184-189.

9. Jain, J. S., Srivastava, R. S., Agarwal, N., Sinha, R. Synthesis and Evaluation of Schiff Bases for Anticonvulsant and Behavioral Depressant Properties. *Central Nervous System Agents in Medicinal Chemistry*. 2007; 7(3): 200-204.

10. Rawat, P., Verma, S. M. Design and synthesis of chroman derivatives with dual anti-breast cancer and antiepileptic activities. *Drug Design, Development and Therapy*. 2016; 10: 2779-2788.

11. Kunda, P. K., Rao, J. V., Mukkanti, K., Induri, M., Reddy, G. D. Synthesis, Anticonvulsant Activity and Insilco Studies of Schiff Bases of 2-Aminothiophenes via Guanidine-Catalyzed Gewald Reaction. *Trop J Pharm Res.* 2013; 12(4): 566-576.

12. Rajput, N., Pandeya, S. N. Synthesis and Anticonvulsant activity of Various Mannich and Schiff bases of 1,5-benzodiazepines. *Der Pharmacia Lettre*. 2012; 4(3): 938-946.

13. Sharma, P. P., Pandeya, S. N., Roy, R. K., Verma, A. K., Gupta, S. Synthesis and Anticonvulsant Activity of Some Novel Isatin Schiff's Bases. *Int. J. ChemTech Res*. 2009; 1(3): 758-763.

14. Bairam, R., Srinivasa, M. M. Synthesis, Characterization and Biological Evaluation of Some Novel Schiff Base 1, 3-thiazine Derivatives. *Panacea Journal of Pharmacy and Pharmaceutical Sciences*. 2018; 7(1): 719-734.

15. Yadav, M. K., Tripathi, L. Synthesis, anticonvulsant (Chemo shock) activity of Isatin Mannich bases of quinazolone derivative. *MIT International Journal of Pharmaceutical Sciences*. 2016; 2: 38-42.

16. Adedirina, O., Uzairub, A. Gideon A., Abechib, S. E. Computational studies on α-aminoacetamide derivatives with anticonvulsant activities. *Beni-Suef University Journal of Basic and Applied Sciences*. 2018; 7: 709–718.

17. "The United State Pharmacopoeia" USP-NF-XXV, The United State Pharmacopoeial Convention Inc. Rockville, M. D. 2002.

18. Yadav, M. K., Tripathi, L., Goswami, D. Synthesis and Anticonvulsant Activity (Chemo-Shock) of some Novel Schiff Bases of substituted 4-amino-5-phenyl-2, 4-dihydro-[1, 2, 4]-triazole-3-thione. *Saudi J. Med. Pharm. Sci*. 2017; 3: 45-54.

19. Reitman, S., Frankel, S. A colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. *American Journal of Clinical Pathology*. 1957; 28(1): 56–63.

20. Siddiqui, N., Ali, R. Preliminary Anticonvulsant and Toxicity Screening of Substituted Benzylidenehydrazinyl – N – (6-substituted benzo [d] thiazol – 2 – yl)propanamides. *The Scientific World Journal*. 2014; 1 – 10.

21. Nilkanth, P. R., Ghorai, S. K., Sathiyanarayanan, A., Dhawale, K., Ahamad, T., Gawande, M. B., Shelke, S. N. Synthesis and Evaluation of Anticonvulsant Activity of Some Schiff Bases of 7-Amino-1,3-dihydro-2H-1,4-benzodiazepin-2-one. *Chem Biodivers.* 2020; 17(9): 342 – 352.

22. Osman, H. M., Elsaman, T., Yousef, B. A., Elhadi, Eltayib, E. M., Mohamed, M. A. Schiff Bases of Isatin and Adamantane-1-Carbohydrazide: Synthesis, Characterization and Anticonvulsant Activity. *Journal of Chemistry*. 2021; 1 – 11.

23. Hsu, P. K., Tsai, Y. T., Lin, Y. C., Kuan, C. M. Assessement of the acute and sub – acute toxicity of the ethanolic extract of the aerial parts of Crassocephalumrabens (Asteraceae) in rats. *Toxicity Report*. 2022; 9: 58 – 63.

24. Tchekalarova, J., Todorov, P., Stoyanova, T., Atanasova, M. Comparative Analysis of Anticonvulsant Activity of Trans and Cis 5,5′-Diphenylhydantoin Schiff Bases. *Int. J. Mol. Sci*. 2023; 24(22): 160 - 171.

25. Tiglani, D., Mazumder, A., Ahsan, M. J. Synthesis Anticonvulsant and Cytotoxic Evaluation of Benzimidazole-Quinoline Hybrids Schiff Base Analogs. *Polycyclic Aromatic Compounds*. 2024; 44(2): 960 – 980.

26. Ngu, C. P., Sahu, R., Shah, K., Paliwal, D., Sah, A. K., Prajapati, B. G. Recent Development in Synthesis and Anticonvulsant Activity of Promising Schiff Base Derivatives. *Journal of Heterocyclic Chemistry*. 2025; 62(10): 1264 – 1284.