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ANTICONVULSANT EVALUATION OF SOME NOVEL 1, 3-THIAZINE DERIVATIVES

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ABSTRACT: In the present study, a series of novel biologically active 8-benzylidene-6-tert-butyl-4-phenyl- 5, 6, 7, 8-tetrahydro-benzo-1, 3-thiazin – 2 -imines (TB_1-TB_{12}) were synthesized and evaluated for their biological activities. Initially, 2,6-dibenzylidene-4-tert-butylcyclohexanones were synthesized by Claisen-Schmidt condensation of 4-tert-butylcyclohexanone with various aromatic aldehydes in the presence of dilute sodium hydroxide. Further these compounds were subjected to cyclocondensation with thiourea, in isopropyl alcohol, catalyzed by aqueous potassium hydroxide to form 4-aryl-8-arylidene-5,6,7,8-tetrahydro-1H-benzo[d][1,3]thiazin-2(4H)-imines (TB_1-TB_{12}). The structures of the newly synthesized compounds have been established on the basis of their spectral data and elemental analysis. Anticonvulsant activity was performed by Maximal electroshocks (MES) method by using Diazepam as standard reference. These compounds were subjected to molecular properties prediction, drug-likeness, lipophilicity and solubility parameters determination using Molinspiration, Osiris program was used for prediction of the toxicity, and also Molsoft and ALOGPS 2.1 softwares. Among all compounds TB_5 and TB_8 containing lipophilic methoxyl and isopropyl group were more potent whereas TB_{12} containing hydroxyl groups were least potent among the series.

INTRODUCTION: Epilepsy is one of the most common neurological diseases. It is commonly accepted that a significant proportion of patients suffering from seizures are resistant to drug treatment.

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The ratio of intractable patients has not been reduced despite of the marketing of several new anticonvulsant agents in recent years.

Research in pharmaceutical chemistry renders a vital role in the discovery of newer therapeutic agents. The heterocyclic compounds [1] which contain nitrogen, sulfur and oxygen possess an enormous significance in the field of medicinal chemistry. Heterocyclic compounds are abundant in nature and have acquired more importance because they form the structural subunits of many natural products such as Vitamins, hormones,

antibiotics and drug molecules. 1, 3- thiazines which contains nitrogen and sulfur as a part of six membered heterocyclic ring (N-C-S). This ring also present in some of the medicinally important compounds like Xylazine (agonist at the α_2-adrenergic receptor is used for sedation, anesthesia, muscle relaxation, and analgesia in animals [2]) and Chlormezanone (used as an anxiolytic and a muscle relaxant [3]). Thiazine derivatives were reported to possess diverse biological activities including anticonvulsant [4], antimicrobial [5, 6, 7, 8, 9 10], analgesic [11], anti-inflammatory and ulcerogenic [12, 13], anticancer [14, 15, 16, 17, 18], antidiabetic [19], immunotropic [20], antianxiety [21], insecticidal and pesticidal [22], antitubercular [23], anthelmintic [24], anesthetic [25] and antiviral [26].

In the present study we intended to synthesize some novel hydrophobic 1,3-thiazine derivatives for evaluating the anticonvulsant activity. Most of the anticonvulsant agents in therapy comprise a hydrophobic group with urea or urea like functionality (**Fig. 1**).

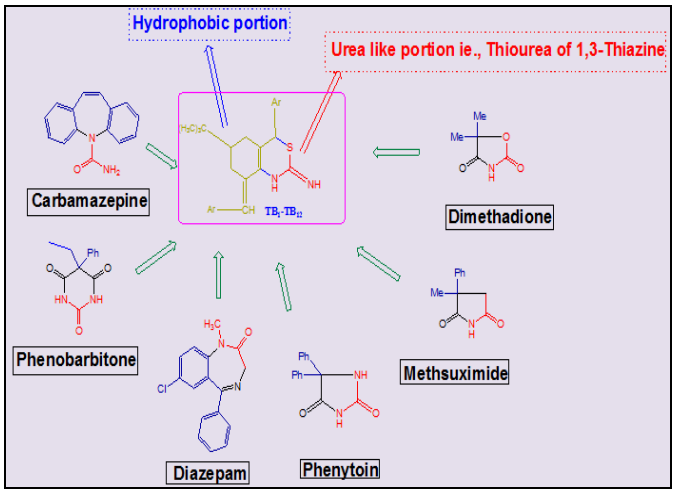


FIG. 1: DESIGN OF HYDROPHOBIC GROUP LINKED 1, 3- THIAZINE ANALOGUES FOR THEIR ANTICONVULSANT ACTIVITY. HYDROPHOBIC PORTION SHOWN IN BLUE WHERE AS POLAR UREA OR UREA LIKE PORTION IN RED

Hydrophobicity assists the molecule to reach the brain by crossing blood brain barrier and also to interact with the target site via the hydrophobic interactions whereas the urea or urea like functionality for the polar interactions. These structural features of the existing drugs have become the rationale for designing novel anticonvulsant agent-containing hydrophobic substituted cyclohexyl portions along with polar thiourea moieties. We reported the synthesis of novel hydrophobic 1,3-thiazine derivatives containing electron withdrawing and electron-releasing substituents on the aryl and arylidene rings and without any modification on the thiourea portion to study the effect of such substitutions on anticonvulsant activity.

2. MATERIALS AND METHODS:

2.1 General: All the chemicals and solvents were purchased from Sigma-Aldrich and Merck, India. The melting points were determined in open capillary tubes and are uncorrected. The IR spectra of the compounds were recorded on Perkin Elmer FTIR spectrometer in KBr discs method. ^{1}H & ^{13}C, NMR spectra were recorded on Bruker 400 MHZ AVANCE ^{1}H NMR in $CDCl_3$ at 400 MHz. The chemical shifts are reported in parts per million downfield from tetramethylsilane. Mass spectra were recorded on LC-MS Schimadzu 2010A using dimethyl sulfoxide as solvent. Elemental analyses were carried out in a Coleman automatic carbon, hydrogen and nitrogen analyzer.

All the compounds gave satisfactory chemical analysis. The homogeneity of the compounds was checked by TLC on aluminum foil packed precoated silica gel plates using n-hexane and ethyl acetate (8:2) as mobile phase and visualized by iodine vapors. All the compounds have been purified by column chromatography performed on silica gel (100-200 mesh, Merck) as stationary phase, and ethyl acetate: hexane mixture as solvent system. Diazepam was obtained from Taj Pharmaceuticals Ltd., Mumbai and was dissolved in normal saline. The healthy albino rats (Wistar, 100–150 g, 5–6 weeks) were obtained from Mahaveer enterprises, Kolkata.

2.2 Chemistry:

2.2.1 General method of synthesis of 2, 6-dibenzylidene-4-tert-butylcyclohexanone [3]: The substituted aromatic aldehyde (0.02mol) was added to reaction mixture of 30 ml of 10% sodium hydroxide and 4-tert-butylcyclohexanone (0.01mol) in 50 ml of ethyl alcohol, which was maintained at 20-25 ^{0}C. This reaction mixture was magnetically stirred at 500 rpm for a period of 2 hrs. Subsequently the reaction mixture was stored at a temperature of 0-4 ^{0}C for a period of 8 hrs to obtain a residue. Finally, the residue was filtered, washed

thoroughly with ice cold water followed by ice-cold ethanol, dried and recrystallized from dimethyl formamide to form yellow crystals. Yield = 95%, mp. 126.6–127.0. UV (λ_{max}): 396 nm; IR (KBr, cm^{-1}): 1657cm^{-1} (C=O) 1598, 1554, 1506, 1462 cm^{-1} (aromatic), 834 cm^{-1} (C=C); ^{1}H NMR: ($CDCl_3$): δ 0.75 (m, 2× -CH_3, 6H), 0.78 (d, -CH_3, 3H), 1.32-1.34 (d, -CH-, 1H), 1.83-1.87 (m, -CH_2-, 2H), 2.88 (s, -CH_2-, 2H), 7.28-7.41 (m, Ar-H, 10H). Mass of 3a: MS (ESI): *m/z* = 388.9 [M^1 +1] [27, 28].

2.2.2 General procedure for the preparation of 8-benzylidene-6-tert-butyl-4-phenyl-5, 6, 7, 8-tetrahydro-1H-benzo-1, 3-thiazin-2-imine: A reaction mixture containing 2, 6-dibenzylidene-4-tert-butylcyclohexanone (0.01mol), thiourea (0.015mol) and potassium hydroxide (0.01mol) was dissolved in specified amount (5 ml) of water and refluxed in isopropyl alcohol (100 ml) for a period 16 hrs. Formation of the product was confirmed by TLC method of estimation. Finally the solvent was removed by distillation and the residue obtained was treated with ice cold water, filtered, dried and the dried residue was purified by silica gel column chromatography (ethyl acetate/hexane) to give respective compound 1,3-thiazine derivative [29, 28, 30].

The final product was recrystallized from ethyl acetate and hexane. The same procedure was followed for the synthesis of all the compounds TB_1-TB_{12}.

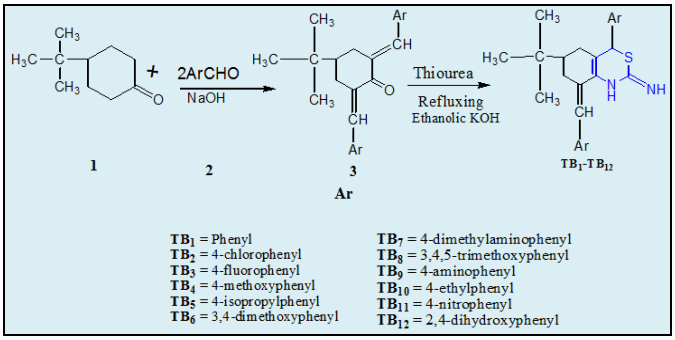


FIG. 2: REACTION SCHEME FOR SYNTHESIS OF TITLE COMPOUNDS

2.2.3 8-benzylidene-6-tert-butyl-4-phenyl-5, 6, 7, 8-tetrahydro-1H-benzo[d][1,3]thiazin-2-(4H)-imine (TB_1): Yellow solid, Yield 90%; mp. 184-186 ^oC; R_f : 0.7; IR (KBr, cm^{-1}): 3399.9 (Imine NH-), 3207.0 (cyclic NH-), 1609.3 (C=N), 1077 (C-N), 1476.2 (CH_3-); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 0.75 (m, 2× CH_3, 6H), 0.78 (d, CH_3, 3H), 1.32-1.34 (d, CH, 1H), 1.83-1.87 (m, -CH_2-, 2H), 2.88 (s, CH_2, 2H), 4.93 (s, CH-S, 1H), 6.51 (s, imine NH, 1H), 6.64 (s, cyclic NH, 1H), 7.25-7.27 (d, Ar-H, 1H), 7.28-7.41(m, Ar-H, 9H), 7.55 (s, methine H, 1H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 178.47, 152.03, 151.79. 151.68, 148.41, 142.58, 139.67, 139.26, 137.58, 135.88, 134.53, 133.84, 132.36, 127.53 (C-2' and C-6'), 127.41, (C-3" and C-5"), 125.82, 125.42, 118.87, 44.15, (-CH2-, C of isobutyl group at C-4"); 43.95, 36.1, 35.9; 31.54 (-CH-, C of isobutyl group at C-4"); MS (ESI): m/z = 388.9 [M^1 +1]; Anal. Calcd for: $C_{19}H_{19}ClO$: C, 76.37; H, 6.41; Found: C, 76.40; H, 6.44.

2.2.4 8-(4-chlorobenzylidene)-6-tert-butyl-4-(4-chlorophenyl)-5, 6, 7, 8-tetrahydro-1H-benzo [d] [1,3] thiazin-2(4H)-imine (TB_2): White solid, Yield 80%; mp. 222-224 ^oC; R_f : 0.7; IR (KBr, cm^{-1}): 3424.0 (Imine NH-), 3275.5 (cyclic NH-), 1615.0 (C=N), 1089.0 (C-N), 1487.0 (CH_3-); Ar-Cl-809.0; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 0.75 (m, 2× CH_3, 6H), 0.78 (d, CH_3, 3H), 1.32-1.34 (d, CH, 1H), 1.83-1.87 (m, -CH_2-, 2H), 2.88 (s, CH_2, 2H), 4.93 (s, CH-S, 1H), 6.51 (s, imine NH, 1H), 6.64 (s, cyclic NH, 1H), 7.25-7.27 (d, Ar-H, 1H), 7.28-7.41(m, Ar-H, 9H), 7.55 (s, methine H, 1H); MS (ESI): m/z = 456.7 [M^1 +1]. Anal. Calcd for: $C_{25}H_{26}Cl_2N_2S$, %: C, 65.64; H, 5.73; N, 6.12. Found, %: C, 65.45; H, 5.55; N, 6.10.

2.2.5 8-(4-fluorobenzylidene)-6-tert-butyl-4-(4-fluorophenyl)-5, 6, 7, 8-tetrahydro-1H-benzo [d] [1, 3] thiazin-2(4H)-imine (TB_3): White solid, Yield 82%; mp. 243-245 ^oC; R_f : 0.6; IR (KBr, cm^{-1}): 3256.0 (Imine NH-), 2921.0 (cyclic NH-), 1693.0 (C=N), 1115.0 (C-N), 1412.0 (CH_3-), Ar-F-934.9; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 0.75-0.78 (m, 3 × CH_3, 9H), 1.32-1.34 (d, CH, 1H), 1.61 (d, CH_2 2H), 1.83-2.03 (m, CH_2, 2H), 4.93 (s, CH-S, 1H), 6.51 (s, imine NH, 1H), 6.64 (s, cyclic NH, 1H), 7.25-7.39 (m, Ar-H, 8H), 7.59 (s, methine H, 1H). ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 190.28 (C-1), 184.30, 172.64, 163.28, 154.62, 150.37, 131.99, 128.19, 127.95, 112.70, 107.50, 59.33, 44.33, 40.57, 39.94, 27.88; MS (ESI): m/z = 425.1 [M^1 +1]. Anal. Calcd for: $C_{25}H_{26}F_2N_2S$, %: C, 70.73; H, 6.17; N, 6.60. Found, %: C, 70.40; H, 6.01; N, 6.51.

2.2.6 8-(4-methoxybenzylidene)-6-tert-butyl-4-(4-methoxyphenyl)-5, 6, 7, 8 - tetrahydro-1H-benzo[d] [1, 3] thiazin - 2(4H) – imine (TB_4): Yellowish solid, Yield 89%; mp. 189-192 ^oC; R_f : 0.4; IR (KBr, cm^{-1}): 3384.0 (Imine NH-), 3201.0 (cyclic NH-), 1601.1 (C=N), 1031.0 (C-N), 1248(C-O-C), 1476.0 (CH_3-); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 0.76-0.79 (m, 3 × CH_3, 9H), 0.84-0.92 (m, CH_2, 2H), 1.28-1.34 (m, CH_2, 1H), 1.81-1.89 (m, CH_2 1H), 1.96-2.02 (m, CH,1H), 3.81-3.83((m, 2× OCH_3, 6H), 6.44 (s, CH-S, 1H), 6.61 (s, imine NH, 1H), 6.88 (s, cyclic NH, 1H), 6.88-6.92 (m, Ar-H, 4H), 7.19-7.23(m, Ar-H, 4H), 7.57 (s, methine H, 1H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm):174.37, 149.14, 148.90, 135.27, 135.27, 128.50, 122.75, 119.33, 114.36, 111.98, 58.33, 55.78, 43.73, 39.55, 27.87; MS (ESI): m/z = 448.9 [M^1 +1]. Anal. Calcd for: $C_{27}H_{32}N_2O_2S$, %: C, 72.29; H, 7.19; N, 6.24. Found, %: C, 72.26; H, 7.15; N, 6.21.

2.2.7 8-(4-isopropylbenzylidene)- 6 - tert-butyl-4-(4-isopropylphenyl)-5,6,7,8-tetrahydro - 1H – benzo[d][1,3]thiazin-2(4H)-imine (TB_5): White solid, Yield 88%; mp. 180-182 ^oC; R_f : 0.5; IR (KBr, cm^{-1}): 3426.0 (Imine NH-), 3064.0 (cyclic NH-), 1650.0 (C=N), 1159.0 (C-N), 1457.0 (CH_3-); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 0.71-0.86 (m, 3 × CH_3, 9H), 0.87-0.97 (m, CH_2, 2H), 1.28-1.34 (m, CH_2, 1H), 1.81-1.89 (m, CH_2 1H), 1.96-2.40 (m, CH,1H), 2.40-2.44 (m, CH,1H), 3.13-3.89((m, 2× CH_3, 6H), 4.84 (s, CH-S, 1H), 6.4 (s, imine NH, 1H), 6.88 (s, cyclic NH, 1H), 6.88-6.92 (m, Ar-H, 4H), 7.61-7.73(m, Ar-H, 4H), 7.75 (s, methine H, 1H); MS (ESI): m/z = 742.7 [M^1 +1]. Anal. Calcd for: $C_{31}H_{40}N_2S$, %: C, 78.76; H, 8.53; N, 5.93. Found, %: C, 78.56; H, 8.44; N, 5.71.

2.2.8 8-(3,4-dimethoxybenzylidene) – 6 - tert-butyl-4-(3,4-dimethoxyphenyl)-5, 6, 7, 8 - tetra hydro-1H-benzo[d][1, 3] thiazin - 2 (4H) -imine (TB_6): Yellowish solid, Yield 78%; mp 166-168 ^oC. R_f: 0.7; IR (KBr, cm^{-1}): 3426.0 (Imine NH-), 3098.0 (cyclic NH-), 1655.0 (C=N), 1067.0 (C-N), 1250.0 (C-O-C), 1458.0 (CH_3-). ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 0.67-0.86 (m,3× CH_3, 9H), 1.20-1.23 (d, CH,1H), 1.82-1.88 (m, CH_2, 2H), 1.93-2.07 (m, CH_2 2H), 3.41-3.88 (m (4× OCH_3, 12H),18H), 4.72 (s, CH-S, 1H), 6.72 (s, imine NH, 1H), 6.74 (s, cyclic NH, 1H), 6.74-6.98 (m, Ar-H, 6H), 8.93 (s, methine, 1H). ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 174.47, 148.47, 147.25, 140.413, 135.05, 129.58, 128.41, 127.44, 126.58, 122.88, 114.66, 58.42, 43.62, 40.61, 39.98, 27.49. MS (ESI): m/z = 509.1[M^1 +1]. Anal. Calcd for: $C_{29}H_{36}N_2O_4S$, %: C, 68.47; H, 7.13; N, 5.51. Found, %: C, 68.40; H, 7.20; N, 5.49.

2.2.9 4-((6-tert-butyl - 4 - (4-(dimethyl amino) phenyl)-2-imino-1,2,6,7-tetrahydro-4H-benzo[d] [1,3]thiazin-8(5H)-ylidene)methyl)-N,N-dimethyl benzenamine (TB_7): Light brown solid, Yield 69%; mp. 142-144 ^oC; R_f : 0.7; IR (KBr, cm^{-1}): 3425.0 (Imine NH-), 3069.0 (cyclic NH-), 1654.0 (C=N), 1116.0 (C-N), 1454.0 (CH_3-); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 0.67-0.74 (m, CH_3, 3H), 0.81-2.23 (m, 2× CH_3, 6H), 1.25 (d, CH, 1H),2.79-2.82 (m, 2× CH_2, 4H), 2.84-3.07 (m, 4× NCH_3, 12H), 5.38 (s, CH-S, 1H), 6.55 (s, imine NH, 1H), 6.66 (d, cyclic NH, 1H), 6.67-7.65 (m, Ar-H, 8H), 7.67 (s, methine H, 1H); MS (ESI): m/z = 473.2[M^1 +1]. Anal. Calcd for: $C_{29}H_{38}N_4S$, %: C, 73.37; H, 8.07; N, 11.80. Found, %: C, 73.31; H, 8.00; N, 11.78.

2.2.10 8-(3,4,5-trimethoxybenzylidene) – 6 -tert-butyl-4-(3,4,5-trimethoxyphenyl)-5, 6, 7, 8-tetra hydro-1H-benzo[d][1,3] thiazin - 2(4H) - imine (TB_8): Yellowish solid, Yield 94%; mp. 210-212 ^oC; R_f : 0.5; IR (KBr, cm^{-1}): 3663.0 (Imine NH-), 3291.0 (cyclic NH-), 1673.0(C=N), 1065.0 (C-N), 1263.0 (C-O-C), 1419.0 (CH_3-); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 0.71-0.79 (m, $(CH_3)_2$, 6H), 0.81-0.83 (d, CH_3, 3H), 1.22 (s, CH, 1H), 1.24-1.99 (m, CH_2 2H), 2.08-2.99 (s, CH_2, 1H), 2.48 (s, CH_2, 1H), 3.61-3.84 (m (6× OCH_3, 18H), 4.44 (s, CH-S, 1H), 6.57 (s, imine NH, 1H), 6.58 (s, cyclic NH, 1H), 6.62-6.64 (m, Ar-H, 4H), 8.96 (s, methine H, 1H); MS (ESI): m/z = 569.1 [M^1 +1]. Anal. Calcd for: $C_{31}H_{40}N_2O_6S$, %: C, 65.47; H, 7.07; N, 4.93. Found, %: C, 65.42; H, 6.98; N, 4.51.

2.2.11 4-((4-(4-aminophenyl)-6-tert – butyl - 2-imino-1,2,6,7-tetrahydro-4H - benzo [d][1,3] thiazin - 8 (5H)- ylidene) methyl) benzenamine (TB_9): Light brown solid, Yield 70%; mp. 156-158 ^oC; R_f : 0.6; IR (KBr, cm^{-1}): 3441.0 (Imine NH-), 3063.0 (cyclic NH-), 1653.0(C=N), 1092.0 (C-N), 1456.0 (CH_3-), 735.6 (Ar-NH_2); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 0.67-0.75 (m, 3× CH_3, 9H), 1.13-1.19 (d, CH, 1H), 1.73-1.79 (m, CH_2, 2H), 1.95-2.05 (m, CH_2, 1H), 2.812.85 (d, CH_2, 1H),

4.81-4.84 (d, CH-S, 1H), 6.96 (s, imine NH, 1H), 7.06 (s, cyclic NH, 1H),7.16-7.73 (m, Ar-H, 8H), 9.04 (s, methine H, 1H). ^{13}C NMR ((400 MHz, DMSO-d_6, δ ppm): 174.48, 163.36, 162.52, 160.94, 139.22, 133.88, 131.51, 129.56, 128.31, 122.14, 116.08, 114.83, 57.89, 43.69, 39.35, 27.96; MS (ESI): m/z = 418.5 [M^1 +1]. Anal. Calcd for: $C_{25}H_{30}N_4S$, %: C, 71.73; H, 7.22; N, 13.38. Found, %: C, 71.68; H, 7.15; N, 13.11.

2.2.12 8-(4-ethylbenzylidene)-6-tert-butyl-4 - (4-ethylphenyl)-5, 6, 7, 8-tetrahydro-1H-benzo[d] [1,3]thiazin-2(4H)-imine (TB_{10}): Yield yellow solid, 76%; mp. 178-180 ^oC; R_f: 0.6; IR (KBr, cm^{-1}): 3380.0 (Imine NH-), 3201.0 (cyclic NH-), 2934.0 (Ar-C_2H_5). 1611.0 (C=N), 1060.0(C-N), 1475.0 (CH_3-), 735.6 (Ar-NH_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 0.76-0.79 (m, 3× CH_3, 9H), 0.82-0.86 (m, 2× CH_2, 4H), 0.92 (m, CH, 1H), 1.21-1.27 (m, 2× CH_2, 4H), 2.62-2.69 (m, 2× CH_3, 6H), 6.47(s, CH-S, 1H), 6.59 (s, imine NH, 1H), 7.20 (s, cyclic NH, 1H), 7.20-7.22 (m, Ar-H, 8H), 7.58 (s, methine H, 1H); ^{13}C NMR (400 MHz, DMSO-d_6, *δ,* ppm): 174.48, 163.36, 162.52, 160.94, 139.22, 133.88, 131.51, 129.56, 128.31, 122.14, 116.08, 114.83, 57.89, 43.69, 39.35, 27.96; MS (ESI): m/z = 444.9 [M^1 +1]. Anal. Calcd for: $C_{29}H_{36}N_2S$, %: C, 78.33; H, 8.16; N, 6.30. Found, %: C, 78.310; H, 8.13; N, 6.21.

2.2.13 8-(3-Nitrobenzylidene)-6-tert-butyl-4-(4-nitrophenyl)-5, 6, 7, 8-tetrahydro - 1H-benzo [d] [1,3]thiazin-2(4H)-imine (TB_{11}): Light brown solid, Yield 65%; mp. 182-184 ^oC; R_f : 0.7; IR (KBr, cm^{-1}): 3418.0 (Imine NH-), 3087.0 (cyclic NH-), 1601.0 (C=N), 1060.0 (C-N), 1462.0 (CH_3-), 832.1 (Ar-NO_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 0.63-0.65 (d, CH_3, 3H), 0.70-0.92 (m, 2× CH_3, 6H), 1.00-1.02 (d, CH, 1H), 1.12-1.16 (m, CH_2, 2H), 1.71-2.37 (m, CH_2, 2H), 4.96-5.21 (m, CH-S, 1H), 6.42 (t, imine NH, 1H), 6.57 (s, cyclic NH, 1H), 6.66-6.91 (d, Ar-H, 1H), 7.07-7.23 (d, Ar-H, 1H), 7.65-7.89(m, Ar-H, 4H),7.92(d, Ar-H, 2H), 8.31 (s, methine H, 1H). MS (ESI): m/z = 478.56 [M^1 +1]. Anal. Calcd for: $C_{25}H_{26}N_4O_4S$, %: C, 62.74; H, 5.48; N, 11.71. Found, %: C, 62.71; H, 5.38; N, 11.65.

2.2.14 4-((6-tert-butyl-4-(3,4-dihydroxyphenyl)-2-imino-1,2,6,7-tetrahydro- 4H - benzo [d] [1,3] thiazin-8(5H)-ylidene)methyl)benzene-1,2-diol (TB_{12}): Light white solid, Yield 65%; mp. 177-179 ^oC; R_f : 0.5; IR (KBr, cm^{-1}): 3444.0 (Imine NH-), 2951.0 (cyclic NH-), 1593.0(C=N), 1027.0 (C-N), 1466.0 (CH_3-), 774.3 (Di-substituted); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.5-2.4 (m, $(CH_2)_2$, 4H), 2.4 (m, CH_2, 2H), 4.3 (s, CH-S, 1H), 6.9 (s, imine, 1H), 6.9 (s, cyclic NH, 1H), 7.0-7.7(m, Ar-H, 8H), 7.7 (s, methine, 1H); ^{13}C NMR (400 MHz, DMSO-d_6, *δ* ppm): 174.65, 153.45, 138.29, 137.57, 136.85, 133.03, 131.93, 129.40, 128.57, 123.04, 114.60, 107.09, 104.71, 60.52, 58.60, 43.73, 40.60, 39.97, 27.40; MS (ESI): m/z = 451.4 [M^1 +1]. Anal. Calcd for: $C_{25}H_{28}N_2O_4S$, %: C, 66.35; H, 6.24; N, 6.19. Found, %: C, 66.29; H, 6.18; N, 6.06.

2.3 Biology General procedure:

2.3.1 Anticonvulsant activity: All the experimental protocols and procedures described here upon were prior approved by Institutional Animal Ethics Committee. Maximal electroshock (MES) method was employed for studying anticonvulsant activity [33, 34]. Diazepam (10 mg/kg) was dissolved in normal saline. The healthy albino rats (Wistar, 100–150 g, 5–6 weeks) were kept under standard laboratory conditions (room temperature: 23+28 ^0C; relative humidity: 60+5 %; illumination: 12 hrs light/dark cycle) and had freely access to food pellets and fresh water except for the short time duration when animals were removed for pharmacological testing. All experiments were performed between 9.00 AM and 2.00 PM.

2.3.2 Acute oral toxicity studies: Acute oral toxicity study was done as per OECD/OCDE/425 guideline 423. A group of three Wistar rats of either sex selected randomly and were used for acute toxicity study. The experimental protocol for the pharmacological screening was done in accordance with the guidelines prescribed by an Institutional Animal Ethics Committee (CPCSEA No: 1292/ac/09/CPCSEA). The synthesized compounds were administered orally at the dose level of 5 mg/kg body weight to the animals and observed for 14 days. Since no mortality was observed, the procedure was repeated for further higher doses of 50, 300 and 2000 mg/ kg body weight. The compounds showed no mortality at doses up to 2000 mg/kg. Hence 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of this dose were selected as the dose levels for the study.

2.3.3. Computationally predicted toxicity, lipophilicity and drug score profiles: Shredding of each molecule at every rotatable bond lead to a set of nucleus fragments. These in turn were used to rebuild all possible larger fragments which could be the substructure of the original fragment. Afterwards, a search procedure of substructure determined the occurrence frequency of every one of the fragment (constructed and core fragments) surrounded by all traded drugs of 3300 in addition to 15,000 commercially available chemicals (Fluka) to predict toxicity, C Log P, and drug score.

2.3.4 Electrically-induced seizures: In the electrically-induced seizure experiment, the Maximal electroshock (MES) method described previously by Swinyard was employed. In brief, tonic convulsions of the hind extremities of the rats were induced by passing alternating electrical current of 50 Hz and 150 mA for 0.2 sec through corneal electrodes. The animals were divided randomly into 5 groups containing 4 animals each. Group I served as control group containing normal saline; groups II served as standard drug containing diazepam 10mg/kg, III, IV and V served as test groups treated with test compounds (50, 100 and 200 mg/kg, i.p., 30 min), respectively prior to the induction of convulsion. In MES test values represent number of mice protected divided by number of mice tested [35].

3. RESULTS AND DISCUSSIONS:

3.1 Chemistry: Twelve novel 1,3-thiazine derivatives (TB_1-TB_{12}) were synthesized by the condensation of different 2, 6-dibenzylidene-4-tert-butylcyclohexanones with thiourea in the presence of ethanol by refluxation. The yields of the compounds were in between 65-94%. All the compounds were purified by column chromatography and characterized by spectral methods including IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis. Formation of 1,3-thiazine scaffold is confirmed by the characteristic IR absorption bands in the ranges of 3250-3450 cm^{-1} (Imine NH-), 2950-3200 (cyclic NH-), 1590-1680 (C=N) and 1020-1120 (C-N) respectively. Two diagnostic singlet peaks in the ^{1}H NMR spectrums around δ 6.4-7.2 ppm helped to unravel the structures of the compounds. The ^{13}C NMR spectrum of compounds exhibited the characteristic signals in the range of δ 170-190 ppm which corresponds to C=N, apart from the peaks corresponding to the other carbons. The Mass spectra obtained by positive mode ionization method revealed the $[M+H]^+$ ions representing the molecular weight of the compounds. The results of elemental analysis were also in close agreement within ± 0.4 % of the calculated values.

3.2 Biology:

Anticonvulsant activity: 1,3-thiazine derivatives were tested for their anticonvulsant activity by following maximal electroshock method. The results are summarized in **Table 1**.

TABLE 1: MAXIMAL ELECTROSHOCK WITH SINGLE DOSE BY MES IN ALBINO RATS

Compounds	Dose in mg/kg	Quantal protection	Mean on set of seizures(seconds)	Mean recovery time (minute)	% of animals protected	% of mortality
TB_1	200	3/4	5.94±0.88	6.02±0.16	75	25
TB_2	200	3/4	6.74±0.18	5.26±0.16	75	25
TB_3	200	3/4	6.86±0.22	4.22±0.12	75	25
TB_4	200	3/4	7.18±0.34	3.72±0.18	75	25
TB_5	200	3/4	7.76±0.18	3.08±0.34	75	25
TB_6	200	3/4	7.21±0.22	3.68±0.14	75	25
TB_7	200	3/4	6.24±0.94	5.56±0.94	75	25
TB_8	200	3/4	7.93±0.14	3.15±0.08	75	25
TB_9	200	3/4	5.72±1.16	6.39±0.09	75	25
TB_{10}	200	3/4	6.92±0.94	4.39±0.16	75	25
TB_{11}	200	3/4	6.12±0.94	5.28±0.94	75	25
TB_{12}	200	3/4	5.62±0.93	6.14±0.94	75	25
Control	200	0/4	5.91±1.62	6.28±1.54	0	100
Diazepam	10	4/4	---------	---------	100	0

Effect of test compounds on maximal electroshock (MES) - induced seizures in rats

"Values are expressed as mean ± SEM of each group (n=4) and are significant when done ONE-WAY ANOVA with followed by Dunnett's multiple comparison test. *** p <0.001 when compared with disease control."

From the results it can be clearly notified that 1,3-thiazines possess significant anticonvulsant activity but less than the standard diazepam. Compound TB_8 containing 3,4,5-trimethoxyphenyl moiety was most potent of the series. This is followed by TB_5 with 4-isopropylphenyl substitution. The third and fourth in the activity is seen in TB_6 and TB_4 with 3,4,-dimethoxyphenyl and 4-methoxyphenyl scaffolds respectively. Compound TB10 with 4-ethylphenyl moiety also possess significant anticonvulsant activity. Halogenated thiazines were also exhibited good activity. TB_1 which possess no substitution on the phenyl ring was found to be more active than the compound TB_{12} with dihydroxyphenyl substitution and also TB_{12} is the least active of the series.

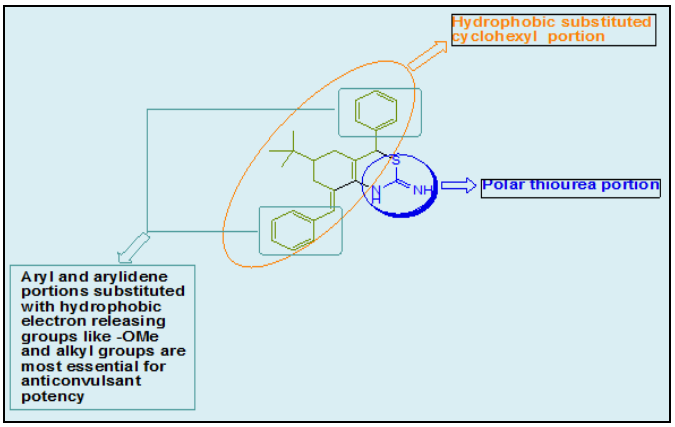


FIG. 3: SUMMARY OF THE STRUCTURE ACTIVITY RELATIONSHIPS OF 1,3-THIAZINES FOR ANTICONVULSANT ACTIVITY

Structure-activity relationships (**Fig. 3)** based on the above results suggests that 1,3-thiazine scaffolds fused with hydrophobic substituted cyclohexyl portion is essential for the activity. The substituted aryl and arylidene moieties at positions-4 and 8 plays a crucial role in the activity. In particular it was found that if these rings are substituted with electron releasing hydrophobic groups like methoxyl and alkyl groups are essential for the activity. If more number of such groups are present, there is a greater increase in the potency. Presence of hydrophilic hydroxyl groups on the aromatic rings has reduced the activity. These SAR studies suggest that for thiazines to be act as potent anticonvulsant agents hydrophobicity is crucial factor.

3.3 Statistics: The statistical analysis of the results obtained for diazepam and the synthesized compounds were done. The significance of differences between the different dosing groups was calculated by the Steel test (seizure score) or by one-way analysis of variance (ANOVA) followed by post hoc comparison using Dunnett's test. $p < 0.05$ was considered statistically significant.

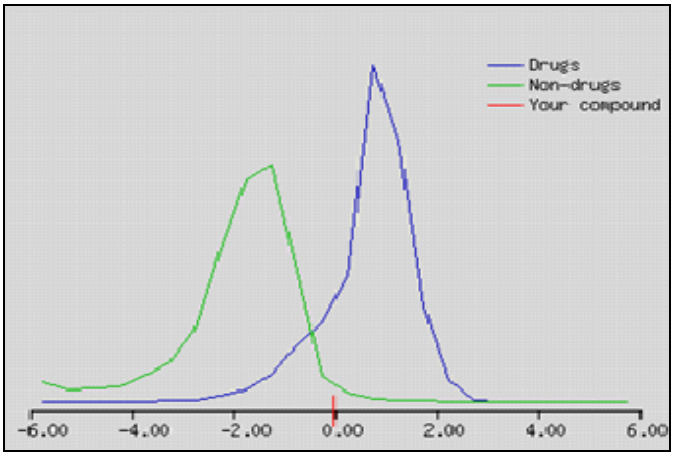
3.4. Assessment of Toxicity, Lipophilicity and Drug Score Profiles: Osiris program was used for prediction of the toxicity of the synthesized compounds. The prediction relies on a substructure look for route determining the occurrence frequency of any fragment (constructed and core fragments) within any of toxicity classes. The drug score combines C log P, molecular weight and toxicity risks in one handy value that may be used to judge the compounds overall potential to meet the requirements for a drug. Osiris program was used for prophecy of the C log P of compounds. C log P is a well established parameter to determine the hydrophilicity of the synthesized compounds. Compounds show reasonable probability of being well absorbed, when they have C log P value around 5.0. All synthesized 1, 3-thiazin-2-imine derivatives have showed low Insilco possible toxicity risks. From the table, it was observed that all compounds have revealed C log P around 5.0 indicating that the synthesized compounds could be potential drug candidates. Prophecy of C log P, drug score and toxicity for 1, 3-thiazin-2-imine derivatives are prearranged in **Table 2** and almost all the compounds possess good values of drug score, C log P, and low probable toxicity risks as revealed by computational in silico studies.

TABLE 2: COMPUTATIONALLY PREDICTED TOXICITY RISKS, LIPOPHILICITY DRUG SCORES

Entry	Clog P*	Drug score range	Toxicity risks**
TB_1	6.12	0.27	Negative
TB_2	7.35	0.25	Negative
TB_3	6.24	0.21	Negative
TB_4	5.91	0.37	Negative
TB_5	8.31	0.08	Negative
TB_6	5.38	0.33	Negative
TB_7	5.49	0.31	Negative
TB_8	5.53	0.37	Negative
TB_9	4.93	0.42	Negative
TB_{10}	7.47	0.21	Negative

* Indicating Clog P valves Calculated for the lipophilicity
** Indicating the Mutagenicity, Tumorigenicity, Irritancy & Reproductive effects

Drug-likeness model score graph: -0.02 & Non toxic fragments:



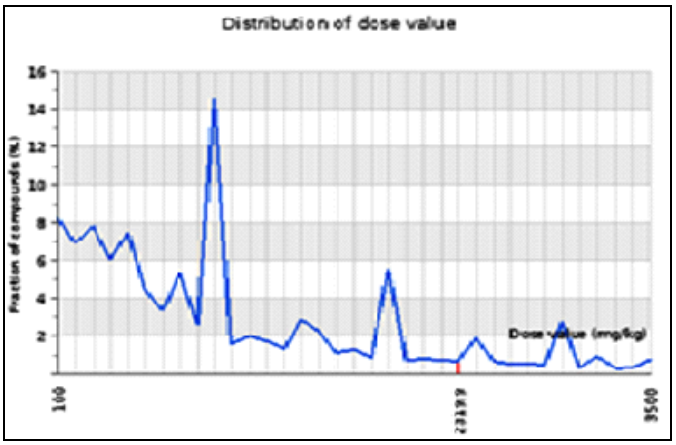


FIG. 4: DRUG-LIKENESS MODEL SCORE OF TB3 MOLECULE & TOXICITY PROFILE

CONCLUSION: In our study, we have synthesized and characterized a series of 1,3-thiazines. Anticonvulsant activity was performed by MES methods. All the compounds exhibited some anticonvulsant activity. The observations of biological results of all the new thiazines reveal that the % of protection increases with various substitutions in the following order -OMe > -iPr > Et- > Cl- > F-. Further substitution of the 4-substituted-aryl-8-substitutedarylidene-2-imino - 5, 6-dihydro-4H,7H-(3,1)-benzothiazines with an increase in the number of hydrophobic electron releasing or a combination of electron releasing and electron donating groups at different positions of the aryl and arylidene rings to further increase the anticonvulsant activity.

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CONFLICT OF INTERESTS: The authors declare that they have no conflicts of interest.

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