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SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF SOME NEW 6-(3,5-SUBSTITUTED-2-BROMO/HYDROXY PHENYL)-1,2,4-TRIAZINE DERIVATIVES AS ANTICONVULSANT AGENTS

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ABSTRACT: 6-(2-Amino-3,5-substituted phenyl)-1,2,4-triazines were prepared by refluxing semicarbazones or thiosemicarbazones in presence of sodium hydroxide solution. 6-(3,5-Substituted-2-bromophenyl)-1,2,4-triazine derivatives and 6-(3,5-substituted-2-hydroxyphenyl)-1,2,4-triazine derivatives were prepared from 6-(2-amino-3,5-substituted phenyl)-1,2,4-triazine derivatives by sodium nitrite, hydrobromic acid, copper (I) bromide and sodium nitrite, sulfuric acid, respectively. The structures of the synthesized compounds were confirmed on the basis of their elemental analysis and spectral data (FT-IR and H^1-NMR). Amongst synthesized compounds, some displayed significantly active profile against the electrically induced seizures at a dose of 30 mg/kg after 0.5 h. At the same dose level, one compound also showed activity after 4.0 h. Two compounds exhibited protection at dose level of 100 mg/kg after 0.5 h. At 4.0 h, two compounds persisted to reveal anti-MES protection at same dose, whereas two other compounds indicated to prevent seizure spread at a higher dose of 300 mg/kg after 4h. One compound displayed protection at 300 mg/kg after 0.5 h. The neurotoxicity screening data revealed that two compounds were exhibited neurotoxicity at a dose level of 300 mg/kg after 0.5 h. Screened compounds displayed 40.59, 56.88 and 66.61 % increase in immobility time. Some compounds showed excellent anticonvulsant activity with no neurotoxicity and little CNS depressant effect and does not violated Lipinski's rule, making them potentially promising agents for treatment of epilepsy

INTRODUCTION: Epilepsy is one of the most common serious CNS disorder and characterized by the recurrent, unprovoked seizures.[1-2] A global campaign against epilepsy conducted by World Health Organization showed that about 50 million people worldwide are suffering from this CNS disorder. Every year about 2.4 million new cases are added to this figures.[3-4] The currently available antiepileptic drugs (AEDs) in clinical practice produce effective seizure control only in 70% of patients.[5] Moreover, several new AEDs (such as levetiracetam, pregabaline, topiramate) have proven to be effective in reducing seizures but their therapeutic efficacy is limited due to undesirable side effects such as anorexia, ataxia, headache, hepatotoxicity, gastrointestinal disturbance, hirsutism, and nausea.[6-7] Lamotrigine (containing phenyl 1,2,4-triazine basic moiety) is a broad spectrum antiepileptic drug acts by prolonging

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inactivation of voltage-sensitive Na^+ channels.[8] Aryl semicarbazones (like 4-bromobenzaldhyde semicarbazones) and various aryloxy semicarbazones revealed activity comparable with or exceeding that of phenytoin in the maximal electroshock (MES) induced seizure test in mice.[9-10]

Based on the above facts, new 6-(3,5-substituted-2-bromo/hydroxy phenyl)-1,2,4-triazine derivatives represented by the following structure, were synthesized with anticipation of improvement in the anticonvulsant activity with little toxicity (**Fig.1**).

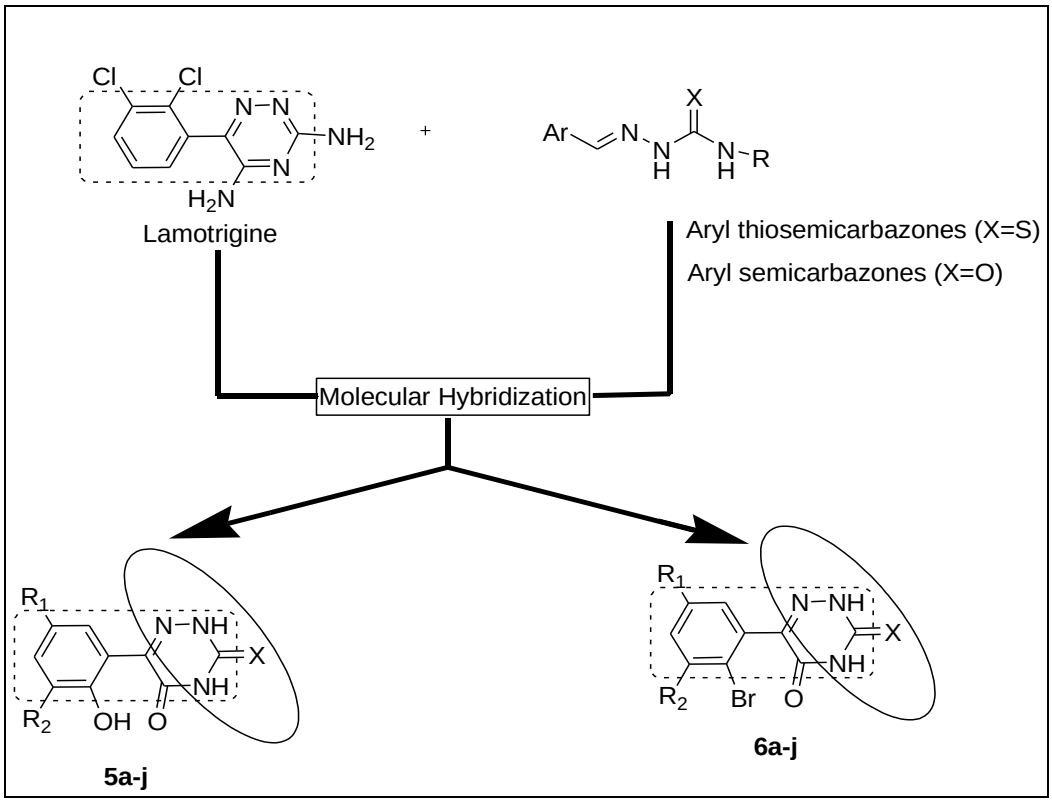


FIG.1: RATIONAL CONCEPT TO DESIGN NEW 6-(3,5-SUBSTITUTED-2-BROMO/HYDROXY PHENYL)-1,2,4-TRIAZINE DERIVATIVES (5a-j AND 6a-j).

MATERIAL AND METHODS:

Chemicals and solvents for synthesis were purchased by Merck (India), Spectrochem chemicals (India) and S.D. Fine Chemicals (India). Thin layer chromatography (TLC) was run throughout the reactions to optimize the reactions for purity and completion. Developing solvent for TLC was ethyl acetate and n-hexane (50 %). Iodine vapors or UV light was used as visualizing agents. Digital melting point apparatus was used for determine melting points of newly synthesized compounds, were uncorrected.

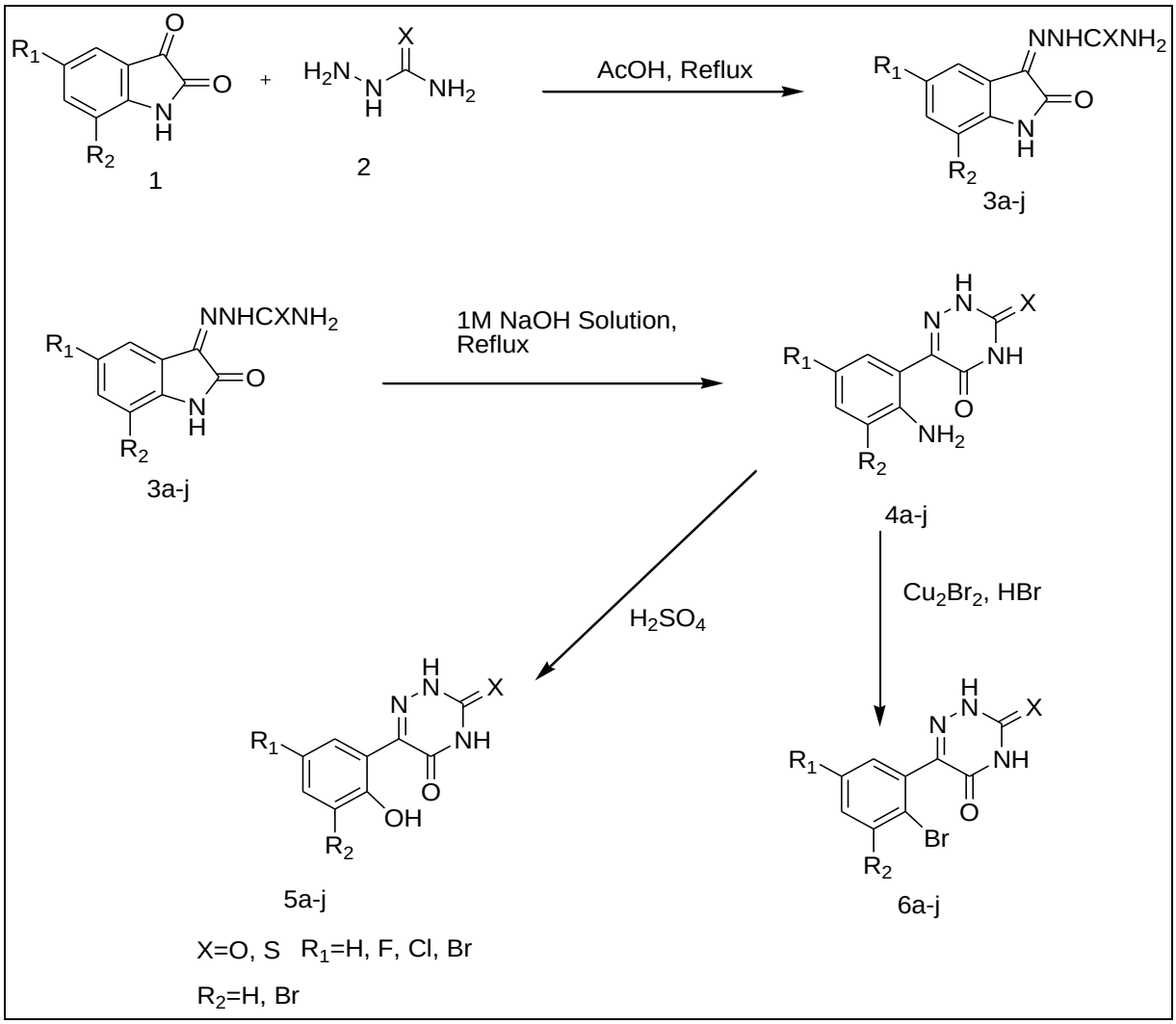
FT-IR (KBr) spectra of the synthesized compounds were recorded on a Nicolet 5PC FTIR spectrophotometer (λ-max in cm^{-1}) and ^1H NMR was observed on a Brucker Model-300 NMR Spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as the internal reference (chemical shifts in δ ppm). The details of physical properties of the synthesized compounds were presented in **Table 1**.

a. Synthesis of isatin semicarbazones or isatin thiosemicarbazones (**3a-j**): Semicarbazones and thiosemicarbazones (**3a-j**) were synthesized according to the procedure reported in literature.[11-12]

b. Synthesis of 6-(2-amino-3,5-substituted phenyl)-1,2,4-triazines (**4a-j**): 6-(2-Amino-3,5-substituted phenyl)-1,2,4-triazines (**4a-j**) were synthesized according to the procedure reported in literature.[11, 13]

c. General procedure for synthesis of 6-(3,5-substituted-2-hydroxy phenyl)-1,2,4-triazine derivatives (**5a-j**): 6-(3,5-Substituted-2-hydroxy phenyl)-1,2,4-triazine derivatives **5a-j** were

synthesized according to the procedure specified in literature with some modifications as shown in synthetic **Scheme 1**.[11] Appropriate triazines (**4a-j**, 0.6 mmol) were dissolved in sodium hydroxide solution (10%, 5 mL) with heating. After cooling sodium nitrite (0.8 mmol) was added, the mixture was cooled in an ice bath to 5 °C, it was added drop-wise to the cold solution of sulphuric acid (96%, 3.5 mL) and water (3.5 mL), and stirring in an ice bath was continued for 15 min. The mixture was filtered and boiled for 30 min. The precipitate was collected through filtration, washed with water and dried.



SCHEME I: FOR SYNTHESIS OF 6-(3,5-SUBSTITUTED-2-BROMO/HYDROXY PHENYL)-1,2,4-TRIAZINE DERIVATIVES (5a-j and 6a-j)

TABLE 1: PHYSICAL DATA OF COMPOUNDS 5a-j AND 6a-j

Code No.	R_1	R_2	X	Mol. For.	% yield	M.P.(^{0}C)	R_f
5a	H	H	O	$C_9H_7N_3O_3$	40	273-276	0.56
5b	H	H	S	$C_9H_7N_3O_2S$	38	240-242	0.61
5c	F	H	O	$C_9H_6FN_3O_3$	50	289-291	0.49
5d	F	H	S	$C_9H_6FN_3O_2S$	45	257-259	0.72
5e	Cl	H	O	$C_9H_6ClN_3O_3$	36	244-246	0.61
5f	Cl	H	S	$C_9H_6ClN_3O_2S$	48	228-230	0.69
5g	Br	H	O	$C_9H_6BrN_3O_3$	52	203-205	0.48
5h	Br	H	S	$C_9H_6BrN_3O_2S$	44	268-271	0.59
5i	Br	Br	O	$C_9H_5Br_2N_3O_3$	47	200-202	0.55
5j	Br	Br	S	$C_9H_5Br_2N_3O_2S$	61	146-148	0.73
6a	H	H	O	$C_9H_6BrN_3O_2$	46	212-15	0.39

6b	H	H	S	$C_9H_6BrN_3OS$	54	238-41	0.46
6c	F	H	O	$C_9H_5BrFN_3O_2$	38	194-97	0.52
6d	F	H	S	$C_9H_5BrFN_3OS$	42	230-33	0.36
6e	Cl	H	O	$C_9H_5BrClN_3O_2$	43	210-12	0.43
6f	Cl	H	S	$C_9H_5BrClN_3OS$	51	173-75	0.56
6g	Br	H	O	$C_9H_5Br_2N_3O_2$	48	255-58	0.62
6h	Br	H	S	$C_9H_5Br_2N_3OS$	55	172-74	0.47
6i	Br	Br	O	$C_9H_4Br_3N_3O_2$	61	190-92	0.52
6j	Br	Br	S	$C_9H_4Br_3N_3OS$	64	182-85	0.67

6-(2-hydroxyphenyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione 5a: IR (KBr): ν_{max} = 3431 ($OH_{Phenolic}$), 3224 ($NH_{Triazine}$), 1736 (C=O, Amide), 1610 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ =5.70 (br, s, 1H, OH), 6.68-7.24 (m, 4H, ArH), 12.13 (s, 1H, =NNH), 12.47 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 332.6 (M^++1). Anal. calcd for $C_9H_7N_3O_3$ (205.0487): C 52.67, H 3.41, N 20.46; found C 52.65, H 3.43, N 20.42.

3,4-dihydro-6-(2-hydroxyphenyl)-3-thioxo-1,2,4-triazin-5(2*H*)-one 5b: IR (KBr): ν_{max} = 3386 ($OH_{Phenolic}$), 3176 ($NH_{Triazine}$), 1733 (C=O, Amide), 1616 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ =5.34 (br, s, 1H, OH), 6.92-7.50 (m, 4H, ArH), 12.77 (s, 1H, =NNH), 14.61 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 222.3 (M^++1). Anal. calcd for $C_9H_7N_3O_2S$ (221.0259): C 48.84, H 3.21, N 18.97; found C 48.82, H 3.23, N 18.95.

6-(5-fluoro-2- hydroxyphenyl) - 1, 2, 4 -triazine-3,5(2*H*,4*H*)-dione 5c: IR (KBr): ν_{max} = 3453 ($OH_{Phenolic}$), 3226 ($NH_{Triazine}$), 1742 (C=O, Amide), 1605 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ =5.70 (br, s, 1H OH), 6.68-7.24 (m, 3H, ArH), 12.10 (s, 1H, =NNH), 12.48 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 223.1 (M^++1). Anal. calcd for $C_9H_6FN_3O_3$ (223.0393): C 48.46, H 2.69, N 18.80; found C 48.49, H 2.67, N 18.78.

6-(5-fluoro-2-hydroxyphenyl)-3, 4 – dihydro - 3-thioxo-1,2,4-triazin-5(2*H*)-one 5d: IR (KBr): ν_{max} = 3386 ($OH_{Phenolic}$), 3176 ($NH_{Triazine}$), 1730 (C=O, Amide), 1615 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ =5.34 (br, s, 1H, OH), 6.93-7.50 (m, 3H, ArH), 12.75 (s, 1H, =NNH), 14.65 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 240.2 (M^++1). Anal. calcd for $C_9H_6FN_3O_2S$ (239.0165): C 45.21, H 2.55, N 17.59; found C 45.23, H 2.52, N 17.61.

6-(5-chloro-2-hydroxyphenyl)-1, 2, 4 - triazine-3,5(2*H*,4*H*)-dione 5e: IR (KBr): ν_{max} = 3396 ($OH_{Phenolic}$), 3191 ($NH_{Triazine}$), 1741 (C=O, Amide), 1642 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ =5.79 (br, s, 1H, OH) 7.68-7.84 (m, 3H, ArH), 11.97 (s, 1H, NH), 12.79 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 240.2 (M^++1). Anal. calcd for $C_9H_6ClN_3O_3$ (239.0098): C 45.14, H 2.54, N 17.52; found C 45.16, H 2.57, N 17.50.

6-(5-chloro-2-hydroxyphenyl)- 3, 4 - dihydro-3-thioxo-1,2,4-triazin-5(2*H*)-one 5f: IR (KBr): ν_{max} = 3363 ($OH_{Phenolic}$), 3197 ($NH_{Triazine}$), 1733 (C=O, Amide), 1629 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ =5.54 (br, s, 1H, OH), 7.12-7.82 (m, 3H, ArH), 12.75 (s, 1H, =NNH), 14.11 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 256.6 (M^++1). Anal. calcd for $C_9H_6ClN_3O_2S$ (254.9869): C 42.26, H 2.41, N 16.41; found C 42.24, H 2.39, N 16.45.

6-(5-bromo-2-hydroxyphenyl)-1, 2, 4 - triazine - 3,5(2*H*,4*H*)-dione 5g: IR (KBr): ν_{max} = 3396 ($OH_{Phenolic}$), 3187 ($NH_{Triazine}$), 1741 (C=O, Amide), 1643 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ =5.21 (br, s, 1H, OH), 7.68-7.84 (m, 3H, ArH), 11.97 (s, 1H, NH), 12.76 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 285.6 (M^++1). Anal. calcd for $C_9H_6BrN_3O_3$ (282.9593): C 38.10, H 2.15, N 14.75; found C 38.12, H 2.13, N 14.77.

6-(5-bromo-2-hydroxyphenyl)-3, 4 - dihydro - 3-thioxo-1,2,4-triazin-5(2*H*)-one 5h: IR (KBr): ν_{max} = 3362 ($OH_{Phenolic}$), 3193 ($NH_{Triazine}$), 1739 (C=O, Amide), 1625 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ =5.12 (br, s, 1H, OH), 7.13-7.82 (m, 3H, ArH), 12.76 (s, 1H, =NNH), 14.08 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 300.6 (M^++1). Anal. calcd for $C_9H_7ClN_4OS$ (298.9364): C 36.06, H 2.08, N 14.02; found C 36.08, H 2.12, N 13.98.

6-(3,5-dibromo-2-hydroxyphenyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione 5i: IR (KBr): ν_{max} = 3367 ($OH_{Phenolic}$), 3167 ($NH_{Triazine}$), 1734 (C=O, Amide), 1638 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ =5.20 (br, s, 1H, OH), 6.79-7.15 (m, 2H, ArH), 12.33 (s, 1H, =NNH), 12.68 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 362.9 (M^++1). Anal. calcd for $C_9H_6Br_2N_4O_2$ (360.8698): C 29.82, H 1.41, N 13.24; found C 29.84, H 1.43, N 13.26.

6-(3,5-dibromo-2-hydroxyphenyl)-3, 4 - dihydro-3-thioxo-1,2,4-triazin-5(2*H*)-one 5j: IR (KBr): ν_{max} = 3357 ($OH_{Phenolic}$), 3123 ($NH_{Triazine}$), 1726 (C=O, Amide), 1608 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 5.10 (br, s, H, OH), 6.80-7.31 (m, 2 H, ArH), 12.88 (s, 1H, =NNH), 13.90 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 378.0 (M^++1). Anal. calcd for $C_9H_6Br_2N_4OS$ (376.8469): C 28.50, H 1.35, N 11.02; found C 28.48, H 1.37, N 11.05.

d. General procedure for synthesis of 6-(3, 5-substituted - 2 - bromophenyl) - 1, 2, 4 - triazine derivatives (**6a-j**):

6-(3,5-Substituted-2-bromophenyl)-1,2,4-triazine derivatives (**6a-j**) were synthesized according to the procedure specified in literature with some modifications as shown in synthetic Scheme 1.[11] Triazines (**4a-j**, 0.8 mmol) were dissolved in sodium hydroxide solution (10%, 5 mL) with heating. After cooling sodium nitrite (0.9 mmol) was added, the resultant mixture was cooled in an ice bath to 5 °C and added drop-wise to an ice cold mixture of hydrobromic acid (2.5 mL), water (2.5 mL) and toluene (2.5 mL). After stirring for 15 min, the resulting diazonium salt solution was carefully added to a solution of copper (I) bromide (0.5 mmol) in hydrobromic acid (48%, 5 mL) and water (5 mL). The mixture was stirred at room temperature overnight. The formed product was filtered, washed with water and dried.

6-(2-bromophenyl)-1,2,4-triazine-3, 5 (2*H*,4*H*) - dione 6a: IR (KBr): ν_{max} = 3168 ($NH_{Triazine}$), 1735 (C=O, Amide), 1620 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 6.78-7.14 (m, 4H, ArH), 12.32 (s, 1H, =NNH), 12.69 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 266.9 (M^++1). Anal. calcd for $C_9H_6BrN_3O_2$ (366.9643): C 40.30, H 2.23, N 15.55; found C 40.28, H 2.21, N 15.57.

6-(2-bromophenyl)-3,4-dihydro-3-thioxo -1, 2, 4-triazin-5(2*H*)-one 6b: IR (KBr): ν_{max} = 3129 ($NH_{Triazine}$), 1723 (C=O, Amide), 1631 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 6.82-7.34 (m, 4H, ArH), 12.83 (s, 1H, =NNH), 13.89 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 285.1 (M^++1). Anal. calcd for $C_9H_6BrN_3OS$ (282.9415): C 38.10, H 2.15, N 14.81; found C 38.12, H 2.16, N 14.83.

6-(2-bromo-5-fluorophenyl)-1, 2, 4- triazine -3, 5 (2*H*, 4*H*)-dione 6c: IR (KBr): ν_{max} = 3226 ($NH_{Triazine}$), 1739 (C=O, Amide), 1660 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 6.68-7.24 (m, 3H, ArH), 12.09 (s, 1H, =NNH), 12.43 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 286.1 (M^++1). Anal. calcd for $C_9H_5BrFN_3O_2$ (284.9549): C 37.77, H 1.78, N 14.72; found C 37.75, H 1.80, N 14.74.

6-(2-bromo – 5 - fluorophenyl)-3, 4-dihydro-3-thioxo-1,2,4-triazin-5(2*H*)-one 6d: IR (KBr): ν_{max} = 3171 ($NH_{Triazine}$), 1722 (C=O, Amide), 1610 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 6.96-7.57 (m, 3H, ArH), 12.71 (s, 1H, =NNH), 14.54 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 303.1 (M^++1). Anal. calcd for $C_9H_5BrFN_3OS$ (300.9321): C 35.76, H 1.65, N 13.93; found C 35.74, H 1.63, N 13.95.

6-(2-bromo-5-chlorophenyl)-1, 2, 4 - triazine- 3, 5(2*H*,4*H*)-dione 6e: IR (KBr): ν_{max} = 3185 ($NH_{Triazine}$), 1726 (C=O, Amide), 1640 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 7.68-7.86 (m, 3H, ArH), 11.96 (s, 1H, NH), 12.74 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 303.5 (M^++1). Anal. calcd for $C_9H_5BrClN_3O_2$ (238.6305): C 35.71, H 1.69, N 13.87; found C 35.70, H 1.71, N 13.86.

6-(2-bromo-5-chlorophenyl) - 3, 4- dihydro - 3-thioxo-1,2,4-triazin-5(2*H*)-one 6f: IR (KBr): ν_{max} = 3193 ($NH_{Triazine}$), 1735 (C=O, Amide), 1619 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 7.12-7.83 (m, 3H, ArH), 12.74 (s, 1H, =NNH), 14.10 (s, 1H, NH), ppm. Mass (ES+) *m/z*= 319.5 (M^++1). Anal. calcd for $C_9H_5BrClN_3OS$ (316.9025): C 33.90, H 1.60, N 13.21; found C 33.91, H 1.62, N 13.23.

6-(2,5-dibromophenyl)-1,2,4-triazine-3,5(2*H*,4*H*) - dione 6g: IR (KBr): ν_{max} = 3116 ($NH_{Triazine}$), 1726

(C=O, Amide), 1642 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 6.56 -7.27 (m, 3H, ArH), 12.18 (s, 1H, =NNH), 12.93 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 347.8 (M^++1). Anal. calcd for $C_9H_5Br_2N_3O_2$ (344.9629): C 31.20, H 1.42, N 12.13; found C 31.22, H 1.40, N 12.12.

6-(2,5-dibromophenyl)-3,4-dihydro – 3 - thioxo-1,2,4-triazin-5(2*H*)-one 6h: IR (KBr): ν_{max} = 3184 ($NH_{Triazine}$), 1736 (C=O, Amide), 1651 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 6.72-7.33 (m, 3H, ArH), 12.37 (s, 1H, =NNH), 14.10 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 364.2 (M^++1). Anal. calcd for $C_9H_5Br_2N_3OS$ (360.8520): C 29.80, H 1.41, N 11.55; found C 29.82, H 1.43, N 11.53.

6-(2,3,5-tribromophenyl)-1,2,4 – triazine - 3, 5 (2*H*,4*H*)-dione 6i: IR (KBr): ν_{max} = 3167 ($NH_{Triazine}$), 1730 (C=O, Amide), 1630 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) 6.75-7.15 (m, 2H, ArH), 12.38 (s, 1H, =NNH), 12.65 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 426.4 (M^++1). Anal. calcd for $C_9H_4Br_3N_3O_2$ (422.7854): C 25.40, H 0.98, N 9.85; found C 25.42, H 0.97, N 9.83.

6-(2,3,5-tribromophenyl)-3, 4 -dihydro-3-thioxo-1,2,4-triazin-5(2*H*)-one 6j: IR (KBr): ν_{max} = 3125 ($NH_{Triazine}$), 1724 (C=O, Amide), 1631 (C=N str) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ = 6.81-7.31 (m, 2H, ArH), 12.87 (s, 1H, =NNH), 13.97 (s, 1H, NH) ppm. Mass (ES+) *m/z*= 442.6 (M^++1). Anal. calcd for $C_9H_4Br_3N_3OS$ (438.7625): C 24.44, H 0.94, N 9.53; found C 24.42, H 0.95, N 9.55.

Pharmacology: Male albino mice (CF-1 strain, 18-25 g) were used in groups as experimental animals. The synthesized compounds and standard drug were suspended in polyethylene glycol, administered intraperitoneally. All experimental protocols were carried out with permission from the Institutional Animal Ethics Committee (838/ac/04/CPCSEA). Animals were obtained from the Central Animal House Facility, Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar, Ghaziabad, Uttar Pradesh, India.

a. Anticonvulsant screening: Synthesized compounds (**5a-j** and **6a-j**) were evaluated for their anticonvulsant activity by using maximal electroshock seizure (MES) model.[14-15] In MES screening, an electrical stimulus of 0.2 s in duration (50 mA at 60 Hz of alternating current) in mice was delivered by corneal electrodes. In anticonvulsant screening, each compound was administered intraperitoneal at three dose levels 30, 100, 300 mg/kg. In this test, anticonvulsant activity of the synthesized compounds was performed after 0.5 and 4h intervals after test sample administration. Protection against the spread of MES-induced seizures displayed by the abolition of the hind limb and tonic maximal extension component of the seizure.

b. Neurotoxicity screening: Neurological toxicity induced by a compound was detected in mice by the rotarod test.[16] The mice were trained to stay on rotating rotarod (3.2 cm rod diameter, 6 rpm speed). The motor impairment effect was observed by the inability of the animal to maintain equilibrium for a given time (1 min) in each of the trials.

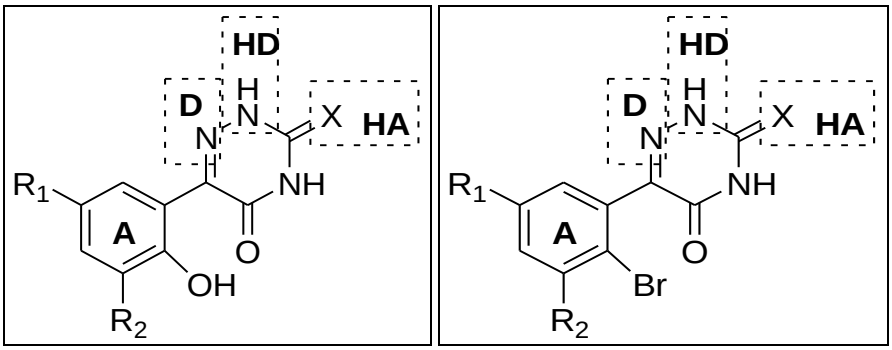
c. CNS depression study: The CNS depression effect of some anticonvulsant active compounds is observed by screening the immobility time of the mice during swimming phase following the Porsolt's swim pool test.[17] Mice were placed in chamber (height 20 cm, diameter 45 cm) containing water up to a height of 15 cm at 25±2° C. Two swim sessions were performed, an initial 15 min pre-test, followed by a 5 min test session 24 h later. Then test compounds were administered through an intraperitoneal route (100 mg kg^{-1}) in the trained animals, before 30 min the test session. The duration of passive floating without struggling was recorded during the 5 min test period as immobility time.

Computational study: (Calculation of physicochemical parameters): A computational study for prediction of ADME properties of titled compounds (**5a-j** and **6a-j**) was performed. The degree of absorption is expressed by the percentage of absorption. Percentage absorption (% ABS) was calculated by using: % ABS = 109-(0.345 × TPSA).[18] Violations of Lipinski's rule of five,[19] milog P, number of rotatable bonds, polar surface area (TPSA),[20] molecular volume, number of

hydrogen donor and acceptor atoms were computed by MolSoft software.[21] The drug-score, drug-likeness and theoretical toxicity risks were computed by Osiris Property Explorer.[22]

RESULT AND DISCUSSION:

a. Designing of the novel anticonvulsant compounds: Substitution on the aryl ring by halogens has been found to increase potency in the MES screen.[23] According to Unverferth et al. study[24] essential pharmacophoric elements that are necessary for good anticonvulsant activity through blocking of voltage-gated sodium channels are (i) aryl ring center or the lipophilic group (A), (ii) an electron donor atom (D), (iii) a hydrogen bond acceptor (HA) and (iv) a hydrogen bond donor (HD) unit (**Fig.2**). [25-26]



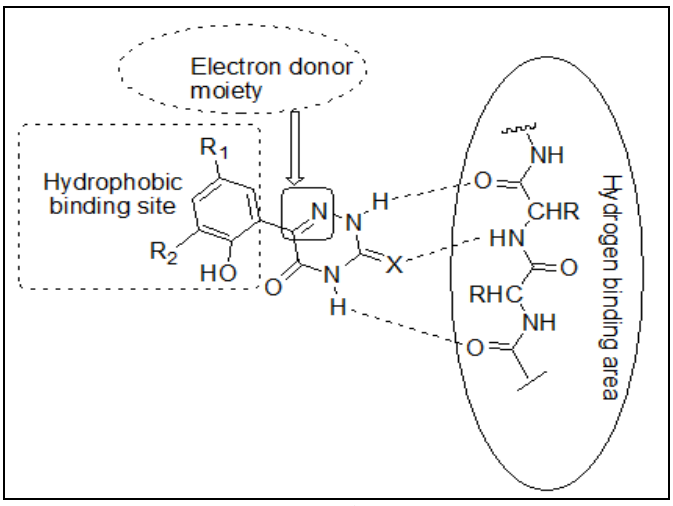
5a-j **6a-j**

FIG. 2: STRUCTURES OF PROPOSED GENERAL PHARMACOPHORE MODEL OF THE DESIGNED COMPOUNDS AND REPORTED MARKETED DRUGS.

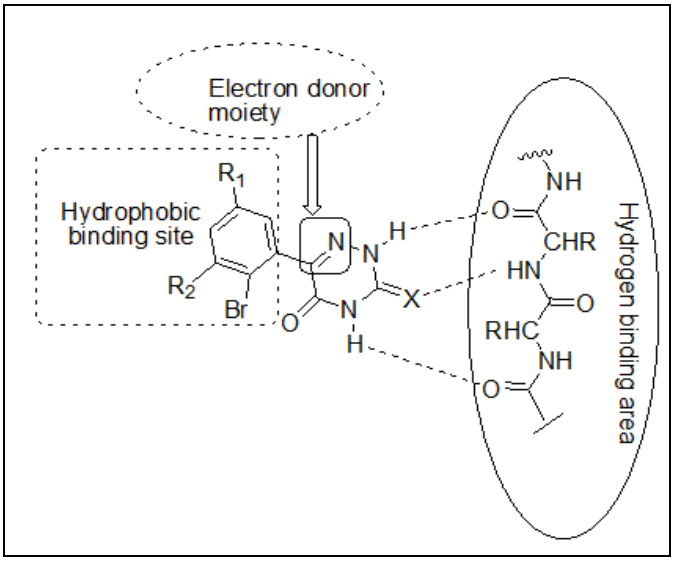
b. Anticonvulsant and CNS depressant activities: The MES model for generalized tonic-clonic seizure evaluation keyed out some clinical candidates that forbade seizure spread (**Table 2**). Amongst the plethora of the synthesized compounds, two compounds **5c** and **6i** displayed significantly active profile against the electrically induced seizures at a dose of 30 mg/kg after 0.5 h. At the same dose level, compound **6i** also showed activity after 4.0 h. Compounds **5j** and **6h** exhibited protection at the dose level of 100 mg/kg after 0.5 h. At 4.0 h, compounds **5e** and **6g** persisted to reveal anti-MES protection at the same dose, whereas, compounds **5b** and **6b** indicated to prevent the seizure spread at a higher dose of 300 mg/kg after 4h. Compound **6c** displayed protection at 300 mg/kg after 0.5 h. The remaining compounds were found to be inactive at the maximum administered dose of 300 mg/kg at both times (0.5 h and 4.0 h).

The neurotoxicity screening data revealed that two compounds **5a** and **6f** exhibited neurotoxicity at a dose level of 300 mg/kg after 0.5 h. Compounds **5f** and **6d** displayed neurotoxicity at a maximum dose level of 300 mg/kg after 4.0 h. whereas rest the compounds were without any neurotoxicity.

Significantly anticonvulsant active compounds (**5c, 6i** and **6j**) were further evaluated for their CNS depressant activity. With respect to control, screened compounds (**5c, 6i** and **6j**) displayed 40.59, 56.88 and 66.61 % increase in immobility time, where as standard drug Carbamazepine revealed 58.63 % increase in the immobility time (**Table 3**). Putative binding site theory used for postulating the interaction of anticonvulsant compounds at a specific binding site, the molecule observed to interact with the protein receptor as shown in **Fig. 3**. [1]



5a-j



6a-j

c. Prediction of ADME properties: Computation study of the all compounds showed that none of the titled compounds violated lipinski's rules and making them potentially promising agents for anticonvulsant therapy. All compounds exhibited a great % ABS ranging from 74.89 to 87.76% (**Table 4**). Positive drug likeness (1.06 to 5.22) and drug score (0.32 to 0.94) values of compounds were found to be similar or even better than some of the drugs currently used on the market [drug likeness (-0.88 to 4.20) and drug score (0.19 to 0.51) values] (**Table 5**). Theoretical toxicity risks data displayed that compounds (**5b**, **5d, 5f, 5h, 5j, 6b**, **6d, 6f, 6h** and **6j**) showed only mutagenic and reproductive effective toxicities.

TABLE 2: ANTICONVULSANT AND MOTOR IMPAIRMENT SCREENING OF SYNTHESIZED COMPOUNDS (5a-j and 6a-j) USING MAXIMAL ELECTROSHOCK SEIZURE AND ROTAROD MODELS.

Code No.	MES[a]		Motor Impairment[a]	
	0.5h	4.0h	0.5 h	4.0 h
5a	-	-	300	-
5b	-	300	-	-
5c	30	-	-	-
5d	-	-	-	-
5e	-	100	-	-
5f	-	-	-	300
5g	-	-	-	-
5h	-	-	-	-
5i	-	-	-	-
5j	100	-	-	-
6a	300	-	-	-
6b	-	300	-	-
6c	300	-	-	-
6d	-	-	-	300
6e	-	-	-	-
6f	-	-	300	-
6g	-	100	-	-
6h	100	-	-	-
6i	30	30	-	-
6j	-	30	-	-
Control	-	-	-	-
Phenytoin[b]	30	30	100	100
Carbamazepine[b]	30	100	100	300

[a]Doses of 30, 100, and 300 mg/kg were administered to albino mice through intraperitoneal route. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of mice. The dash (-) indicates an absence of activity at maximum dose administered (300 mg/kg). [b]Data of Phenytoin and Carbamazepine (reference drugs) were obtained from the references.[1, 27]

TABLE 3: DATA OF CNS DEPRESSANT ACTIVITY OF THE SELECTED COMPOUNDS (5c, 6i and 6j) PERFORMED IN MICE USING FORCED SWIM TEST.

Compound Code	Duration of immobility(sec) (mean ± SEM)	% Increased of immobility
5c	74.57±1.43	40.59
6i	83.21±1.12	56.88
6j	88.37±1.87	66.61

Carbamazepine	84.14±1.33	58.63
Control	53.04±2.47	-

The compounds were tested at a dose of 100 mg/kg. Each value represents the mean ± SEM of six mice. The CNS depressant effect was compared with respect to standard drug. *$p < 0.0001$. Data was analyzed by unpaired student's *t* test.

TABLE 4: PHARMACOKINETIC PARAMETERS IMPORTANT FOR GOOD ORAL BIOAVAILABILITY OF COMPOUNDS 5a-j and 6a-j.

Code No.	% ABS	TPSA (A^2)	n-ROTB	MW	MV	miLogP	n-OHNH donors	n-ON acceptors	Lipinski's Violations
Rule	-	-	-	<500	-	<5	<5	<10	<1
5a	74.898	98.844	1	205.173	167.252	0.193	3	6	0
5b	80.788	81.773	1	221.241	176.13	0.536	3	5	0
5c	74.898	98.844	1	223.163	172.183	0.333	3	6	0
5d	80.788	81.773	1	239.231	181.061	0.676	3	5	0
5e	74.898	98.844	1	239.618	180.788	0.847	3	6	0
5f	80.788	81.773	1	255.686	189.666	1.19	3	5	0
5g	74.898	98.844	1	284.069	185.137	0.978	3	6	0
5h	80.788	81.773	1	300.137	194.015	1.321	3	5	0
5i	74.898	98.844	1	362.965	203.023	1.716	3	6	0
5j	80.788	81.773	1	379.033	211.901	2.058	3	5	0
6a	81.877	78.616	1	268.07	177.119	1.222	2	5	0
6b	87.766	61.545	1	284.138	185.997	1.564	2	4	0
6c	81.877	78.616	1	286.06	182.051	1.361	2	5	0
6d	87.766	61.545	1	302.128	190.929	1.704	2	4	0
6e	81.877	78.616	1	302.515	190.655	1.876	2	5	0
6f	87.766	61.545	1	318.583	199.533	2.218	2	4	0
6g	81.877	78.616	1	346.966	195.005	2.007	2	5	0
6h	87.766	61.545	1	363.034	203.883	2.349	2	4	0
6i	81.877	78.616	1	425.862	212.89	2.744	2	5	0
6j	87.766	61.545	1	441.93	221.768	3.086	2	4	0
Lamotrigine	77.70	90.722	1	256.096	192.632	2.040	4	5	0
Phenytoin	88.92	58.196	2	252.273	223.886	2.178	2	4	0
Carbamazepine	92.43	48.028	0	236.274	215.083	2.840	2	3	0

% ABS, percentage of absorption; TPSA, topological polar surface area; n-ROTB, number of rotatable bonds; MW, molecular weight; MV, molecular volume; n-OHNH, number of hydrogen bond donors; n-ON, number of hydrogen bond acceptors; miLogP, logarithm of compound partition coefficient between n-octanol and water.

TABLE 5: DRUGLIKENESS, DRUG-SCORE AND IN SILICO TOXICITY RISKS OF TITLED COMPOUNDS 5a-j and 6a-j.

Code No.	Druglikeness	Drug-Score	Mutagenic	Tumorigenic	Irritant	Reproductive Effective
5a	5.22	0.94	1	1	1	1
5b	4.15	0.59	2	1	1	2
5c	4.19	0.91	1	1	1	1
5d	3.14	0.57	2	1	1	2
5e	5.91	0.89	1	1	1	1
5f	4.88	0.56	1	1	1	1
5g	3.74	0.69	2	1	1	1
5h	2.69	0.53	2	1	1	2
5i	3.74	0.74	1	1	1	1
5j	2.69	0.44	2	1	1	2
6a	1.66	0.78	1	1	1	1
6b	0.57	0.43	2	1	1	2
6c	2.15	0.77	1	1	1	1
6d	1.06	0.44	2	1	1	2
6e	3.87	0.75	1	1	1	1
6f	2.81	0.45	2	1	1	2
6g	3.49	0.71	1	1	1	1
6h	2.4	0.42	2	1	1	2

6i	3.88	0.56	1	1	1	1
6j	2.83	0.32	2	1	1	2
Phenytoin	4.20	0.19	3	3	1	3
Carbamazepine	2.80	0.23	3	1	1	3
Lamotrigine	-0.88	0.51	1	1	1	1

CONCLUSION: A computational study was also performed for the prediction of pharmacokinetic properties. Among the all screened compounds, compounds (**5c**), (**6i**) and (**6j**) revealed excellent protection against the electrically induced seizures at a dose of 30 mg/kg without neurotoxicity in rotarod test. Furthermore, all compounds also did not show Lipinski's violation, making them potentially promising agent for epilepsy treatment.

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