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Synthesis, Characterization and Anticonvulsant Activity of Some 1, 2, 4-Triazinone Derivatives

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ABSTRACT

This presented study aims to design the drugs which reduce various types of side effect and toxicity and also give the potent anticonvulsant activity. Epilepsy is a central nervous system (CNS) disorder characterized by the existence of more than one epileptic seizure which is associated with neurobiological, cognitive, psychological, and social disturbances. The medicine used to treat epilepsy in the past had various side effects such as ataxia, drowsiness, gastrointestinal disturbances etc. Owing to these side effects, we had an interest in the development of anticonvulsant drugs. To design and synthesize some N-containing heterocyclic moieties i.e. triazinone derivatives which may enhance the anticonvulsant activity of compounds to some extent. A series of 2-(2-amino-3-phenylpropanoyl)-5-((1, 5-disubstituted-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one derivatives were designed and synthesized using the appropriate synthetic route and keeping all the view in the structural requirements of pharmacophoric pattern and evaluated for anticonvulsant activity and CNS activities.

Keywords: *Triazinone, derivatives, synthesis, MES-screening, anticonvulsant, activity*

INTRODUCTION

Epilepsy is defined as a group of symptoms originating from dysfunction of the brain neurons that is characterized by recurrent seizures produced by peroxisomal excessive neuronal discharges [1, 2].

It is estimated that unresponsiveness to antiepileptic drugs has been found in about 25% of the epileptic population. Conventional antiepileptic agents exhibit an unfavorable side effect profile and fail to adequately control seizures. Hence, there is an urgent need to develop novel antiepileptic agents.

Triazinone is a nitrogen-containing heterocyclic compound. Triazinone derivatives continued to attract widespread interest for a long time due to their diverse biological activities as anticancer [3-5], antiulcer [6], anti-inflammatory [7], antimicrobial [8-10], antiviral [11], treatments of CNS disorders [12-13], antimalarial [14-15] and others [16-17]. Based on the knowledge of pharmacophoric pattern for anticonvulsant activity,

We designed and synthesized 2-(2-amino-3-phenylpropanoyl)-5-((1, 5-disubstituted-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one derivatives as a part of the refinement of lamotriazine, an aromatic amino acid triazine derivatives.

All the synthesized titled compounds have been fulfilling all the essential pharmacophoric criteria for anticonvulsant activity [18]. The fundamental structural features which could be responsible for interaction with the active site of ion channel are

a) The hydrophobic groups

b) Electron donor group

c) Hydrogen donor or acceptor group. Keeping all these into consideration, we tried to develop and explore potential anticonvulsant agents.

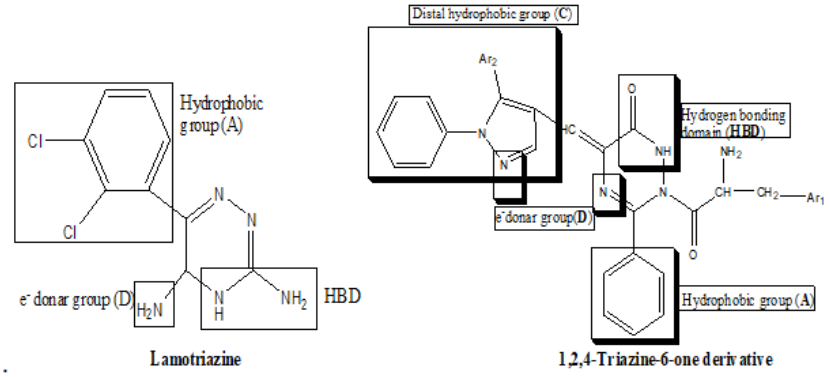
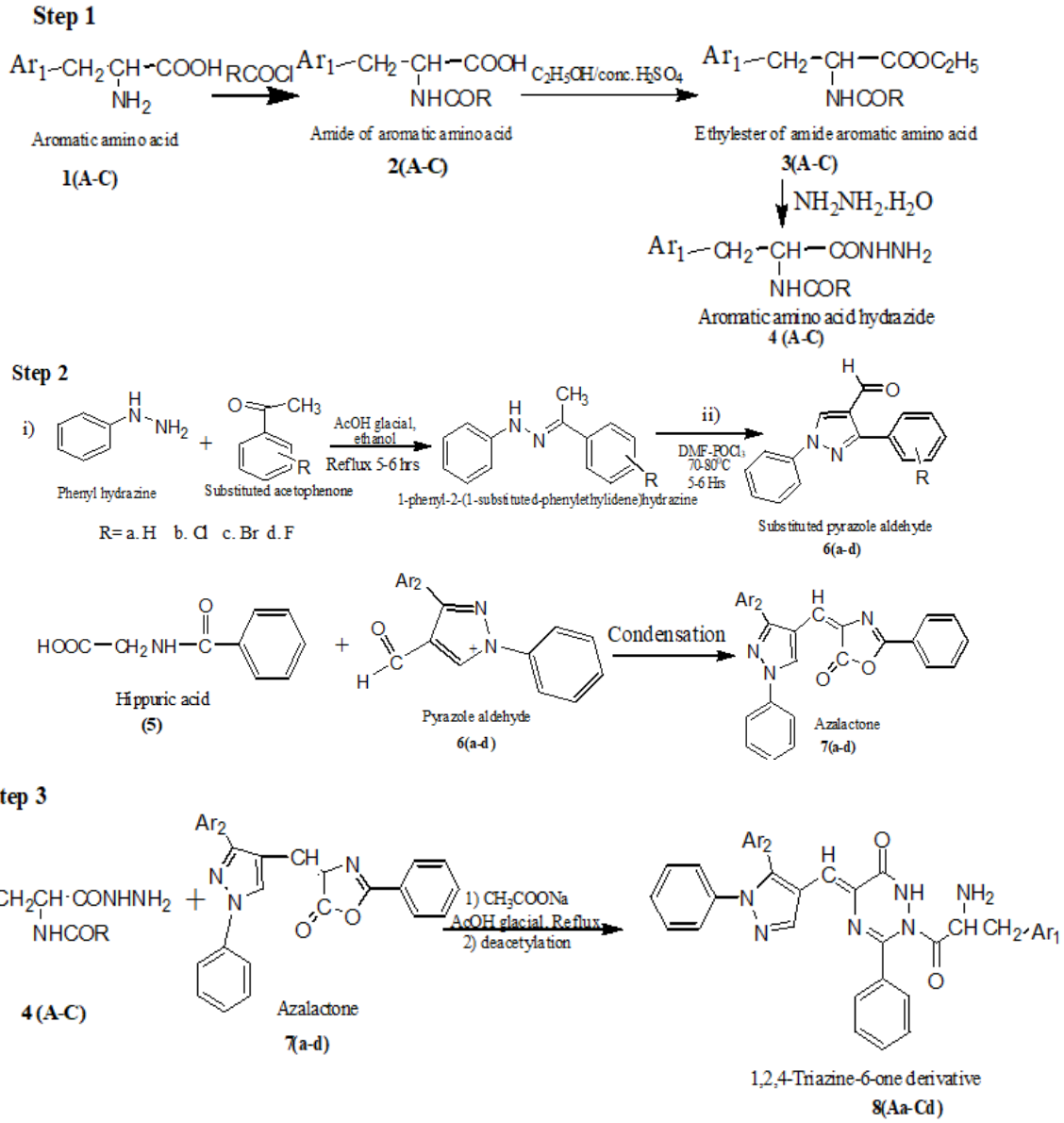


Figure 1: Essential pharmacophoric features for the anticonvulsant activity of synthesized Triazinone derivatives.

Owing to pharmacophoric model of lamotriazine as an anticonvulsant agent, we had found that the synthesized compounds showing all essential pharmacophoric features are delineated in (Figure 1) which could be responsible for interaction with the active site on voltage-gated ion channels. It contains a hydrophobic aryl ring (A), hydrogen bonding domain (HBD) and e^- donor group (D). There is another distal hydrophobic group (C) attached proximally to the aryl ring found to increase the van der wall's interaction within the receptor and increase the potency of any compounds in Figure 2.

MATERIALS AND METHODS

Chemistry



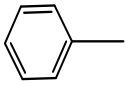
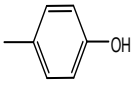
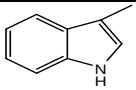
Comp.	8Aa	8Ab	8Ac	8Ad	8Ba	8Bb	8Bc	8Bd	8Ca	8Cb	8Cc	8Cd
Ar_1												
Ar_2	C_6H_5-	p-Cl-C_6H_4-	p-Br-C_6H_4-	p-F-C_6H_4-	C_6H_5-	p-Cl-C_6H_4-	p-Br-C_6H_4-	p-F-C_6H_4-	C_6H_5-	p-Cl-C_6H_4-	p-Br-C_6H_4-	p-F-C_6H_4-

Figure 2: Synthesis of 1, 2, 4-Triazine-6-one derivatives (8Aa-Cd)

General Procedure for blocking of free amino group 2(A-C)

Firstly, tookthe mixture of aromatic amino acid 0.05 mol and zinc dust in acetic acid (60 ml) in a 250 ml round bottom flask. The reaction mixture was gently heated on a water bath for about 2 hours. Then the reaction mixture was poured into a 500 ml beaker containing cold water (200 ml). It was stirred vigorously while cooling down the mixture. The acetyl derivative was separated as solid and collected the crystal of acetyl derivative by filtration. The solid crystals were washed with water, then dried it and recrystallized by methanol [19].

General Procedure for nucleophilic substitution as ester 3(A-C)

Aromatic amino acids 1(a-c) were converted to its ester by using concentrated H_2SO_4in absolute ethanol. The mixture of aromatic amino acid, absolute ethanol and concentrated $H_2SO_4$was refluxed on a water bath for 10-12 hours. After this, half of the alcohol was distilled off on the water bath and diluted the residue with a sufficient quantity of water. Then separated the upper layer of crude ester and extracted the lower aqueous layer with ether. After that, crude amino acid ester and ethereal extract were combined and washed with water, then with saturated sodium bicarbonate until effervescence ceased and finally washed with water. Dried the amino acid ester with anhydrous sodium sulphate and removed the ether on a water bath

General Procedure for the synthesis of Amino acid hydrazide 4(A-C)

The hydrazine hydrate was added in an alcoholic solution of aromatic amino acid ester 3(a-c). The reaction mixture was refluxed on a water bath for 10-12 hours. Distilled off the excess alcohol from the mixture and cooled it. The solid crystals of amino acid hydrazide were separated and collected by filtration. The product was recrystallized from ethanol.

General Procedure for the synthesis of 1-Phenyl-2-(1-substituted-phenylethylidene) hydrazine

Dissolved 0.25 mol of substituted acetophenone in 100 ml of ethanol into 500 ml round bottom flask. To the above flask added 0.25 mol of phenyl hydrazine with 2 ml of glacial acetic acid and refluxed the reaction mixture for 5-6 hours. The progress of the reaction was analyzed by TLC method using silica gel G as the stationary phase and toluene: ethyl acetate (7:3) as a mobile phase. After the completion of the reaction, the reaction mixture was cooled to room temperature and solid crystals were obtained. The product was separated by filtration, washed with ethanol and dried to give substituted acetone phenyl hydrazine.

General Procedure for the synthesis of substituted pyrazole aldehyde 5(a-d)

Taken 50 ml of dry DMF into 500 ml flat bottom flask and then 6 ml of phosphorus oxychloride ($POCl_3$) added drop wise under stirring at 0-5^{0}C. After complete addition of phosphorus oxychloride ($POCl_3$), the reaction mixture was stirred at this temperature for 15- 20 minutes. In the above mixture 0.05 mol of freshly prepared acetophenone hydrazone was added and heated on the water bath for 5-6 hours. The succession of the reaction was monitored by TLC method using silica gel G as the stationary phase and toluene: ethyl acetate (7:3) as a mobile phase. After completion of the reaction, the reaction mixture was cool to room temperature and then poured on crushed ice. The solid crystals were separated and collected by filtration. The product was washed with cold water to remove acidic impurity. It was dried and recrystallized from DMF- Methanol to obtain pure substituted pyrazole aldehyde [20-23].

Weighed accurately 0.5 mol of pyrazole aldehyde, 0.5 mol of hippuric acid, 1.5 mol of acetic anhydride and anhydrous sodium acetate and transferred into 500 mL conical flask. The reaction mixture was heated on an electric hot plate with constant shaking. Instantly the mixture was completely liquefied. Later on, the flask was transferred to a water bath and heater for 2-3 hours. In the above flask mixture, 200 mL of ethanol added slowly and stand the mixture for overnight. Filtered off the product and washed with boiling water and then dried it.

General Procedure for the synthesis of Triazine-6 one derivatives (8Aa-Cd)

Transferred a mixture of azlactone (0.05 mol), amino acid hydrazide (0.05 mol) and sodium acetate (10 gm) in glacial acetic acid in a 250 mL round bottom flask and refluxed it for 8-10 hours. After completion of the reaction, the reaction mixture was poured into crushed ice with continuous stirring. Filtered off the solid product, washed with water and dried it. The synthesized triazinone derivatives were recrystallized from ethanol.

Determination of Log P-value

The partition coefficient of synthesized triazinone derivatives was determined by using octanol and aqueous at room temperature [24].Transferred 10 mg of triazinone derivative into a glass stoppered graduated tube containing 20 mL of octanol and 20 mL phosphate buffer. On the mechanical shaker, the mixture was shaken for 24 hours. Latterly, the mixture was transferred into a separating funnel and allowed to settle for 6 hours. The phosphate buffer and octanol phase was separated and filtered through membrane filtration. The drug content in the phosphate buffer media was analyzed by UV spectroscopy. The phosphate buffer phase carefully diluted with deionized water using pipettes, test tubes and the graduated cylinders to obtain a series of different, but known concentrations to create a calibration curve. The curve establishes a relationship between phosphate buffer concentration and absorbance measured by UV spectroscopy. A sample of each dilution series was pipetted into a cuvette and placed in the UV spectrophotometer. The instrument was set to a wavelength of 268 nm. A cuvette with deionized water was used as a blank to zero the spectrophotometer before measurement of each sample.

Physical and Spectral data of synthesized compounds

2-(2-amino-3-phenylpropanoyl)-5-((1, 5-diphenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Aa)

$C_{34}H_{28}N_6O_2$ (552.63),**mp** (^{0}C) 148-150; **% Yield** 74;**log P**2.97; **IR *v*-max (cm^{-1}):**1756 (amide C=O), 1739 (lactam C=O), 3350 (triazine N-N), 3091 (triazine N-H,), 3285 (1^0 amine N-H),1602 (triazine C=N), 3392 (pyrazole N-N),1639 (pyrazole C=N) 2872 (C-H), 1472 (C-H), 974 (C-C), 2914 (Ar C-H), 1729 (Ar C=C);**^{1}H NMR (400 MHz,DMSO-*d*$_6$) (δ, ppm):** 8.03 (s, 1H, sec. CONH), 8.30(d, 2H, NH_2), 8.62(s, 1H, 1-pyrazole), 7.64-7.94 (m, 4H, Ar-H),7.30-7.42 (m, 4H, Ar-H), 7.12-7.38 (m, 4H, Ar-H), 7.44-7.54 (m, 4H, Ar-H), 7.91 (s, 1H, =CH), 3.70 (CH methine), 3.0-3.37 (CH_2 methylene); **$_{13}$C-NMR (20989.8 Hz, $CDCl_3$): δ**143.20, 139.52, (pyrazole),165.73 (amide), 107.57(ethylene), 56.25(aliphatic -CH), 41.23 (aliphatic –CH_2),130.89,132.32, 133.02, 136.63, 139.52, 127.53, 128.64 (benzene) **Mass spectra:** M^+ (552.23), M+2 (554.18);**Elemental analysis:** C- 73.95%, H- 4.96%, N-15.61%, O-5.89%.

2-(2-amino-3-phenylpropanoyl)-5-((5-(4-chlorophenyl)-1-phenyl-^{1}H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Ab)

$C_{34}H_{26}N_6O_2Cl$ (587.07),**mp** (^{0}C) 169-171; **% Yield** 88;**log P**4.53; **IR *v*-max (cm^{-1}):**1747 (amide C=O), 1695 (lactam C=O), 3318

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(δ,ppm):8.02 (s, 1H, sec. CONH), 8.30(d, 2H, NH_2), 8.73(s, 1H, 1-pyrazole), 7.64-7.94 (m, 4H, Ar-H), 7.55-7.85 (d, 2H, p-Cl-Ar-H),7.33-7.44 (m, 4H, Ar-H), 7.52-7.86 (m, 4H, Ar-H), 7.57 (s, 1H, =CH), 3.43 (CH methine), 3.12-3.41 (CH_2 methylene); **Elemental analysis:** C- 69.44%, H- 4.85%, N-14.34%, O-5.35%, Cl-5.96%.

2-(2-amino-3-phenylpropanoyl)-5-((5-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Ac)

$C_{34}H_{26}N_6O_2Br$ (631.52),**mp** (^{0}C) 184-186; **% Yield** 84;**log P**3.80; **IR *ν*-max (cm^{-1}):**1730 (amide C=O), 1713 (lactam C=O), 3369 (triazine N-N), 3134 (triazine N-H,), 3277 (1^0 amine N-H),1610 (triazine C=N), 3390 (pyrazole N-N),1642 (pyrazole C=N), 2810 (C-H), 1504 (C-H), 1022 (C-C), 2914 (Ar C-H), 1689 (Ar C=C), 852 (para substituted Ar C-H), 756 (Ar C-Br); **Elemental analysis:** C- 64.56%, H- 4.65%, N-13.43%, O-5.06%, Br-11.96%.

2-(2-amino-3-phenylpropanoyl)-5-((5-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1,2-dihydro-1, 2, 4-triazin-6(5H)-one (8Ad)

$C_{34}H_{26}N_6O_2F$ (570.62), **mp** (^{0}C) 197-199; **% Yield** 82;**log P**3.13; **IR *ν*-max (cm^{-1}):**1734 (amide C=O), 1682 (lactam C=O), 3362 (triazine N-N), 3036 (triazine N-H,), 3261 (1^0 amine N-H),1689 (triazine C=N), 3395 (pyrazole N-N),1580 (pyrazole C=N), 2853 (C-H), 1455 (C-H), 1012 (C-C), 2923 (Ar C-H), 1590 (Ar C=C), 879 (para substituted Ar C-H), 1379 (Ar C-F); **Elemental analysis:** C- 71.56%, H- 4.94%, N-14.76%, O-5.60%, F-3.34%.

2-(2-amino-3-(4-hydroxyphenyl) propanoyl)-5-((1, 5-diphenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Ba)

$C_{34}H_{28}N_6O_3$ (568.62), **mp** (^{0}C) 204-206; **% Yield** 76;**log P**2.59; **IR *ν*-max (cm^{-1}):**1732 (amide C=O), 1673 (lactam C=O), 3383 (triazine N-N), 3074 (triazine N-H,), 3242 (1^0 amine N-H),1622 (triazine C=N), 3473 (pyrazole N-N),1567 (pyrazole C=N) 2860 (C-H), 1496 (C-H), 1020 (C-C), 2815 (Ar C-H), 1593 (Ar C=C), 3664 (Ar O-H), 1486 (Ar C-O); **Elemental analysis:** C- 71.69%, H- 5.13%, N-14.63%, O-8.43%.

2-(2-amino-3-(4-hydroxyphenyl) propanoyl)-5-((5-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Bb)

$C_{34}H_{27}N_6O_3Cl$ (603.07),**mp** (^{0}C) 224-226; **% Yield** 72;**log P**3.27; **IR *ν*-max (cm^{-1}):**1716 (amide C=O), 1708 (lactam C=O), 3360 (triazine N-N), 3004 (triazine N-H,), 3258 (1^0 amine N-H),1645 (triazine C=N), 3415 (pyrazole N-N),1609 (pyrazole C=N) 2841 (C-H), 1431 (C-H), 1092 (C-C), 2776 (Ar C-H), 1686 (Ar C=C), 3623 (Ar O-H), 1419 (Ar C-O), 903 (para substituted Ar C-H), 685 (Ar C-Cl); **Mass spectra:** M^+(602.18), M+2 (604.23). **Elemental analysis:** C- 67.60%, H- 4.57%, N-13.86%, O-7.95%, Cl-5.87%.

2-(2-amino-3-(4-hydroxyphenyl) propanoyl)-5-((5-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Bc)

$C_{34}H_{27}N_6O_3Br$ (647.52), **mp** (^{0}C) 195-197; **% Yield** 83;**log P**3.41; **IR *ν*-max (cm^{-1}):**1784 (amide C=O), 1721 (lactam C=O), 3375 (triazine N-N), 2929 (triazine N-H,), 3237 (1^0 amine N-H),1651 (triazine C=N), 3464 (pyrazole N-N),1625 (pyrazole C=N) 2858 (C-H),

1457 (C-H), 1045 (C-C), 2825 (Ar C-H), 1668 (Ar C=C), 3653 (Ar O-H), 1456 (Ar C-O), 957 (para substituted Ar C-H), 791 (Ar C-Br); **^{1}H**

NMR (400 MHz, DMSO-d_6) (δ, ppm): 8.97(s, 1H, OH-Ar), 8.0 (s, 1H, sec. CONH), 8.17 (d, 2H, NH_2), 8.64 (s, 1H, 1-pyrazole), 6.98-7.30 (m, 4H, Ar-H), 7.44-7.49 (d, 2H, p-Br- Ar-H), 7.39-7.67 (m, 4H, Ar-H), 7.51-7.83 (m, 4H, Ar-H), 7.85 (s, 1H, =CH), 4.53 (CH methine), 3.47-3.72 (CH_2 methylene); **Elemental analysis:** C- 62.87%, H- 4.43%, N-12.88%, O-7.52%, Br-12.34%.

2-(2-amino-3-(4-hydroxyphenyl) propanoyl)-5-((5-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Bd)

$C_{34}H_{27}N_6O_3F$ (586.61),**mp** (^{0}C) 230-232; **% Yield** 65;**log P**2.74; **IR *ν*-max (cm^{-1}):**1771 (amide C=O), 1740 (lactam C=O), 3343 (triazine N-N), 3052 (triazine N-H,), 3260 (1^0 amine N-H),1679 (triazine C=N), 3450 (pyrazole N-N),1667 (pyrazole C=N), 2883 (C-H), 1482 (C-H), 1004 (C-C), 2892 (Ar C-H), 1640 (Ar C=C), 3634 (Ar O-H), 1478 (Ar C-O),972 (para substituted Ar C-H), 1309 (Ar C-F); **^{1}H NMR (400 MHz, DMSO-d_6) (δ, ppm):** 9.56 (s, 1H, OH-Ar), 8.05 (s, ^{1}H, sec. CONH), 8.18 (d, 2H, NH_2), 8.67 (s, ^{1}H, 1-pyrazole), 6.94-7.30 (m, 4H, Ar-H), 7.33-7.79 (d, 2H, p-F- Ar-H), 7.52-7.60 (m, 4H, Ar-H), 7.57-8.08 (m, 4H, Ar-H), 7.55 (s, ^{1}H, =CH), 3.44 (CH methine), 3.21-3.25 (CH_2 methylene); **Elemental analysis:** C- 69.45%, H- 4.73%, N-14.36%, O-8.18%, F-3.24%.

2-(2-amino-3-(^{1}H-indol-3-yl) propanoyl)-5-(1,5-diphenyl-1H-pyrazol-4-yl)methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Ca)

$C_{36}H_{29}N_7O_2$ (591.66),**mp** (^{0}C) 185-187; **% Yield** 76;**log P** 2.52; **IR *ν*-max (cm^{-1}):**1760 (amide C=O), 1735 (lactam C=O), 3323 (triazine N-N), 3303 (triazine N-H,), 3271 (1^0 amine N-H),1657 (triazine C=N), 3465 (pyrazole N-N),1640 (pyrazole C=N) 2883 (C-H), 1455 (C-H), 1087 (C-C), 2818 (Ar C-H), 1664 (Ar C=C), 3364 (indole NH); **^{1}H NMR (400 MHz, DMSO-d_6) (δ, ppm):** 10.69 (s, ^{1}H, 3-indole), 6.75-7.30(m, 4H, indole-C), 8.03 (s, ^{1}H, sec. CONH), 7.96 (d, 2H, NH_2), 8.06 (s, 1H, 1-pyrazole), 7.25-7.77 (m, 4H, Ar-H), 7.55-7.69 (m, 4H, Ar-H), 7.42-7.49 (m, 4H, Ar-H), 7.57 (s, ^{1}H, =CH), 3.41 (CH methine), 3.08-3.36 (CH_2 methylene); **Elemental analysis:** C- 72.98%, H- 5.13%, N-16.32%, O-5.38%.

2-(2-amino-3-(^{1}H-indol-3-yl) propanoyl)-5-((5-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1,2-dihydro-1, 2, 4-triazin-6(5H)-one (8Cb)

$C_{36}H_{29}N_7O_2Cl$ (626.11),**mp** (^{0}C) 210-212; **% Yield** 70;**log P** 3.07; **IR *ν*-max (cm^{-1}):**1760 (amide C=O), 1742 (lactam C=O), 3325 (triazine N-N), 3047 (triazine N-H,), 3231 (1^0 amine N-H),1672 (triazine C=N), 3415 (pyrazole N-N),1651 (pyrazole C=N) 2852 (C-H), 1458(C-H), 1021(C-C), 2917 (Ar C-H), 870 (para substituted Ar C-H) 1614 (Ar C=C), 658 (Ar C-Cl), 3362 (indole NH); **Elemental analysis:** C- 68.92%, H- 4.68%, N-15.45%, O-5.18%, Cl- 5.63%.

2-(2-amino-3-(^{1}H-indol-3-yl) propanoyl)-5-((5-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Cc)

$C_{36}H_{28}N7O_2Br$ (670.56), **mp** (0C) 235-237; **% Yield** 81;**log P** 3.34; **IR *ν*-max (cm^{-1}):**1745 (amide C=O), 1715 (lactam C=O),

3351 (triazine N-N), 3064 (triazine N-H,), 3270 (10 amine N-H),1649 (triazine C=N), 3493 (pyrazole N-N),1688 (pyrazole C=N) 2850 (C-H), 1475(C-H), 1074 (C-C), 2921(Ar C-H), 1598 (Ar C=C), 895 (para substituted Ar C-H), 763 (Ar C-Br), 3389 (indole NH); **^{1}H NMR (400 MHz, DMSO-$_{d6}$) (δ, ppm):** 10.69 (s, ^{1}H, 3-indole), 6.74-7.30 (m, 4H, indole-C), 8.0 (s, 1H, sec. CONH), 8.54 (d, 2H, NH_2), 8.16 (s, 1H, 1-pyrazole), 7.42-7.65 (m, 4H, Ar-H), 7.52-7.84 (m, 4H, Ar-H), 7.68-7.71 (d, 2H, p-Br- Ar-H) , 7.59 (s, ^{1}H, =CH), 3.41 (CH methine),

3.16-3.34 (CH2 methylene); **Elemental analysis:** C- 64.36%, H- 4.39%, N-14.54%, O-4.76%, Br-11.86%.

2-(2-amino-3-(^{1}H-indol-3-yl) propanoyl)-5-((5-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one (8Cd)

$C_{36}H_{28}N_7O_2F$ (609.65), **mp** (^{0}C) 245-247; **% Yield** 75;**log P**2.67; **IR *ν*-max (cm−1):**1737 (amide C=O), 1732 (lactam C=O), 3374 (triazine N-N), 3065 (triazine N-H,), 3282 (1^0 amine N-H),1657 (triazine C=N), 3482 (pyrazole N-N),1598 (pyrazole C=N) 2916 (C-H), 1467(C-H), 1087 (C-C), 2827 (Ar C-H), 1672 (Ar C=C), 943 (para substituted Ar C-H), 1347 (Ar C-F), 3394 (indole NH); **Elemental analysis:** C- 70.78%, H- 4.83%, N-15.94%, O-5.27%, F-3.16%.

Pharmacological studies

Acute Toxicity Studies (LD_{50})

The acute toxicity of synthesized triazinone derivatives was determined by using Wistar Albino rats weighing 100-150 gm. The animals were fasted for 24 hours before experimenting and up and down method (OECD Guideline No. 425) of CPCSEA were used for the acute toxicity studies. The acute toxicity is involved in the estimation of LD_{50}. A maximum dose administered up to 1000mg/kg has been tested for mortality. The visual observations while acute toxicity studies included skin changes, mobility, aggressiveness, sensitivity to sound and pain, excitation, tremors as well as respiratory movements and number of survivors was noted after 24 hours. Also, the animals are observed for next 14 days where their weights were recorded. The LD_{50} was then determined at the end of the experiment. From that one dose is selected, 100 mg/kg which is $1/10^{th}$ of the LD_{50} value. After the test, the animal was the sole occupant of the cage with free access to food and water during the observation period of 1-2 hours and thereafter at intervals. The animals which were dead during the experimental work were used for in-vitro studies in other institutional experiments to avoid the sacrificing of live animals.

Anticonvulsant activity

Study of anticonvulsant activity of triazinone derivatives against MES induced convulsions in adult Wistar albino rats.In the present study, MES is selected [25, 26and 27].In MES convulsions, electroshock is applied through the ear electrode. The MES convulsions consist of five phases: (i) tonic flexion (ii) tonic extension (iii) clonic convulsion (iv)stupor (v) recovery / death.A substance is known to acquire anticonvulsant property if it reduces or abolishes the extensor phase of MES convulsions. This procedure may be useful to produce convulsions both in rats and mice.

The male adult Wistar albino rats of weighing 100-150 gm were procured from the animal house (CPCSEA Approved). They were housed in microloan boxes with the standard diet of animals and water ad libitum. The study was conducted after obtaining clearance from the institutional animal ethical committee. Anticonvulsant activity performed under the conscious stage of the animal so no anaesthesia was required in this research work.

Weighed and numbered the animals. Divided them into fourteen groups and each group consist of 6 rats: a) one group is used as the control b) one group for standard drug Phenytoin and c) other groups are for test compounds.A suspension of the test compound was prepared by taking 100 mg in 1% CMC solution (10 mL) to get the dose of 10 mg/mL.

In the control group of test animals, ear electrode was placed on the ears of rats and 150 mA current was applied for 0.2 seconds. On analyzing the result of an anticonvulsant effect, normal response was recorded on test animals of the control group.Phenytoin was administered to the standard group by intraperitoneally with a dose of 30 mg/kg. The test compounds were administered similarly to the other group by intraperitoneally. As the drugs are insoluble in water, CMC was employed for the suspension preparation of test compounds.

At the end of 1 hour, the animals were subjected to electro convulsions. After experimentation, all the animals recovered soon and they were kept in a separate cage with proper care under the supervision of expertiseperson. In this research work no need to use any euthanization method. The time for different responses was noted. The readings are tabulated in Table 1.

Table 1: Anticonvulsant activity of 2-(2-amino-3-phenylpropanoyl)-5-((1, 5-disubstituted-^{1}H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one derivatives by MES induced convulsions in rats**.**

Comp.	Flexion Phase (Seconds)	Extensor Phase (Seconds)	Clonus Phase (Seconds)	Stupor Phase (Seconds)
Control	7.66 ± 0.581	18 ± 0.874	34.66 ± 1.27	112 ± 0.66
Phenytoin	2 ± 0.56***	0.67 ± 0.33***	15.5 ± 0.76***	71.63 ± 0.86***
8Aa	3.4 ± 0.57^{ns}	4.2 ± 0.66***	18.26 ± 0.336***	82.66 ± 0.71**
8Ab	4.76 ± .45^{ns}	6.2 ± 0.89***	30.83 ± 0.44^{ns}	88 ± 0.38***
8Ac	3.9 ± 0.47^{ns}	5.33 ± 0.49***	26.33±0.57***	79.5 ± 1.52***
8Ad	5.66 ± 0.88^{ns}	7.83 ± 0.79***	27 ± 0.47**	90.66 ± 0.36*
8Ba	3.7 ± 0.42^{ns}	6 ± 0.57***	23 ± 2.35^{ns}	83 ± 1.31**
8Bb	4.53± 0.98^{ns}	10.66 ± 1.35^{ns}	24.53 ± 1.3***	69.33 ± 0.67***
8Bc	3.5 ± 0.48^{ns}	7 ± 0.57***	28 ± 0.35^{ns}	79.0 ± 0.31**
8Bd	4.66 ± 0.47^{ns}	9.33 ± 0.81**	24.66± 0.46***	89.83 ± 0.34**
8Ca	9.36 ± 0.49^{ns}	15.33 ± 0.83**	26.5 ± 0.562***	90 ± 0.92**
8Cb	5.33 ± 0.88^{ns}	15.5 ± 1.78^{ns}	20.33± .256***	87 ± 0.77***
8Cc	4.7 ± 0.82^{ns}	7.33 ± 0.67***	21 ± 1.35^{ns}	85 ± 1.51**
8Cd	5.46 ± 0.667^{ns}	10.33 ± 0.84**	23.67± 0.52***	90.81 ± 1.34**

Values are illustrated as Mean ± SEM, animals involved in each group: n=6

Analysis of values by one way ANOVA followed by Tukey- Kramer's test

Where, *Represents mild significant at P<0.05

**Represents moderate significant at P<0.01

****Represents non-significant at P<0.001 vs control

ns Represents non-significant at P<0.05 vs control.

Group I: The solvent control received normal CMC

Group II: Positive control received Phenytoin (30 mg/kg)

Group III: Triazinone derivatives at a dose of 100 mg/kg in 1% w/v CMC was received respectively

RESULTS AND DISCUSSION

Chemistry

The titled compounds 2-(2-amino-3-phenylpropanoyl)-5-((1, 5-disubstituted-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one **(8Aa-8Cd)** were synthesized as presented in Scheme-1 by reaction between N-(1-hydrazinyl-1-oxo-3-substituted-propan-2-yl) acetamide **(4A-C))** which in turn were synthesized from the aromatic amino acid **(2A-C)** via its esterification **(3A-C)** followed by its nucleophilic substitution reaction i.e. hydrazinolysis with hydrazine hydrate and 4-(3-substituted-1-phenyl-1H-pyrazol-4-yl) methylene)-2-phenyloxazol-5(4H)-one **(7a-d)** was synthesized from 3-substituted-1-phenyl-1H-pyrazole-4-carbaldehyde **(6 a-d)** by Vilsmeir Haack reaction[28] presented in Scheme-1.

In this presented research work, the chemical reactivity and unique template biological activities [29, 30] are responsible for the adoption of pyrazolyl moiety in conjugation with triazinone. Pyrazole is five-membered aromatic heterocyclic compounds. It has a pi-excessive heterocyclic system which contains two nitrogen atoms at position 1 and 2. The N- atom at position 2 with two electrons is basic and therefore reacts with electrophiles. The N- atom at position 1 is unreactive but loses its proton in the presence of a base. The aromatic nature in pyrazole systems appears from the unshared pair of the electron at position 2 which acts as good electron donor group and the four pi-electrons. All these structural properties make it applicable in designing the anticonvulsant pharmacophore.

Anticonvulsant activity

A series of 2-(2-amino-3-phenylpropanoyl)-5-((1, 5-disubstituted-1H-pyrazol-4-yl) methylene)-3-phenyl-1, 2-dihydro-1, 2, 4-triazin-6(5H)-one derivatives **(8Aa-8Cd)** were synthesized. In the present study, the synthesized compounds were structurally compared with well-known compounds with anticonvulsant activity i.e. phenytoin and lamotriazine. Both drugs are structurally different but reveal at least one aryl ring (C), one electron donor (D) and a hydrogen bond acceptor/donor unit (HBD) as depicted in (Figure 1). Accordingly, synthesized molecules have all the structural elements essential for anticonvulsant action. The lipophilicity of the synthesized compounds was the first descriptors to be identified as important for CNS penetration.In particular, for drugs to be potent which act on the central nervous system, they have to cross the blood-brain barrier (BBB), for them, their potency has been correlated with optimum lipophilicity (log P). However, increasing lipophilicity increases BBB penetration. Based on log P values of synthesized compounds, it was observed that they have acceptable lipophilic properties for anticonvulsant activity.

All the evaluated compounds exhibited protection against MES test which indicates that they can inhibit the seizure spread. The titled synthesized compounds delayed the onset and reduced duration of convulsion in MES induced convulsion model. In the acute toxicity study, it was found that the selected dose is 100 mg/kg. Upon observing the result in Table 1, 7 out of 12 triazinone derivatives (**8Aa, 8Ab, 8Ac, 8Ad, 8Ba, 8Bc and 8Cc)** were found to show highly significant activity with $p<0.001$ while compounds **8Bd,8Ca and 8Cd** were found to be medium activity with $p<0.01$ and triazinone derivatives **8Bb and 8Cb** were non- significant with $p<0.05$.

CONCLUSION

A series of twelve novels 1, 2, 4- Triazinone derivatives were synthesized, characterized and screened for their anticonvulsant activity by MES model.In the presented research work the relationship between the synthesized compounds and their log P values was studied and it was found that the higher the log P value (lipophilicity) showed better anticonvulsant activity. Compounds **8Aa, 8Ab, 8Ac, 8Ad, 8Ba, 8Bc and 8Cc**displayed highly significant anticonvulsant activity among all 12 synthesized derivatives. On the basis of acute toxicity studies, the synthesized compounds did not exhibit any acute toxicity in the experimental animals. Thus, it may be concluded that

the synthesized 1, 2, 4-triazinone derivatives exhibit the potential and nontoxic anticonvulsant agents.

ABBREVIATIONS

CNS: Central Nervous system; MES: Maximal Electroshock; DMF: Dimethyl formamide; DMSO: Dimethyl sulfoxide; CMC: Carboxy methyl cellulose; BBB: Blood-brain barrier; CPCSEA: *Committee for Control and Supervision of Experiments on Animals*

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the animal ethical committee on research, care and use of experimental animals of Pranveer Singh Institute of Technology, Kanpur, India

HUMAN AND ANIMAL RIGHTS

No humans were used for studies that are base of this research. All the animals were used in accordance the CPCSEA law (CPCSEA/AC/09/1273).

AVAILABILITY OF DATA AND MATERIALS

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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