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Design, Synthesis, Characterisation & Anticonvulsant Activities of Novel Heterocyclic Substituted Isatin Derivatives

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ABSTRACT

Twelve new isoxazole/pyrazole/pyrimidine substituted 5-nitrosation analogues were designed according to the requirements of the anticonvulsant drugs pharmacophore model and synthesised from indole-2,3-dione. Entire prepared compounds chemical structures were established from its IR, proton-NMR, Mass spectrum & microanalysis data. Anticonvulsant potency of final isatin analogues was assessed by MES technique & sc PTZ technique. Besides rotarod test was used to assess the neurotoxicity of all potent title analogues. Title compounds exhibited a varying degree of anticonvulsant potency ranging from mild to good. In the present study, it was concluded that pyrazole derivatives exhibited higher anti-epileptic activity than isoxazole derivatives. However, pyrimidine analogues displayed inferior activity than isoxazole analogues. 4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxindole-3-ylideneamino)phenyl) hydrazone)-1-(4-chlorophenyl)-3-amino-1H-pyrazole-5(4H)-one 7c was established as the most active analog of this series. Hence this derivative can act as a pilot molecule for further progress of new effective anticonvulsant drugs.

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INTRODUCTION

Epilepsy is a neurological illness manifested by the unexpected repeated occurrence of sensory disturbance, convulsions or loss of consciousness. Generally, it was coupled with anomalous electrical activity in the brain (McNamara, 1999). Worldwide approximately about 1 % of people are affected by epilepsy. Among them, almost 90 % are from developing countries (Fisher *et al.*, 2005). In a lifetime, about 5 % of individuals are affected by epilepsy. In the elders, following dementia and cerebrovascular diseases, epilepsy is the third most frequently spread neurological disorder (Krämer, 2001).

Based on the part and area of the brain affected by electrical disturbances, the seizures are categorised into several different types. Till date for epilepsy, no permanent cure is available. Use of AED_S (anti-epileptic drugs) or/ and suppression of seizure through surgery (invasive), electrical stimulation (either directly or indirectly) are the options available currently for the treatment of epilepsy. Phenytoin, carbamazepine, sodium valproate and phenobarbitone are the first generation AED_S used by around 20 % of the epilepsy patients (Levy *et al.*, 1995). Several new AEDs are developed but even

though about 30 % epileptic patients experienced seizures continuously and several other patients faced toxicity which is mainly dose-dependent with severe side effects like megaloblastic anaemia, hepatic failure and minimal brain impairment (Brown and Holmes, 2001). In medicinal chemistry field, there is a lot of scope for the development of novel AED_S with lower toxicity & high selectivity due to the limitations as mentioned above of currently available medications for epilepsy.

From the literature review it was found that the vital pharmacophore responsible for producing anti-epileptic activity are 1) A or hydrophobic domain (A); 2) HAD or hydrogen donor or acceptor unit & 3) D or electron donor atom. These pharmacophores produce activity by interacting with sodium channels (active site of voltage-gated) (Estrada and Peña, 2000; Bruno-Blanch *et al.*, 2003). This pharmacophore pattern was found in many first and second-generation AED_S and preclinical/clinical development stage AEDs (Figure 1). Many new AEDs came into the market as promising anti-epileptic agents in recent years due to the efforts based on the pharmacophore model (Stefan and Feuerstein, 2007). In the synthetic organic chemistry field, heterocyclic compounds were recognised as one of the critical categories. At present, these heterocyclic compounds were widely considered because of their broad applications & significant properties. In recent years medicinal chemists considered isatin as one such noteworthy heterocyclic nucleus mainly owing to their broad spectrum of pharmacological activities (Saravanan *et al.*, 2014; Dweedar *et al.*, 2014). Additionally Schiff (Liu and Hamon, 2019; Alpaslan *et al.*, 2019) and Mannich base (Kulkarni *et al.*, 2017; Mohanvel *et al.*, 2019) exhibited excellent pharmacological properties. Conversely, isoxazoles (Kumar *et al.*, 2015; Tatee *et al.*, 1987), pyrazoles (Alagarsamy and Saravanan, 2013; Selvam *et al.*, 2014; Anush *et al.*, 2018) & pyrimidines (Hanna, 2012; Kuppast and Fahmy, 2016) have gained significance due to their associated pharmacological and physiological potencies. Hence, in the present work, an effort has been made to prepare a series of novel isoxazole/ pyrazole/ pyrimidine nucleus substituted 5-nitroisatin as a potent anti-epileptic agent.

MATERIALS AND METHODS

In open capillaries using melting point apparatus (Thomas Hoover) melting points (mp) are measured & are un-corrected. Using KBr disks IR spectrum (ν, cm^{-1}) were measured on Bruker FT-IR spectrometer. In ppm (parts per million, δ) ^{1}H-NMR spectra were documented at 300 MHz using Bruker FT-NMR spectrophotometer in deuterated chloroform by employing TMS (tetramethylsilane) as an internal standard. A mass spectra were measured in JEOL-SX-102 instrument using FAB (fast atom bombardment) positive. On PerkinElmer 2400 CHN analyser microanalyses were measured. Calculated values are compared against experimental values & were found to be within the acceptable limits ($\pm$ 0.4 %). Using readymade silica gel plates, the reaction progresses were monitored. Compounds were detected using UV lamp & iodine as the developing agent. In present work all reagents & chemicals used was procured from CDH, E.Merck India Ltd., Qualigens, & SD Fine Chem. & were utilised without additional purification.

Synthesis of 5-nitroisatin (2)

As per the protocol documented in the literature, 5-nitroisatin 2 was synthesised (Socca *et al.*, 2014). To sum up, in 500 ml RBF 0.33 mol isatin was slowly added with recurrent shaking to 0.75 mol concentrated H_2SO_4 & 0.50 mol concentrated HNO_3 solution. Crushed ice-cold water was used to cool the obtained solution by immersing the flask. The reflux condenser was fixed in association to flask after adding all isatin. At 60 °C on a water bath for one hour, the above mixture was refluxed to produce the preferred derivative 5-nitroisatin 2. Then from the preferred product to clean out as much acid, the total solutions were added to 500 ml cold water contained in a beaker. From the mixture, the upper acid stratum was taken away when derivative two was matured completely at the underneath. Subsequently, the base stratum was moved to the separating funnel having 50 ml of water & shaken dynamically. Finally, the unwanted substances were gathered, dehydrated with calcium chloride (anhydrous) to get compound 2 in pure form. Yield: 65 %, melting point (in °C): 230-232. IR data: 1348 & 1570 (Nitro), 1732 (Carbonyl), 2996 (Aromatic CH), 3342 (NH). Proton-NMR data: 8.92 (1H, s, NH), 7.02-7.94 (3H, m, Aromatic proton). Molecular weight: 192. Molecular formula: $C_8H_4N_2O_4$. Microanalysis calculated: C, 50.01; H, 2.10; N, 14.58. Found: C, 49.91; H, 2.11; N, 14.62.

Preparation of 3-(4-aminophenylimino)-5-nitroindolin-2-one (3)

4-Amino aniline and 5-nitro isatin 2 in equimolar quantities (0.01 mmol) was mixed in an RBF having 25 ml ethanol & glacial acetic acid (few drops).

In a water bath at 100 °C, the above mixtures were refluxed for three hours.

The resulting mixtures were kept in RT until it cools

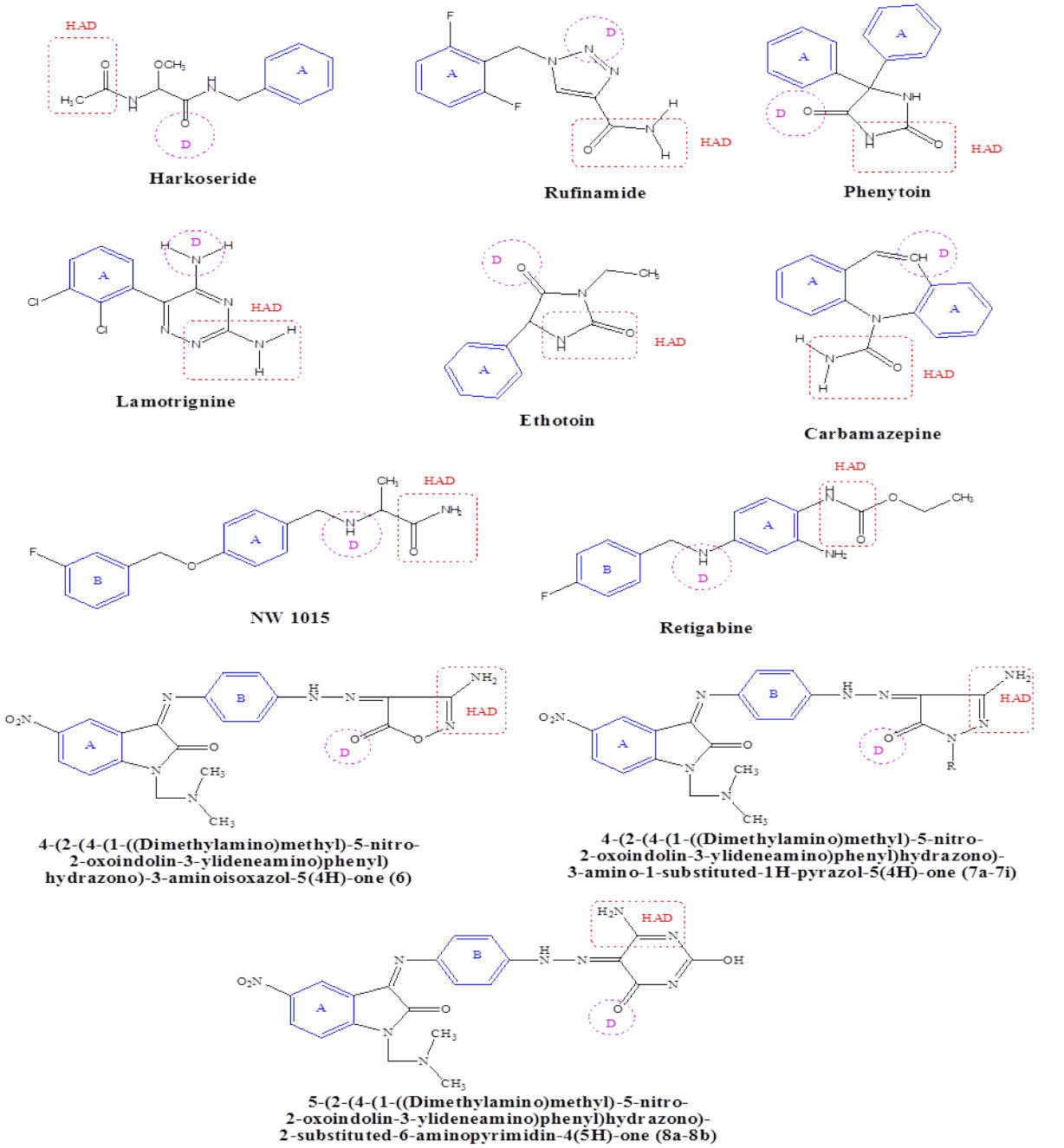


Figure 1: The pharmacophoric pattern of well-known anti-epileptics and title compounds with its vital structural features: (A) hydrophobic aryl ring system, (HAD) hydrogen bond acceptor/donor domain, (D) electron donor moiety and (B) distal aryl ring.

& the resulting compounds were collected.

The collected compound 3 was washed with ethanol & re-crystallised using chloroform & ethanol mixture. Yield: 76 %, melting point (in °C): 189-192. IR data: 1349 & 1515 (Nitro), 1634 (C=C), 1640 (C=N), 1702 (Carbonyl), 3038 (Aromatic CH), 3281 & 3353 (NH). Proton-NMR data: 8.95 (1H, s, NH), 7.19-8.32 (7H, m, Aromatic proton), 4.37 (2H, s, Amine).

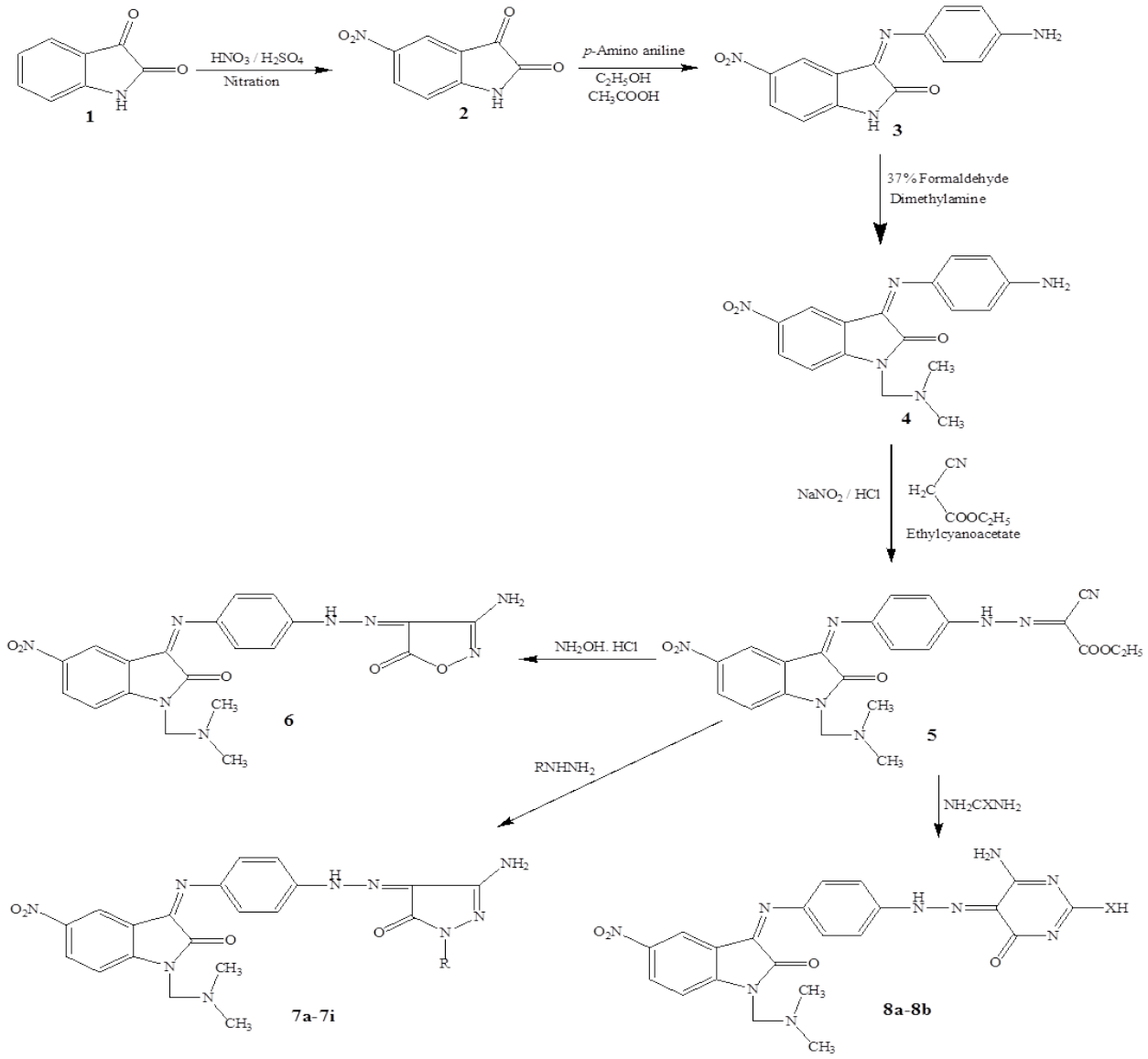
Molecular weight: 282. Molecular formula: $C_{14}H_{10}N_4O_3$. Microanalysis calculated: C, 59.57; H, 3.57; N, 19.85. Found: C, 59.74; H, 3.56; N, 19.81.

Preparation of 3-(4-aminophenylimino)-1 ((dimethylamino)methyl)-5-nitroindolin-2-one (4)

To a mixture of 25 ml ethanol containing 0.01 mol 3-(4-aminophenylimino)-5-nitroindolin-2-one (3), 0.25 ml 37% aqueous formaldehyde was added at once.

To the above mixture, 0.04 mol dimethylamine was portion-wise mixed by stirring slowly.

After adding entire dimethylamine for six hours at



7a) R = H; 7b) R = C_6H_5; 7c) R = *p*-ClC_6H_4; 7d) R = *p*-FC_6H_4; 7e) R = *p*-$OCH_3C_6H_4$; 7f) R = *m*-ClC_6H_4; 7g) R = *m*-FC_6H_4; 7h) R = $CSNH_2$; 7i) R = C_5H_4NCO; 8a) X = O; 8b) X = S

Figure 2: Synthetic protocols ofintermediates and title compounds

RT, the reaction solutions were stirred mechanically & then set aside for 48 hours in the refrigerator to obtain the product. Lastly, the formed crystals were alienated by filtration and dried in vacuum.

To get the desired products in pure form, the crystals are re-crystallised by alcohol. Yield: 70 %, melting point (in °C): 235-237.

IR data: 1320 & 1547 (Nitro), 1621 (C=C), 1654 (C=N), 1732 (Carbonyl), 2976 (Methyl CH), 3013 (Aromatic CH), 3305 & 3389 (NH). Proton-NMR data: 7.02-8.13 (7H, m, Aromatic proton), 4.17 (2H, s, Methylene), 4.04 (2H, s, Amine), 2.59 (6H, s, dimethylamine).

Molecular weight: 339. Molecular formula: $C_{17}H_{17}N_5O_3$. Microanalysis calculated: C, 60.17; H, 5.05; N, 20.64. Found: C, 59.99; H, 5.07; N, 20.71.

Preparation of ethyl 2-cyano-2-(2-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)hydrazono)acetate (5)

A solution composed of each 15 ml of water and concentrated HCl was added to 0.01 mol, i.e., 3.39 g of 3-(4-aminophenylimino)-1-((dimethylamino)methyl)-5-nitroindolin-2-one 4 and dissolved. The solution was submerged in a mixture of water and ice and cooled to 5° C. With stirring, to the above mixture of compound 4, 1.38 g powdered sodium nitrite (0.02 mol) dissolved in 10 ml water was added portion-wise. After total addition of sodium nitrite mixture for further one h, the stirring was continued. To a mixture of 1.13 g ethyl 2-cyanoacetate (0.01 mmol) in 25 ml ethanol,

Table 1: Antiepileptic & neurotoxicity study of derivatives III, IVa-IVb & Va-Vi administered intraperitoneally to mice.

Derivatives	MES test		scPTZ test		NT test	
	0.5 h[1]	4.0 h[1]	0.5 h[1]	4.0 h[1]	0.5 h[1]	4.0 h[1]
6	-	-	-	-	Not Determined	Not Determined
7a	-	300	-	300	Not Determined	Not Determined
7b	300	-	-	300	Not Determined	Not Determined
7c	30	100	100	300	-	-
7d	100	100	300	300	300	-
7e	100	300	300	300	Not Determined	Not Determined
7f	30	100	100	300	-	-
7g	100	100	300	300	300	-
7h	300	300	300	300	Not Determined	Not Determined
7i	300	300	300	-	Not Determined	Not Determined
8a	-	300	-	-	Not Determined	Not Determined
8b	-	-	-	-	Not Determined	Not Determined
Phenytoin[2]	30	30	-	-	100	100
Ethosuximide[3]	-	-	100	300	-	-

[1]After administration of drug test time; [2] (Yogeeswari *et al.*, 2005)[3] (Rajak *et al.*, 2009) At maximum dose tested (300 mg/kg) absence of activity was represented by mdash (-) sign.

Table 2: Antiepileptic & neurotoxicity of derivatives 7c and 7f administered orally(30 mg/kg) to rats

Derivatives	MES					TOX
	0.25 h[1]	0.5 h[1]	1 h[1]	2 h[1]	4 h[1]	
7c	One/Four	Two/Four	Three/Four	Three/Four	Two/Four	Zero/Four (-)[2]
7f	One/Four	Two/Four	Two/Four	Two/Four	Two/Four	Zero/Four (-)[2]
Phenytoin[3]	One/Four	Four/Four	Three/Four	Three/Four	Three/Four	Zero/Four (-)[2]

The data point out: No. of protected animals /No. of tested animals; [1]After administration of drug test time; [2](-)at a tested dose notneurotoxic; [3] (Yogeeswari *et al.*, 2005).

the above-obtained diazonium salt was added with stirring. Further in the water bath for ten h, the above solution was refluxed and bringing back to RT. The solid obtained II, so formed, was accumulated by filtration and re-crystallised from ethanol. Yield = 76 %, melting point (in °C): 157-159. IR data: 1344 & 1521 (Nitro), 1618 (C=C); 1642 (C=N), 1735 (Carbonyl), 2949 (Methyl CH), 3063 (Aromatic CH), 3317 (NH). Proton-NMR data: 6.82-7.95 (7H, m, Aromatic proton), 6.41 (1H, s, NH), 4.10-4.47 (2H, t, Methylene), 4.03 (2H, s, Methylene), 2.29 (6H, s, Dimethylamine), 1.24-1.51 (3H, t, Methyl). Molecular weight: 463 (M^+). Molecular formula: $C_{22}H_{21}N_7O_5$. Microanalysis calculated: C, 57.02; H, 4.57; N, 21.16. Found: C, 57.19; H, 4.55; N, 21.11.

Preparation of 4-(2-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)hydrazono)-3-aminoisoxazol-5(4H)-one (6)

In water bath, 1.04 g of hydroxylamine hydrochloride (0.015 mol) and 4.63 g ethyl 2-cyano-2-(2-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)hydrazone) acetate 5 (0.01 mmol) dissolved in 50 ml alcohol were refluxed overnight.

Excess alcohol was removed using distillation, and the remaining mixture obtained was discharged into crushed ice and mixed vigorously. The compound prepared 6 was filtered, washed using water, dried & re-crystallised from alcohol. Yield = 78 %, melting point (in °C): 182-184. IR data: 1375 & 1546 (Nitro), 1638 (C=C), 1642 (C=N), 1707 (Carbonyl), 2953 (Methyl CH), 3070 (Aromatic CH), 3306 & 3354 (NH). Proton-NMR data: 6.96-8.24 (7H, m, Aromatic proton), 6.79 (1H, s, NH), 4.32 (2H, s, Methylene), 2.50 (6H, s, Dimethylamine), 1.98 (2H, s, Amine). Molecular weight: 450 (M^+). Molecular formula: $C_{20}H_{18}N_8O_5$. Microanalysis calculated: C, 53.33; H, 4.03; N, 24.88. Found: C, 53.16; H, 4.04; N, 24.92.

Preparation of 4-(2-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)hydrazono)-3-amino-1-substituted-1H-pyrazol-5(4H)-one (7a-7i)

In the water bath, a mixture of 4.63 g ethyl 2-cyano-2-(2-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)hydrazono)acetate 5 (0.01 mol) and 0.015 mol of various hydrazine hydrochloride in 50 ml of ethanol were refluxed for 24 h. Excess alcohol was separated using distillation & the remaining solution attained was dispensed into crushed ice and mixed vigorously. The obtained compounds 7a-7i was filtered, dried & re-crystallised from alcohol.

4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-3-amino-1H-pyrazol-5(4H)-one (7a)

Yield = 81 %, melting point (in °C): 256-258. IR data: 1376 & 1521 (Nitro), 1613 (C=C), 1644 (C=N), 1718 (Carbonyl), 2950 (Methyl CH), 3082 (Aromatic CH), 3287 & 3319 (NH). Proton-NMR data: 6.70-8.09 (7H, m, Aromatic proton), 6.83 (1H, s, NH), 6.36 (1H, s, NH), 4.78 (2H, s, Methylene), 2.10 (6H, s, Dimethylamine), 2.02 (s, 2H, Amine). Molecular weight: 449 (M^+). Molecular formula: $C_{20}H_{19}N_9O4$. Micro analysis calculated: C, 53.45; H, 4.26; N, 28.05. Found: C, 53.63; H, 4.27; N, 27.94.

4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-3-amino-1-phenyl-1H-pyrazol-5(4H)-one (7b)

Yield = 75 %, melting point (in °C): 218-220. IR data: 1364 & 1553 (Nitro), 1617 (C=C), 1660 (C=N), 1702 (Carbonyl), 2928 (Methyl CH), 3096 (Aromatic CH), 3301 & 3335 (NH). Proton-NMR data: 7.13-8.07 (12H, m, Aromatic proton), 6.55 (1H, s, NH), 4.58 (s, 2H, Methylene), 2.43 (s, 6H, Dimethylamine), 2.21 (s, 2H, Amine). Molecular weight: 525 (M^+). Molecular formula: $C_{26}H_{23}N_9O_4$. Micro analysis calculated: C, 59.42; H, 4.41; N, 23.99. Found: C, 59.61; H, 4.39; N, 23.93.

4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-1-(4-chlorophenyl)-3-amino-1H-pyrazol-5(4H)-one (7c)

Yield = 77 %, melting point (in °C): 231-232. IR data: 778 (C-Cl), 1363 & 1515 (Nitro), 1602 (C=C), 1666 (C=N), 1709 (Carbonyl), 2971 (Methyl CH), 3038 (Aromatic CH), 3214 & 3297 (NH). Proton-NMR data: 7.05-8.21 (11H, m, Aromatic proton), 6.77 (1H, s, NH), 4.51 (2H, s, Methylene), 2.59 (6H, s, Dimethylamine), 1.94 (2H, s, Amine). Molecular weight: 561 (M^{+2}), 559 (M^+). Molecular formula: $C_{26}H_{22}ClN_9O_4$. Micro analysis calculated: C, 55.77; H, 3.96; N, 22.51. Found: C, 55.90; H, 3.95; N, 22.47.

4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-1-(4-fluorophenyl)-3-amino-1H-pyrazol-5(4H)-one (7d)

Yield = 80 %, melting point (in °C): 208-209. IR data: 1065 (C-F), 1366 & 1547 (Nitro), 1610 (C=C), 1663 (C=N), 1711 (Carbonyl), 2979 (Methyl CH), 3044 (Aromatic CH), 3258 & 3282 (NH). Proton-NMR data: 7.07-8.32 (11H, m, Aromatic proton), 6.88 (1H, s, NH), 4.26 (2H, s, Methylene), 2.35 (6H, s, Dimethylamine), 2.04 (2H, s, Amine). Molecular weight: 543 (M^+). Molecular formula: $C_{26}H_{22}FN_9O_4$. Micro analysis calculated: C, 57.46; H, 4.08; N, 23.19. Found: C, 57.28; H, 4.10; N, 23.27.

4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-1-(4-methoxyphenyl)-3-amino-1H-pyrazol-5(4H)-one (7e)

Yield = 76 %, melting point (in °C): 275-277. IR data: 1035 (C-O-C)), 1371& 1508 (Nitro), 1639 (C=C), 1653 (C=N), 1730 (Carbonyl), 2964 (Methyl CH), 3065 (Aromatic CH), 3242 & 3320 (NH. Proton-NMR data: 7.39-8.46 (11H, m, Aromatic proton), 6.42 (1H, s, NH), 4.30 (2H, s, Methylene), 3.42 (3H, s, Methoxy), 2.44 (6H, s, Dimethylamine), 2.10 (2H, s, Amine). Molecular weight: 555 (M^+). Molecular formula: $C_{27}H_{25}N_9O_5$. Micro analysis calculated: C, 58.37; H, 4.54; N, 22.69. Found: C, 58.18; H, 4.53; N, 22.78.

4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-1-(3-chlorophenyl)-3-amino-1H-pyrazol-5(4H)-one (7f)

Yield = 72 %, melting point (in °C): 222-224. IR data: 758 (C-Cl), 1359 & 1530 (Nitro), 1624 (C=C), 1651 (C=N), 1716 (Carbonyl), 2942 (Methyl CH), 3067 (Aromatic CH), 3213 & 3268 (NH). Proton-NMR data: 7.21-8.48 (11H, m, Aromatic proton), 6.64 (1H, s, NH), 4.49 (2H, s, Methylene), 2.17 (6H, s, Dimethylamine), 1.82 (2H, s, Amine). Molecular weight: 561 (M^{+2}), 559 (M^+). Molecular formula: $C_{26}H_{22}ClN_9O_4$. Micro analysis calculated: C, 55.77; H, 3.96; N, 22.51. Found: C, 55.86; H, 3.98; N, 22.45.

4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-1-(3-fluorophenyl)-3-amino-1H-pyrazol-5(4H)-one (7g)

Yield = 75 %, melting point (in °C): 249-250. IR data: 1069 (C-F), 1357 & 1512 (Nitro), 1617 (C=C), 1638

(C=N), 1724 (Carbonyl), 2989 (Methyl CH), 3041 (Aromatic CH), 3256 & 3283 (NH). Proton-NMR data: 7.08-8.13 (11H, m, Aromatic proton), 6.59 (1H, s, NH), 4.27 (2H, s, Methylene), 2.31 (6H, s, Dimethylamine), 1.85 (2H, s, Amine). Molecular weight: 543 (M^+). Molecular formula: $C_{26}H_{22}FN_9O_4$. Micro analysis calculated: C, 57.46; H, 4.08; N, 23.19. Found: C, 57.61; H, 4.07; N, 23.23.

4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-3-amino-5-oxo-4,5-dihydropyrazole-1-carbothioamide (7h)

Yield = 77 %, melting point (in °C): 236-238. IR data: 1374 & 1551 (Nitro), 1638 (C=C), 1645 (C=N), 1733 (Carbonyl), 2962 (Methyl CH), 3039 (Aromatic CH), 3280 & 3306 (NH). Proton-NMR data: 9.14 (2H, s, Thioamide), 7.15-8.20 (7H, m, Aromatic proton), 6.47 (1H, s, NH), 4.30 (2H, s, Methylene), 2.42 (6H, s, Dimethylamine), 2.18 (2H, s, Amine). Molecular weight: 508 (M^+). Molecular formula: $C_{21}H_{20}N_{10}O_4S$. Micro analysis calculated: C, 49.60; H, 3.96; N, 27.54. Found: C, 49.76; H, 3.97; N, 27.45.

4-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-1-isonicotinoyl-3-amino-1H-pyrazol-5(4H)-one (7i)

Yield = 76 %, melting point (in °C): 264-266. IR data: 1368 & 1526 (Nitro), 1622 (C=C), 1650 (C=N), 1707 (Carbonyl), 2935 (Methyl CH), 3073 (Aromatic CH), 3309 & 3375 (NH). Proton-NMR data: 6.94-7.82 (11H, m, Aromatic proton), 6.60 (1H, s, NH), 4.42 (2H, s, Methylene), 2.68 (6H, s, Dimethylamine), 2.21 (2H, s, Amine). Molecular weight: 554 (M^+). Molecular formula: $C_{26}H_{22}N_{10}O_5$. Micro analysis calculated: C, 56.32; H, 4.00; N, 25.26. Found: C, 56.14; H, 4.02; N, 25.31.

Preparation of 5-(2-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)hydrazono)-2-substituted-6-aminopyrimidin-4(5H)-one (8a-8b)

Using water bath, a mixture of 4.63 g ethyl 2-cyano-2-(2-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)hydrazono)acetate 5 (0.01 mol), 0.015 mol urea or thiourea & 0.2 g K_2CO_3 (0.2 g) was dissolved in 30 ml alcohol and refluxed for 24 h. The obtained mixture was chilled & discharged into cold-water (ice) and mixed vigorously. Using filter paper, the solution was filtered & 10 % acetic acid was used to neutralise the filtrate and the separated analogues 8a-8b were removed by filtration & re-crystallised from ethanol.

5-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-2-hydroxy-6-aminopyrimidin-4(5H)-one (8a)

Yield = 79 %, melting point (in °C): 190-193. IR data: 1350 & 1534 (Nitro), 1621 (C=C), 1679 (C=N), 1735 (Carbonyl), 2912 (Methyl CH), 3057 (Aromatic CH), 3233 & 3396 (NH), 3542 (OH). Proton-NMR data: 7.29-8.56 (7H, m, Aromatic proton), 6.95 (1H, s, NH), 4.47 (2H, s, Methylene), 3.09 (1H, s, Alcohol), 2.51 (6H, s, Dimethylamine), 2.36 (2H, s, Amine). Molecular weight: 477 (M^+). Molecular formula: $C_{21}H_{19}N_9O_5$. Micro analysis calculated: C, 52.83; H, 4.01; N, 26.40. Found: C, 53.02; H, 4.02; N, 26.29.

5-(2-(4-(1-((Dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-2-mercapto-6-aminopyrimidin-4(5H)-one (8b)

Yield = 78 %, melting point (in °C): 205-206. IR data: 1352 & 1539 (Nitro), 1625 (C=C), 1657 (C=N), 1720 (Carbonyl), 2519 (SH), 2986 (Methyl CH), 3023 (Aromatic CH), 3295 & 3361 (NH). Proton-NMR data: 7.18-8.31 (7H, m, Aromatic proton), 6.43 (1H, s, NH), 4.60 (2H, s, Methylene), 2.36 (6H, s, Dimethylamine), 2.09 (2H, s, Amine), 1.93 (1H, s, Thiol). Molecular weight: 493 (M^+). Molecular formula: $C_{21}H_{19}N_9O_4S$. Micro analysis calculated: C, 51.11; H, 3.88; N, 25.54. Found: C, 51.29; H, 3.86; N, 25.45

Biological activities

Pharmacology

Using male 18-25 g Swiss albino mice and 100-150 g Wistar rat entire prepared analogues were screened for their anti-epileptic potencies. In mice, two epilepsy methods such as MES technique and scPTZ technique are used for two primary qualitative estimations. Standardised rotorod method was employed in mice to examine the acute neurological toxicity induced by the prepared analogues. Initially 30 mg/kg, 100 mg/kg and 300 mg/kg, i.p. the dose was used to assess the anti-epileptic potencies of title derivatives using epilepsy models such as MES (induces generalised tonic-clonic seizures) and scPTZ (induces myoclonic seizures) models. The potency was calculated after 0.5 and 4 h of test compounds injection. In general, MES & scPTZ tests are used to identify seizure spread prevention and seizure threshold increment, respectively. Standard animal feed was used to feed animals and were grouped as six animals in all cluster. The animals were preserved at 25 ± 2 °C in colony cages under a 12 h light & dark sequence with 45–55 % relative humidity (Olfert *et al.*, 1993).Weeks before the use

of entire animals were acclimatised. The protocol employed for experimentation is properly approved by the IAEC (Institutional Animal Ethics committee).

Anti-epileptic activity

The MES (maximal electro-shock test)

In this technique, before inserting the corneal electrodes to the eyes of the animal, a drop of a mixture composed of 0.9 % saline (electrolyte) solution and 0.5 % tetracaine HCl (anaesthetic) solution was applied. 50 milli Ampere electrical stimulus was applied for mice at 60 Hz, and 150 milli Ampere electrical stimulus was applied for rats at 60 Hz for 0.2 sec period using similar earlier documented apparatus (Woodbury, 1952; White *et al.*, 1995). The endpoint of MES seizure technique was determined from the elimination of the hindleg tonic extensor phase. For the preliminary estimation mice was used against 30, 100 and 300 mg/kg dose of title analogues by i.p. Route of administration. Initially, 30 mg/kg dose of the synthesised drug was given orally to rats. The results were compared with standard phenytoin.

The scPTZ (subcutaneous pentylenetetrazole seizure) test

In this technique, 85 mg/kg dose (which produce convulsion at 97 % of animal) of pentylenetetrazole (a chemical which induces convulsion) was injected in the midline of the neck into a loose fold of skin of mice present to generate convulsion. Test derivatives were injected by i.p. Injection to the animals before injecting pentylenetetrazole. The stress of animals was minimised by placing the animals in segregation cage & the presence/absence of a seizure in animals were observed for the next thirty minutes. The endpoint scPTZ technique is 3-5 sec incident of clonic spasms of the hind &/or forelimbs, jaws, or vibrissae. The animals are considered protected if it does not meet this condition (Swinyard *et al.*, 1961). For the estimation mice was used against 30, 100 and 300 mg/kg dose of title analogues by i.p. Route of administration. The obtained results were compared with standard ethosuximide.

Acute toxicity-minimal motor impairment

Apparent signs of damaged muscular or neurological functions of animals are monitored to assess the test analogues toxicity (undesirable side effects). The MNI (minimal neurological impairment) and MMI (minimal muscular impairment) in mice were disclosed using rotorod procedure (Dunham and Miya, 1957). The mouse can maintain their equilibrium when rod rotates at 6 rpm speed for long periods when it placed on a rotating rod. During 1 minute if the mouse falls off three times from this rotating rod, then the corresponding dose of tested analogue was considered toxic to animals. Abnormal body posture, a zigzag or circular gait & spread of the legs, loss of placing response, somnolence, catalepsy, lack of exploratory behaviour, stupor, changes in muscle tone, hyperactivity, and tremors also noted in animals in addition to MMI and MNI.

RESULTS AND DISCUSSION

Chemistry

In the current research, a sequence of novel Mannich & Schiff bases of isatin analogues 6, 7a-7i & 8a-8b was prepared by placing different heterocyclic substituted phenylhydrazono moiety at C-3 positions of isatin nucleus. The proposed analogues were prepared as per the etiquette displayed in a synthetic scheme (Figure 2). In the present study at imine nitrogen, different groups are substituted, to synthesise sequences of novel heterocyclic substituted isatin analogues. By a multistep synthesis, various new Mannich & Schiff bases of isatin were prepared from isatin. Initially, by using sulphuric acid & nitric acid, 5-nitro isatin 2 were synthesised from isatin by simple nitration. Schiff base reactions between p-phenylenediamine & 5-nitro isatin produced 3-(4-aminophenylimino)-5-nitroindolin-2-one 3 in glacial acetic acid presence. The prepared analog 3-(4-aminophenylimino)-5-nitroindolin-2-one 3 undergone Mannich reaction with dimethylamine (2° amine) & HCHO to synthesize 3-(4-aminophenylimino)-1-((dimethylamino)methyl)-5-nitroindolin-2-one 4.

In the succeeding stair, sodium nitrite and HCl was used to diazotise prepared amino analogue 4. Subsequently by intramolecular rearrangement ethyl cyanoacetate was treated with the diazotised salt to obtain ethyl 2-cyano-2-(2-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)hydrazono) acetate 5. Corresponding isoxazole 6, pyrazole analogues 7a-7i, and pyrimidine analogues 8a-8b were prepared finally by synthesised keto ester 5 with a range of amine analogues such as hydroxylamine HCl, different hydrazine HCl, and urea/thiourea derivatives, respectively through dehydrative cyclisation. TLC was carried out to optimise the process throughout the synthesis for purity & completion.

Several spectroscopic studies such as IR, NMR, mass spectra, & microanalyses were employed to confirm the allotted structures of the prepared analogues. Presence of a few characteristic absorption peaks in the IR spectrum is used for identification of particular groups in prepared analogues. The

appearance of a peak at 1640 cm^{-1} in IR, which correlates the existence of C=N moiety in compound 3 by response between p-phenylenediamine & 5-nitro isatin 2. The emergence of singlet at δ 2.59 ppm (dimethyl moiety) & δ 4.17 ppm (CH_2 linkage, which connects dimethylamine & isatin) confirms the formation of Mannich base derivative 4. In IR spectrum at 1735 cm^{-1} appearance of the sharp peak corresponds to Carbonyl stretching confirms the formations of keto ester 5. One proton present in NH of hydrazone produces one singlet peak at δ 6.41 also confirms the assigned structures of derivative 5. At δ 4.10-4.47 ppm 2 protons present in CH_2 of C_2H_5 produces tetret peak in NMR spectrum along with triplet peak produced by three protons present in CH_3 of C_2H_5 at δ 1.24-1.51 ppm which also further supports the structure of compound 5. In ^{1}H-NMR spectrum disappearance of triplet & tetret peaks corresponds to the CH_3 & CH_2 of C_2H_5 confirms the conversion of ethyl 2-cyano-2-(2-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)acetate 5 into new isatin derivatives substituted with various heterocycles 6, 7a-7i & 8a-8b. Besides, around δ 2.00 ppm appearance of 2 protons singlet peak corresponds to amine group confirms the assigned structures. In NMR spectrum appearance of other characteristic peaks also supports the proposed structure of synthesised derivatives 6, 7a-7i & 8a-8b. The molecular weight & purity of synthesised analogues was established from their corresponding mass spectral data.

Biological activities

Anti-epileptic activity

The maximal electroshock (MES) technique

MES & scPTZ technique was used to assess the anti-epileptic activity of prepared analogues by administering through i.p. Route in mice. The prepared analogues are considered as a notably valuable compound in the treatment of partial, generalised & even absence seizures if it is found to be good exhibit activity in these challenges of seizure test. All preliminary anti-epileptic data of the prepared candidates are summarised in Table 1.

In MES method at 30 mg/kg (lowest) dose, out of 12 screened analogues compounds 7c and 7f were found to be considerably potent at 0.5 h time interval itself. The activity of derivatives 7c and 7f continued at 100 mg/kg dose at the time interval of 4.0 h. The above statements indicate that these analogues 7c and 7f possess rapid onset and long duration of action. Presence of chlorine in phenyl ring attached at C-1 of pyrazole of analogues 7c and 7f may be responsible for the promising activity. Derivatives 7d, 7e and 7g showed protection at a dose of 100 mg/kg after 0.5 h. This indicates that a relatively lower dose these derivatives are capable of guarding against seizures. Except for 7e rest of other analogues such as 7d and 7g were found to exhibit activity at the same dose of 100 mg/kg after 4.0 h time interval. After 4.0 h the analogue 7e was found to exhibit activity only at 300 mg/kg (higher) dose. Either after 0.5 h time interval and 4.0 h time interval at higher tested (300 mg/kg) dose, derivatives 7a, 7b, 7h, 7i and 8a was found to protect animals from seizure. Remaining of test derivatives 6 & 8b doesn't display protection at any dose tested.

The subcutaneous pentylenetetrazole (scPTZ) technique

In the scPTZ evaluation, it was found that many of the tested analogues were found to exhibit moderate to good anti-epileptic potency. The seizure threshold is increased by the derivatives which revealed guard in scPTZ method. At 100 mg/kg (lowest) dose, out of 12 screened analogues compounds 7c and 7f were found to be considerably potent at 0.5 h time interval. The activity of derivatives 7c and 7f continued at 300 mg/kg dose at the time interval of 4.0 h. The above statements indicate that these analogues 7c and 7f possess rapid onset and long duration of action. The same degree of activity was observed in the case of reference drug ethosuximide which is familiar as standard AED for scPTZ method. Derivatives 7d, 7e, 7g and 7h showed protection at a dose of 300 mg/kg after 0.5 h and 4.0 h. This indicates that a relatively higher dose these derivatives are capable of guarding against chemically induced seizures. Either after 0.5 h time interval or 4.0 h time interval rest of derivatives 7a, 7b and 7i except 6, 8a and 8b were found to be active at higher tested dose, i.e., 300 mg/kg. At C-1 of pyrazole ring, the most active compounds possess chlorine substituted phenyl ring which may be responsible for increased anti-epileptic potency of these derivatives.

Almost all derivatives except analogues 6, 7a, 8a & 8b, displayed anti-epileptic activity at any one dose tested in any one of the preliminary epilepsy tests after 0.5 h. 83 % of the test derivatives protected animals from an epileptic seizure in MES screening, and 75 % of the test derivatives protected animals from an epileptic seizure in scPTZ screening. From the study, it was found that MES selectivity observed in theses series compared to scPTZ selectivity.

Minimal motor impairment (Acute toxicity)

Rotorod methods were employed to screen the neurotoxicity of prepared derivatives in mice. From the study, it was found that many title candidates dis-

played neurotoxicity merely at higher doses compared to usually given drugs like ethosuximide or phenytoin. The separation between anti-epileptic dose & neurotoxic dose is enviable when screening anti-epileptic potencies of test analogues. From the preliminary anti-epileptic testing, four derivatives 7c-7d & 7f-7g were selected for neurotoxicity screening due to their excellent potency. At 300 mg/kg dose derivatives, 7d and 7g were established to be neurotoxic, whereas remaining tested analogues 7c and 7f were found to be non-neurotoxic at all dose tested.

The MES (maximal electro-shock) test of selected compounds by oral route

The valuable property of AED is their ability to inhibit epilepsy when administered through oral route. The approximate TPE (time of peak effect), duration of neurotoxicity or anti-epileptic potency and the time of onset was disclosed in this type of test. From the initial screen, two derivatives 7c and 7f were identified as a potent compound. Hence further, these analogues were tested for their oral availability by acute MES seizure test & neurotoxicity test at 30 mg/kg fixed-dose in rats. Table 2 summarises the obtained data.

The most active derivative out of these two analogues is 7c which protected three rats out of 4 at 1 h and 2 h time points. It protected two rats out of 4 at 0.5 h & 4 h time interval, and it protected only one rat out of 4 at a time interval of 0.25 h. Similar to standard phenytoin, this derivative exhibited satisfactory activity. Whereas, in rat MES oral screen analogue 7f was found to exhibit moderate activity as it protected maximum two rats out of four at one h, two h and four h time interval. Besides at 0.25 h time interval, it protected only one animal out of four animals. At 30 mg/kg oral dose, these two analogues were found to be non-toxic. From GIT absorption of derivatives and its penetration to CNS was confirmed from this obtained in vivo data. The most flexible anti-epileptic mechanism of standard drug phenytoin is their influence on voltage depended on sodium channels. Like phenytoin test analogue 7c also protected the animals from electrically induced seizure; hence derivative 7c may also exhibit their action through influence on voltage depended on sodium channels.

Structure-activity relationship

When comparing the pharmacological potency of prepared derivatives with their chemical structures, it was found that two analogues 7c and 7f displayed better anti-epileptic potency out of a range of synthesised derivatives, in MES and scPTZ method. These analogues 7c and 7f possess pyrazolone nucleus coupled isatin hydrazone. The anti-epileptic activity was greatly influenced by the nature of substituted ring on C-3 of isatin ring; the pyrazolone derivatives 7a-7i showed higher anti-epileptic potency compared to isoxazolone derivatives 6 and pyridimidinone derivatives 8a-8b. Among pyrazolone substituted compounds, phenyl substituted compounds 7b-7g exhibited better activity than unsubstituted derivatives 7a, carbothioamide derivatives 7h and isonicotinoyl analogues 7i. Within phenyl substituted pyrazolone derivatives Vb-Vg, test compound 7c and 7f exhibited the highest potency. Presence of chlorine on the phenyl ring of derivatives 7c and 7f is responsible for the increased anti-epileptic potency which may be due to additional bonding with the binding site; whereas in rest of analogues, chlorine is absent in phenyl ring. In generally substituted phenyl ring containing pyrazolone analogues, 7b-7g exhibited the highest activity followed by unsubstituted phenyl ring containing pyrazolone analogues 7b, carbothioamide derivatives 7h and isonicotinoyl analogues 7i.

CONCLUSIONS

In conclusion, a series of new isoxazole/ pyrazole/ pyrimidine substituted indole-2,3-dione derivatives were prepared & characterised using IR, NMR, mass spectral & microanalysis data. MES and scPTZ tests were employed to assess the anti-epileptic activity of all title derivatives. Neurotoxicity of potent compounds was also estimated. Entire derivatives showed various degrees of anti-epileptic and neurotoxicity activities. From the present study, it was found that the pyrazolone derivatives showed superior anti-epileptic potency than the isoxazolone derivatives and pyridimidinone derivatives. Among pyrazolone substituted compounds, phenyl substituted compounds exhibited better activity than unsubstituted derivatives, carbothioamide derivatives and isonicotinoyl analogues. Within phenyl substituted pyrazolone derivatives chlorine substituted phenyl ring containing pyrazolone analogues exhibited the highest activity followed by other group substituted phenyl ring containing pyrazolone analogues, unsubstituted phenyl ring containing pyrazolone analogues. From the study, we identified two compounds 7c and 7f as potent compounds. Hence these two analogues were further screened at 30 mg/kg, p.o. Dose using rats in MES acute seizure test & neurotoxicity. Like standard drug phenytoin, in oral dose compound 7c exhibited almost equal anti-epileptic potency. The most active compound among entire tested derivatives was 4-(2-(4-(1-((dimethylamino)methyl)-

5-nitro-2-oxoindolin-3-ylideneamino)phenyl) hydrazono)-1-(4-chlorophenyl)-3-amino-1H-pyrazole-5(4H)-one 7c that revealed protection at a dose of 30 mg/kg (i.p.) and 100 mg/kg (i.p.) dose after 0.5 h and 4 h, respectively in MES test. In the scPTZ test at 100 mg/kg and 300 mg/kg dose of this compound also protected 0.5 h and 4 h, respectively. Hence, the derivative 7c emerged out as the lead compound without any neurotoxicity & a wide spectrum of anti-epileptic activity.

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Conflicts of interest

The authors declare that they have no conflicts of interest for this study.

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