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INDOLE DERIVATIVES WITH ANTICONVULSANT ACTIVITY AGAINST TWO SEIZURE MODELS

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ABSTRACT

A series of indole derivatives (3-6 & 8a) were synthesized from 2-phenyl-1*H*-indole (1) with an aim to get promising anticonvulsant agents. The compounds were characterized on the basis of their IR, ^{1}H NMR and Mass spectral data results. The compounds were tested for their anticonvulsant activity in MES and scPTZ animal models. A few compounds showed appreciable activity against both the animal models whereas one compound, 5, showed significant anticonvulsant activity and could be considered for further investigations.

Keywords: Indoles, MES, scPTZ, Anticonvulsant.

INTRODUCTION

Antiepileptic drugs belong to structurally different classes of compounds.[1,2] The common structural elements of the older generation of clinically active antiepileptic drugs, derived from hydantoins, oxazolidinediones, succinimides and glutarimides, can be defined as a nitrogen containing heteroatomic system bearing one or two phenyl rings and at least one carbonyl group.[3,4] A literature survey revealed that heterocyclic compounds bearing iodole moiety are endowed with important biological activities including anticonvulsant activities.[5-9] On the other hand, imidazolones and oxazolones have also been a subject of intensive investigations, because of their pharmacological actions including anticonvulsant.[9-12]

Epilepsy is a chronic neurological disorder that is characterized by recurrent unprovoked seizures.[13] There are different types of epilepsy and they are not based on a single underlying mechanism but of multifactorial in origin. Epilepsy is usually controlled but not cured with medication however; over 30% of people with epilepsy do not have seizure control even with the best available medications.[14,15] Therefore, search for new anticonvulsant drugs continues to be an active area of medicinal chemistry research.

In the present investigations, some new indole derivatives having imidazolone/oxazolone ring were synthesized and tested for their anticonvulsant activities in mice.

MATERIALS AND METHODS

Chemistry

Melting points were recorded in open capillary tubes in a paraffin bath and are uncorrected. ^{1}H-

NMR spectra were recorded on Varian E-360 MHz or Bruker spectropsin DPX-300MHz with tetramethylsilane (TMS) as an internal standard. The splitting pattern abbreviations are as follows: s, singlet; br, broad signal; d, doublet; dd, double doublet; t, triplet; m, multiplet. Mass spectra were recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Spectral data are consistent with the assigned structures. Microanalysis of the compounds was done on Perkin-Elmer model 240 analyzer and the values were found within ±0.4% of the theoretical values. Thin-layer chromatography was carried out to monitor the reactions using silica gel G as stationary phase and iodine chamber and UV lamp were used for visualization of TLC spots.

Synthesis of 2-Phenyl-1H-indole (1)

Condensation of acetophenone with phenyl hydrazine HCl in presence of fused zinc by `Fischer indole reaction' conditions[16] followed by crystallization from benzene gave 2-phenyl-1*H*-indole (1) as colourless shining flakes, yield 76%, *Rf* (Benzene: Petroleum ether, 8:2) 0.73, m.p. 188-189 °C; IR (cm^{-1}, KBr): 3312 (NH), 3092, 1607, 1550; ^{1}H-NMR ($CDCl_3$, ppm): δ 6.73 (s, 1H, H-3), 6.91-7.55 (m, 8H, H-4,5,6 of indole + 5H of phenyl), 8.20 (br, 1H, H-7); MS: m/z 193(M^+), 194 (M+1), 165 [M-(HCN+H)], 89.

Synthesis of 2-Phenyl-1H-indole-3-carbaldehyde (2)

Compound (1) on treatment with dimethylformamide and phosphorous oxychloride following 'Vilsmeier Haack reaction' conditions[17] followed by crystallization from acetone gave colourless platelets, yield 88%, *Rf* (Benzene: Petroleum ether, 8:2) 0.31, m.p. 247 °C; IR (cm^{-1}, KBr): 3300 (NH), 3037, 1702 (C=O), 1590, 1560; ^{1}H-NMR (DMSO-d_6, ppm): δ 7.33-7.76 (m, 8H, H-4,5,6 of indole + 5H of phenyl), 8.15 (br, 1H, H-7), 9.95 (s, 1H, CHO). MS: m/z 221 (M^+), 220 (M-1), 191 (M-CHO), 165 [M-(HCN+H)].

Synthesis of 2-Phenyl-4-[(2-phenyl-indolin-3-yl) methylene) oxazol-5(4H)-one (3)

A mixture of compound (2) (0.01 mol, 2.22 gm), benzoylglycine (0.01 mol, 1.79 gm), fused sodium acetate (0.015 mol, 1.22 gm) and acetic anhydride (7.15 ml) was placed in a round bottom flask fitted with a $CaCl_2$ guard tube and heated on a burner with constant shaking. When the reaction mixture liquefied completely, it was transferred to a water bath and heated for another 2h. Ethanol (10 ml) was added slowly to the cooled reaction mixture and the contents allowed to stand overnight. A solid which separated out was filtered, washed with hot water and crystallized from acetone; Orange needles, yield 55%, *Rf* (Toluene: Ethyl acetate: Formic acid, 5:4:1) 0.82, mp 210 °C; IR (cm^{-1}, KBr): 3294 (NH), 3020, 1625 (C=O), 1631,1568; ^{1}H-NMR (δ) ($CDCl_3$); 7.45-7.66 (m, 13H, H-4,5,6 of indole+10H of two phenyl rings), 7.90 (br, 1H, H-7), 8.13 (s, 1H, olefinic proton). MS: (m/z) 364 (M^+), 231, 220, 165.

Ethyl-2-(3-[(-oxo-2-phenyloxazol-4(5H)-ylidene) methyl]-2-phenylindolin-1-yl) acetate (4)

Equimolar quantities of (3) and ethyl chloroacetate (0.01 mol each; 3.65 gm and 1.2 ml, respectively) were refluxed in presence of dry acetone (50 ml) and anhyd. K_2CO_3 (8.0 gm) for 24 h on a water bath. The contents were then cooled and filtered to remove undissolved solid. The filtrate was concentrated to a small volume and left in a refrigerator overnight. A solid mass which separated out was crystallized from acetone-ethanol mixture; Orange fibrous crystals, yield 56%, *Rf* (Toluene: Ethyl acetate: Formic acid, 5:4:1) 0.78, mp 130 °C; IR (cm^{-1}, KBr): 3294 (NH), 3001, 1737 (C=O), 1698, 1632, 1554; ^{1}H-NMR ($CDCl_3$): δ 1.34 (t, 3H, CH_3), 4.23 (q, 2H, OCH_2), 4.76 (s, 2H, NCH_2), 7.21-7.78 (m, 13H, H-4,5,6 of indole+10H of two phenyl rings), 8.11 (br, 1H, H-7), 8.20 (s, 1H, olefinic proton); MS: (m/z) 450, 364, 77.

2-(3-[(1-Amino-5-oxo-2-phenyl-1H-imidazol-4(5H)-ylidene) methyl]-2-phenyl-1H-indol-1-yl)acetohydrazide (5)

Compound (13) (0.01 mol, 4.5 gm) on refluxing with hydrazine hydrate (0.5 mL) in abs. ethanol (50 mL) gave (5); Pale yellow crystals, yield 48%, *Rf* (Toluene: Ethyl acetate: Formic acid, 5:4:1) 0.32, mp 145 °C; IR (cm^{-1}, KBr): 3472, 3304 (NH), 2986, 1737 (C=O), 1641, 1483; ^{1}H-NMR (DMSO-d_6): δ 4.52 (br, 4H, 2xNH_2), 4.84 (s, 2H, CH_2), 7.08-7.73 (m, 13H, H-4,5,6 of indole+10H of two phenyl rings), 8.11 (br, 1H, H-7), 8.26 (s, 1H, olefinic proton), 9.43 (br, 1H, NH, amide); MS: (m/z) 450, 290, 91.

General procedure for synthesis of 1,2,4-Trisubstituted-1H-imidazol-5(4H)-one (6,7)

Equimolar quantities of (**3**) (0.01mol, 3.6 gm) and 1-amino-2-propanol/hydrazine hydrate (0.01 mol) were fused together in an oil bath for 1h. After cooling to room temperature, crushed ice was added to the reaction mixture to give a solid mass which was crystallized from a solvent/solvent mixture to give TLC pure crystals.

1-(2-Hydroxypropyl)-2-phenyl-4-[(2-phenylindolin-3-yl)methylene]-1H-imidazol-5(4H)-one (6)

Pale yellow crystals, yield 64%, *Rf* (Toluene: Ethyl acetate: Formic acid, 5:4:1) 0.58, mp 200-202 °C; IR (cm^{-1}, KBr): 3273 (NH), 3002, 1728 (C=O), 1631, 1496; ^{1}H-NMR (DMSO-d_6): δ 1.48 (d, 3H, CH_3), 3.46 (t, 2H, -N-CH_2-), 3.99 (m, 1H, -CH-OH), 6.93-7.74 (m, 13H, H-4,5,6 of indole+10H of two phenyl rings), 7.82 (br, 1H, H-7), 8.13 (s, 1H, olefinic proton); MS: (m/z) 421 (M^+), 231, 165, 148, 77.

1-Amino-2-phenyl-4-[(2-phenylindolin-3-yl)methylene]-1H-imidazol-5(4H)-one (7)

Red crystals, yield 48%, *Rf* (Benzene: Petroleum ether, 8:2) 0.71, mp 214-216 °C; IR (cm^{-1}, KBr): 3451, 3276 (NH), 3004, 1726 (C=O), 1633, 1514; ^{1}H-NMR (DMSO-d_6): δ 5.99 (br, 2H, NH_2), 7.12-7.65 (m, 13H, H-4,5,6 of indole+10H of two phenyl rings), 7.82 (br, 1H, H-7), 8.15 (s, 1H, olefinic proton); MS: (m/z) 378 (M^+), 361, 191, 157, 77.

Attempted synthesis of imidazolone (8) which resulted in a dimer of methyl-2-benzamido-3-(2-phenylindolin-3-yl)acrylate (8a)

A mixture of (**3**) (0.01 mol, 3.6 gm), ethylene glycol (0.01 mol, 0.6 ml) and sulfanilamide (0.01 mol, 1.72 gm) was refluxed at 175-180 °C in an oil-bath for 2.5 h. After cooling to room temperature, the contents were poured onto crushed ice to give a crude solid mass which was crystallized from ethanol and methanol mixture (1:1); Yellow rhombic crystals, yield 83%, *Rf* (Toluene: Ethyl acetate: Formic acid, 5:4:1) 0.65, mp 180 °C; IR (cm^{-1}, KBr): 3276 (NH), 2983, 1730 (C=O), 1705, 1622, 1508, 840; ^{1}H-NMR ($CDCl_3$): δ 4.72 (s, 4H, 2xCH_2 groups carrying oxygen), 7.17-7.81 (m, 26H, 2xH-4,5,6 of indole+20H of four phenyl rings), 8.03 (br, 2H, 2xH-7), 8.20 (s, 2H, 2xolefinic proton); MS: (m/z) 790 (Molecular ion peak missing), 426, 364, 305.

Pharmacology

Anticonvulsant evaluation of the compounds (4-7 & 8a) was done using Swiss albino mice of either sex weighing 25-30 g. The animals were housed at room temperature of 25±2 °C under 12 h light/12 h dark cycle with free access to food and water *ad libitum*. The studies were undertaken with prior approval from the Institutional Animal Ethics Committee (IAEC) and utmost care was taken to ensure that the animals were treated in the most humane and acceptable manner. Food and water were withdrawn prior to the experiments. All the compounds were dissolved in polyethylene glycol. The compounds were administered ip at doses of 30, 100 and 300 mg/kg to mice. Activity was established using the MES and scPTZ tests according to the protocol by Antiepileptic Drug Development Program (ADD), Epilepsy Branch, National Institute of Health, Bethesda, MD, USA[18,19]. The

anticonvulsant activity data is presented in table1.

Maximal Electroshock Seizure (MES) Test

Mice were prescreened 24 h before by delivering maximal electroshock 50 mA; 60 Hz and 0.2s duration by means of corneal electrodes. A drop of 0.9% sodium chloride was instilled in each eye prior to the application of electrodes in order to prevent death of the animal. Abolition of hind limb tonic extensor component of the seizure in half or more of the animals is defined as protection.

Subcutaneous Pentylenetetrazole Test (scPTZ)

The scPTZ test utilized a dose of pentylenetetrazole 70 mg/kg. This produced clonic seizures lasting for a period of at least five seconds. The test compounds administered at the three graded doses i.e. 30, 100 and 300 mg/kg ip. At the anticipated time the convulsant was administered subcutaneously. Animals were observed over a period of 30 min. Absence of clonic spasm in half or more of the animals in the observed time period indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold.

RESULTS AND DISCUSSION

Chemistry

A series of indolyl-oxazolone/imidazolone derivatives were synthesized as presented in Scheme 1. The starting material, 2-phenyl-1H-indole (1), was synthesized in good yield by condensation of acetophenone with phenyl hydrazine HCl in presence of fused zinc by well known `Fischer indole reaction.[16] 2-Phenyl-1H-indole (1) on treatment with dimethylformamide (DMF) and phosphorous-oxy-chloride following 'Vilsmeier Haack reaction' conditions[17] gave 2-phenyl-1H-indole-3-carbaldehyde (2). Reaction of compound (2) with benzoyl glycine in acetic anhydride furnished 2-phenyl-4-[(2-phenyl-indolin-3-yl) methylene) oxazol-5(4H)-one (3). Compound (3) on treatment with ethylchloroacetate yielded compound (4), which on treatment with hydrazine hydrate furnished compound (5). Further, compound (3) on fusion with hydrazine hydrate or 3-amino-1-propanol gave two 1,2,4-trisubstituted-1H-imidazol-5(4H)-ones (6 & 7). In one case, on attempting the desired condensation of sulfanilamide with compound (3) in presence of ethylene glycol as solvent the oxazolone ring got opened up and instead of the expected compound (8) corresponding ester of ethylene glycol (8a) was obtained. The structures of the synthesized compounds were confirmed on the basis of their spectral data (IR, ^{1}H NMR & mass) results.

In general, the IR spectra of the compounds showed peaks each around 1650 (C=O), 3200 (NH stretching) cm^{-1}. In the nuclear magnetic resonance spectra (^{1}H-NMR, ppm), the signals of the respective protons of the compounds were verified on the basis of their chemical shifts and showed characteristic peaks at appropriate δ values. The structure of the compounds was further supported by mass spectral data.

Pharmacology

Preliminary evaluation of all the synthesized compounds were performed against two seizure models viz. MES and scPTZ. All the compounds showed encouraging anticonvulsant activity. Compounds 5 and 6 were found to be active against MES test at a dose level 100 mg/kg at 0.5 h & 4 h time interval. Compounds that exhibited moderate protection against MES model at 300 mg/kg included 4, 7 and 8g at 0.5 h. Compound 4 was also active at the dose level 300 mg/kg at 4 h in MES test. In chemo-shock study, those compounds that exhibited considerable activity in MES test, chosen for scPTZ study. Compound 6 exhibited good protection against scPTZ model at 100 mg/kg at 0.5 h. Another compound, 5, showed protection at 300 mg/kg against scPTZ model at 4h. Thus majority of the compounds showed appreciable anticonvulsant activity in both the models of seizure (Table 1).

CONCLUSION

In conclusion, the indole derivatives having oxazolone/imidazolone moieties exhibited appreciable anticonvulsant activity. Compounds 5 and 6 were good in their action in MES test. In scPTZ test, a compound, 6, was found to be active. Thus, Among the newer derivatives one compounds, 1-(2-Hydroxypropyl)-2-phenyl-4-[(2-phenylindolin-3-yl)methylene]-1*H*-imidazol-5(4*H*)-one (6) emerged as lead compound and could be considered for further investigations. It is conceivable that these derivatives can be further chemically modified to get potential anticonvulsant agents.

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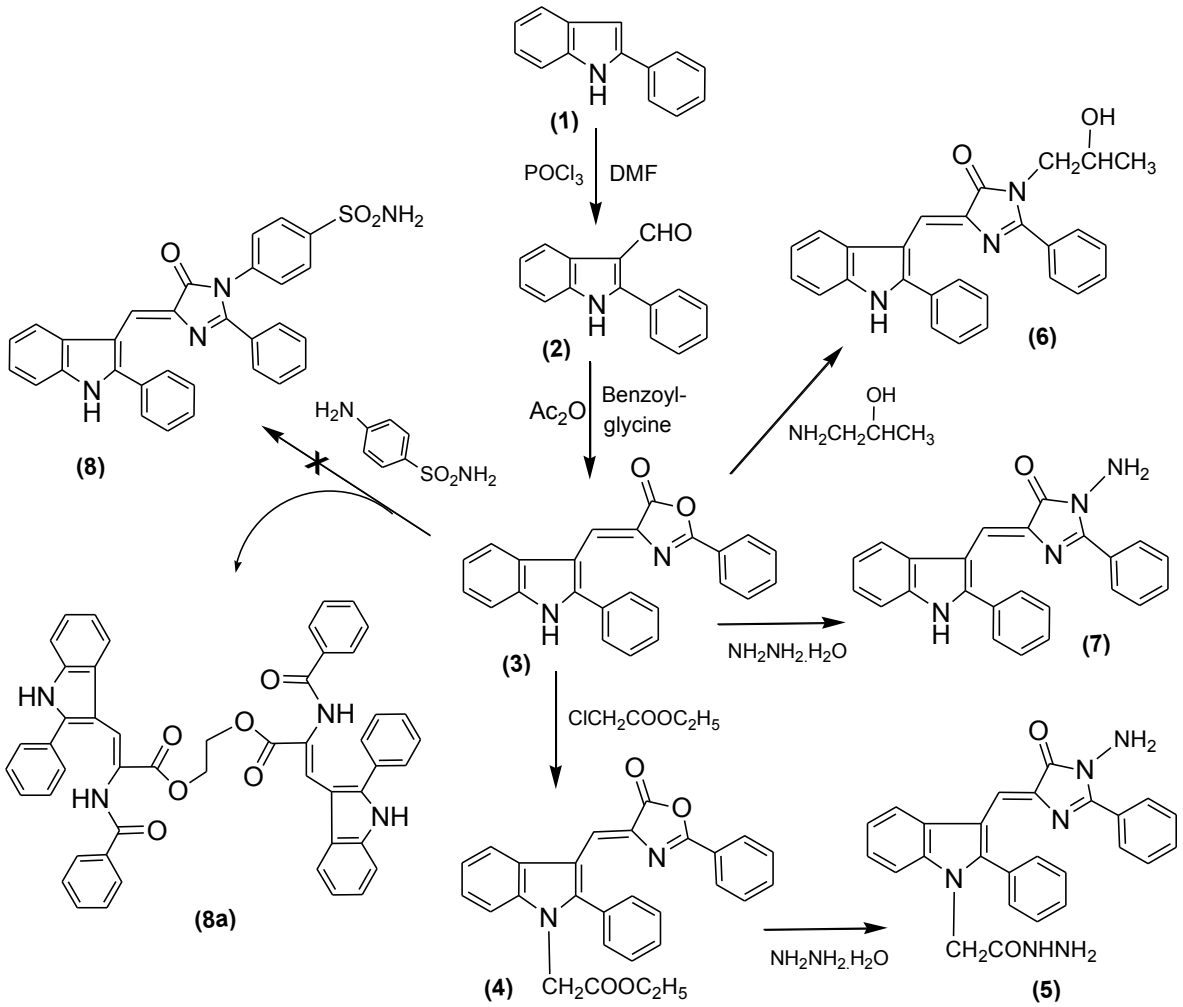

Scheme 1. Protocol for synthesis of title compounds.

Table 1: Anticonvulsant activity data of the title compounds (**4-6 & 8a**).

Compound	MES		scPTZ	
	0.5 h	4 h	0.5 h	4 h
4	300	300	300	(-)
5	100	100	300	300
6	100	100	100	300
7	300	(-)	nt	nt
8a	300	(-)	nt	nt
Phenytoin[a]	30	30	(-)	(-)

- Dose of 30, 100 and 300 mg/kg were administered i.p. The figures indicate the minimum dose whereby bioactivity was demonstrated in half or more mice. The (-) indicates an absence of activity at maximum dose administered (300 mg/kg). The (nt) indicates not tested. [a]Data from reference[20].

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