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Synthesis and *In Vivo* Anticonvulsant Screening of Coumarin Incorporated Schiff Bases of 1,3,4-Oxadiazoles

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A series of coumarin incorporated Schiff bases of 1,3,4-oxadiazoles (**1-18**) was synthesized. The structures of the synthesized compounds were confirmed on the basis of spectral data and elemental analysis. The anticonvulsant and neurotoxicity was studied by maximal electroshock seizure (MES) and rotorod method, respectively. A majority of the compounds were active in MES test. All the compounds were less neurotoxic than the standard drug phenytoin.

Keywords: Coumarin; MES test; 1,3,4-Oxadiazoles; Schiff bases.

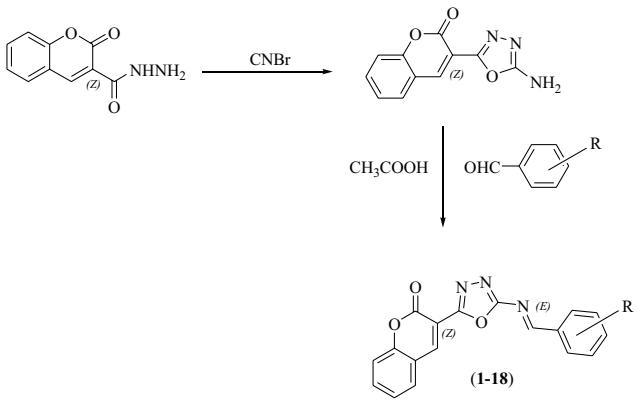
INTRODUCTION

Epilepsy, one of the most frequent neurological disorders, is a major public health issue, affecting about 4% of individuals over their lifetime. Despite the increasing understanding of the pathogenesis of seizures and epilepsy, the cellular basis of human epilepsy remains a mystery. In the absence of specific etiological understanding, approaches to drug therapy for epilepsy must necessarily be directed towards the control of symptoms, that is the suppression of seizures by chronic administration of Antiepileptic Drugs (AEDs). However, seizures remain uncontrolled in at least 30% of all epilepsies, even when an adequate AED therapy is administered. During recent years, a large number of new AEDs have been marketed worldwide, but the proportion of patients failing to respond to drug treatment has not been changed in a significant extent.

This disease affects a large number of populations of different age groups and sex. The classical antiepileptic drugs comprise phenobarbital, available since 1911; phenytoin, marketed in 1939; carbamazepine, used for epilepsy in Europe from the mid 1960s; and valproic acid,[1] available in several European countries as of the late 1960s. All currently approved antiepileptic drugs have dose-related toxicity and idiosyncratic side effects.[2] In response to the premise that major medical breakthroughs in non-pharmacologic therapies for the treatment of epilepsy in the near future seem remote, the search for new antiepileptic drugs with lower toxicities and fewer side effects continues.

In our previous researches on anticonvulsants, different moieties were selected for the synthesis of anticonvulsant agent e.g., sulphonamides, benzothiazoles, semicarbazones coumarin etc.[3-6] In the present investigation we have synthesized coumarin incorporated Schiff bases of 1,3,4-oxadizoles (Scheme I). The compounds were evaluated *in vivo* for anticonvulsant activity by MES test and neurotoxicity by rota-rod method.

Scheme I



1: R = 3-NO_2; **2**: R = 3,4-$(OCH_3)_2$; **3**: R = 4-OH; **4**: R = 2-OH; **5**: R = 2-NO_2; **6**: R = 3-OH; **7**: R = 4-$N(CH_3)_2$; **8**: R = 4-F; **9**: R = 4-OCH_3; **10**: R = 2-Cl; **11**: R = 3-Cl; **12**: R = 4-Cl; **13**: R = H; **14**: R = 4-NO_2; **15**: R = 3-F; **16**: R = 2-F; **17**: R = 2-OCH_3; **18**: R = 3-OCH_3.

EXPERIMENTAL SECTION

Compounds 3-(5-{[(1E)-(substituted phenyl)methylene]amino}-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-ones were synthesized by reacting coumarin hydrazide with cyanogen bromide to form 3-(5-amino-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one (S*tep II*). In IR spectrum bands at

3000 cm^{-1}, 1622 cm^{-1}, 1600 cm^{-1}, 1275 cm^{-1} confirmed the presence of C-H, C=O (coumarin), C=C and C-O groups, respectively. The ^{1}H-NMR spectra showed multiplet at δ 6.99-8.71 for five aromatic protons. A singlet appears at δ 11.38 for two NH protons. The compound 3-(5-amino-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one was reacted with 3-nitrobenzaldehyde in presence of acetic acid to form Schiff base. In IR spectrum bands at 3000 cm^{-1}, 2700 cm^{-1}, 1699 cm^{-1}, 1348 cm^{-1}, 1268 cm^{-1} confirm the presence of C-H, N=CH, C=O (coumarin), C-N and C-O groups, respectively. The ^{1}H NMR spectra showed the multiplet at δ 6.96-8.3 with J = 10 Hz for nine aromatic protons. A singlet appears at 11.33 for one N=CH proton. The synthesized compounds were characterized by elemental analysis, FT-IR and ^{1}H-NMR. The IR spectra revealed the following bands for N=CH, C=O (coumarin), C-N, C-O groups at 2700 cm^{-1}, 1721-1619 cm^{-1}, 1662-1348 cm^{-1}, 1304-1268 cm^{-1}, respectively. The ^{1}H-NMR spectra confirm the presence of aromatic protons at δ 6.9-8.5. The singlet for N=CH-Ar appears at δ 11.0-11.4.

MATERIALS AND METHODS

Animals

Male albino mice (Swiss, 18-25 g) were used as experimental animals. The test compounds were suspended in polyethylene glycol (PEG). The animals were maintained on an adequate diet and allowed free access to food and water except during the short time they were removed from cages for testing. The animals were maintained at room temperature (25-30 °C). All the experimental protocols were carried out with the permission from Institutional Animal Ethics Committee (IAEC), form no. 304. Animals were obtained from Central Animal House Facility, Hamdard University, New Delhi-110062, India. Registration number and date of registration of Animal House Facility: (173/CPCSEA, 28, JAN-2000).

Chemistry

All the solvents were of LR grade and were obtained from Merck, CDH and s. d. fine chemicals. Melting points were determined in open capillary tubes and are uncorrected. All the compounds were subjected to elemental analysis (CHN) and the measured values agreed within ±0.4% with the calculated ones. Thin layer chromatography was performed on Silica gel G (Merck). The spots were developed in iodine chamber and visualized with an ultraviolet lamp. The IR spectra were recorded in KBr pellets on (BIO-RAD FTS 135) WIN-IR spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker model DPX 300 FT-NMR spectrometer in ($CDCl_3$) using tetramethylsilane (Me_4Si) TMS as an internal standard. The chemical shifts are reported in δ ppm scale.

Synthesis of 3-(5-{[(1E)-(substituted phenyl)methylene]amino}-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-ones

Step I: **2-Oxo-2*H*-chromene-3-carbohydrazide**

Ethyl-2-oxo-2*H*-chromene-3-carboxylate (0.01 mol) and hydrazine hydrate 99% (0.01 mol) were dissolved in ethanol (50 mL) to give clear solution and refluxed for 10 h. The contents were concentrated to half volume and allowed to cool. The solid mass which separated out on cooling was retained by filtering and washed with small amount of ice-cooled ethanol (90%). (m.p. = 136-138 °C).

Step II: **3-(5-amino-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one**

To an ethanolic solution of 2-oxo-2*H*-chromene-3-carbohydrazide (0.01 mol), cyanogen bromide (0.01 mol) was added. The reaction mixture was warmed at 55-60 °C for 90 min. The resulting solution was cooled and neutralized by sodium bicarbonate ($NaHCO_3$) solution. The solid was filtered out. (m.p. = 200 °C).

Step III: **3-(5-{[(1E)-(3-nitrophenyl)methylene]amino}-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one (1)**

3-(5-Amino-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one (0.01 mol), 3-nitrobenzaldehyde (0.01 mol) and glacial acetic acid (2 mL) were refluxed in 1,4-dioxan (40 mL) for 8 h. The solvent was distilled off at reduced pressure. The product was obtained by pouring the reaction mixture in ice-cold water. It was recrystallized from ethanol. (m.p. = 105 °C). Similarly other compounds (**2-18**) of the series were synthesized.

The physico-chemical characteristics of the compounds are presented in (Table 1). The data of elemental analysis agree with the results of analytical calculations according to molecular formulas.

Anticonvulsant screening

Electroshock-induced seizures (MES test)

Albino mice (20-25 g) were used in this test. Animals were divided in groups of six and were stimulated through corneal electrodes to 50 mA current at a pulse of 60 Hz al-

Table 1. Physical and spectral properties of the tested compounds **(1-18)**

Compd	M.p (°C)	Molecular formula*	Spectral Data
1	105	$C_{18}H_{10}N_4O_5$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700; (C=O, coumarin) str. 1669; (C-N) str. 1348; (C=C) str. 1527, (C-O) str. 1268; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.2-7.6 (m, 8H, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.3 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 116.4, 119.2, 120.5, 120.9, 122.3, 123.8, 125.2, 125.9, 126.4, 128.3, 130.5, 134.3, 142.1, 145.2, 155.0, 156.4, 163.7, 168.5.
2	180	$C_{20}H_{12}N_3O_5$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700; (C=O, coumarin) str. 1658; (C-O) str. 1304; (C-N) str. 1492; (=C-H) out of plane 789, 824; ^{1}H-NMR ($CDCl_3$) δ ppm: 3.4 (s, 6H, 2 × OCH_3), 7.3-8.7 (m, 8H, *J* = 10 Hz, Ar-H), 11.3 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 56.0, 112.3, 112.6, 116.1, 117.3, 119.2, 120.2, 122.4, 123.8, 124.3, 125.4, 134.6, 140.2, 142.4, 143.6, 155.2, 156.2, 163.5, 168.4.
3	182	$C_{18}H_{11}N_3O_4$	IR (ν, cm^{-1}): (-OH) str. 3650; (C-H) str. 3000; (N=C-H) str. 2700; (C=O, coumarin) str. 1620, 1574; (C-N) str. 1484; (C-O) str. 1271; ^{1}H-NMR ($CDCl_3$) δ ppm: 6.9-7.7 (m, 8H, *J* = 10 Hz, Ar-H), 8.5 (s, 1H, Ar-H, H-4), 8.7 (s, 1H, Ar-OH), 11.3 (s, 1H, -N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 112.4, 116.2, 119.4, 120.4, 122.5, 123.6, 124.1, 125.4, 126.6, 134.4, 143.9, 154.2, 155.3, 156.3, 163.5, 168.5.
4	190	$C_{18}H_{11}N_3O_4$	IR (ν, cm^{-1}): (-OH) str. 3600; (C-H) str. 3046; (N=C-H) str. 2700; (C=O, coumarin) str. 1619; (C=C) str. 1573; (C-N) str. 1485; (C-O) str. 1271; ^{1}H-NMR ($CDCl_3$) δ ppm: 6.9-7.7 (m, 8H, *J* = 10 Hz), 8.5 (s, 1H, Ar-H, H-4), 8.7 (s, 1H, Ar-OH), 11.3 (s, 1H, -N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 111.2, 113.5, 115.4, 115.7, 120.1, 123.5, 124.6, 125.4, 126.5, 127.3, 129.3, 134.3, 144.1, 152.4, 155.2, 156.2, 163.6, 168.4.
5	360 (d°)	$C_{18}H_{10}N_4O_5$	IR (ν, cm^{-1}): (C-H) str. 3246; (N=C-H) str. 2700, (C=O, coumarin) str. 1619; (C=C) str. 1500; (C-N) str. 1457; (C-O) str. 1270; (=C-H) out of plane; ^{1}H-NMR ($CDCl_3$) δ ppm: 750; 7.2-7.6 (m, 8H, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.3 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 115.5, 119.8, 121.2, 122.3, 122.6, 123.8, 124.3, 125.5, 126.0, 128.1, 130.4, 134.4, 144.3, 155.1, 156.4, 163.2, 167.9.
6	160	$C_{18}H_{11}N_3O_4$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700, (C=O, coumarin) str. 1622; (C=C) str. 1486, 1573, (C-N) str. 1382; (C-O) str. 1271; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.0-7.3 (m, 8H, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 8.7 (s, 1H, Ar-OH), 11.4 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 112.4, 115.0, 119.3, 119.6, 123.2, 124.3, 125.2, 127.4, 126.9, 125.3, 129.3, 134.6, 144.8, 155.4, 156.1, 160.7, 163.4, 168.4.
7	315 (d°)	$C_{20}H_{17}N_4O_3$	IR (ν, cm^{-1}): (C-H) str. 2900; (N=C-H) str. 2700; (C=O, coumarin) str. 1619; (C=C) str. 1523, 1486, (C-N) str. 1366; (C-O) str. 1270; ^{1}H-NMR ($CDCl_3$) δ ppm: 1.5 (s, 6H, 2 × NCH_3), 7.0-7.3 (m, 8H, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.3 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 45.4, 109.2, 116.7, 118.3, 122.2, 122.6, 123.8, 125.1, 126.6, 127.4, 134.7, 142.9, 144.8, 157.0, 161.4, 164.1, 168.8.
8	100	$C_{18}H_{10}N_3O_3F$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700; (C=O, coumarin) str. 1619; (C=C) str. 1550, 1486, (C-N) str. 1400; (C-O) str. 1270; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.0-7.6 (m, 8H, *J* = 10 Hz, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.2 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 111.3, 116.4, 121.3, 122.6, 123.7, 124.8, 125.1, 126.3, 128.6, 133.3, 144.7, 156.3, 159.7, 160.8, 164.4, 168.6.
9	140	$C_{19}H_{13}N_3O_4$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700, (C=O, coumarin) str. 1622; (C=C) str. 1486, 1573, (C-N) str. 1382; (C-O) str. 1271; ^{1}H-NMR ($CDCl_3$) δ ppm: 3.2 (s, 3H, OCH_3), 7.2-7.7 (m, 8H, *J* = 10 Hz, Ar-H), 8.5 (s, 1H, Ar-H, H-4), 11.3 (s, 1H, -N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 55.1, 110.1, 116.4, 119.4, 121.3, 122.6, 123.6, 124.1, 125.3, 127.1, 134.6, 144.8, 158.1, 159.7, 162.2, 164.6, 168.5.

10	160	$C_{18}H_{10}N_3O_3Cl$	IR (ν, cm^{-1}): (C-H) str. 2900; (N=C-H) str. 2700; (C=O, coumarin) str. 1619; (C=C) str. 1572, 1484; (C-N) str. 1374; (C-O) str. 1270; (=C-H) out of plane 731; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.0-7.3 (m, 8H, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.3 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 116.1, 121.2, 122.3, 123.7, 125.6, 126.1, 127.0, 127.4, 128.3, 129.4, 130.2, 131.3, 134.4, 144.8, 157.0, 160.6, 164.1, 168.5.
11	140	$C_{18}H_{10}N_3O_3Cl$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700; (C=O, coumarin) str. 1721; (C-N) str. 1662; (C=C) str. 1572; (C-O) str. 1272; (=C-H) out of plane 680, 750; ^{1}H-NMR ($CDCl_3$) δ ppm: 6.9-7.3 (m, 8H, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.3 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 116.3, 121.2, 122.4, 123.5, 124.1, 125.7, 126.1, 127.5, 127.9, 128.4, 129.2, 130.6, 131.9, 136.5, 144.6, 160.4, 164.4, 168.6.
12	130	$C_{18}H_{10}N_3O_3Cl$	IR (ν, cm^{-1}): (C=O, coumarin) str. 1622; (C-O) str. 1269; (C-N) str. 1486; (C=C) str. 1572; (N=C-H) str. 2700; (C-H) str. 3000; ^{1}H-NMR ($CDCl_3$) δ ppm: 6.9-7.8 (m, 8H, J = 10 Hz, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.3 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 116.4, 121.1, 122.6, 123.8, 124.2, 125.0, 125.5, 126.3, 127.4, 132.1, 136.6, 144.8, 157.4, 160.7, 164.6, 168.8.
13	165	$C_{18}H_{11}N_3O_3$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700; (C=O, coumarin) str. 1619; (C=C) str. 1573; (C-N) str. 1480; (C-O) str. 1270 (=C-H) out of plane 731, 748; 7.0-7.4 (m, 9H, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.3 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 116.3, 121.2, 123.5, 124.7, 125.1, 125.8, 127.3, 127.8, 128.7, 129.1, 136.5, 144.8, 159.8, 160.7, 164.6, 168.7.
14	120	$C_{18}H_{10}N_4O_5$	IR (ν, cm^{-1}): (C-H) str. 3246; (N=C-H) str. 2700, (C=O, coumarin) str. 1619; (C=C) str. 1500; (C-N) str. 1457; (C-O) str. 1270; (=C-H) out of plane 750; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.2-7.6 (m, 8H, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.3 (s, 1H, N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 116.2, 121.1, 123.2, 124.6, 125.7, 127.1, 127.6, 128.4, 129.5, 136.6, 144.7, 146.8, 159.0, 160.5, 164.1, 168.5.
15	125	$C_{18}H_{10}N_3O_3F$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700; (C=O, coumarin) str. 1619; (C=C) str. 1550, 1486, (C-N) str. 1400; (C-O) str. 1270; (=C-H) out of plane 750, 740; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.2-7.9 (m, 8H, Ar-H), 8.7 (s, 1H, Ar-H, C-4), 11.2 (s, 1H, N=CH-); ^{13}C-NMR ($CDCl_3$) δ ppm: 112.1, 113.8, 116.5, 121.5, 122.3, 123.6, 124.7, 125.4, 126.3, 127.3, 128.5, 137.6, 146.8, 157.0, 159.2, 160.7, 163.9, 167.8.
16	90	$C_{18}H_{10}N_3O_3F$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700; (C=O, coumarin) str. 1619; (C=C) str. 1550, 1486, (C-N) str. 1400; (C-O) str. 1270; (=C-H) out of plane 750, 740; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.2-7.6 (m, 8H, Ar-H), 8.5 (s, 1H, Ar-H, C-4), 11.0 (s, 1H, N=CH-); ^{13}C-NMR ($CDCl_3$) δ ppm: 111.5, 113.2, 116.1, 121.3, 122.6, 124.5, 125.3, 126.1, 127.4, 128.1, 129.4, 136.4, 144.7, 157.3, 158.6, 163.9, 164.8, 168.9.
17	150	$C_{19}H_{13}N_3O_4$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700, (C=O, coumarin) str. 1622; (C=C) str. 1486, 1573, (C-N) str. 1382; (C-O) str. 1271; ^{1}H-NMR ($CDCl_3$) δ ppm: 3.1 (s, 3H, OCH_3), 7.1-7.4 (m, 8H, J = 10 Hz, Ar-H), 8.5 (s, 1H, Ar-H, H-4), 11.3 (s, 1H, -N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 54.6, 110.1, 112.4, 116.9, 117.3, 121.2, 122.5, 123.5, 124.1, 125.9, 127.1, 128.8, 136.5, 144.7, 157.1, 159.5, 160.7, 163.9, 168.0.
18	55	$C_{19}H_{13}N_3O_4$	IR (ν, cm^{-1}): (C-H) str. 3000; (N=C-H) str. 2700, (C=O, coumarin) str. 1622; (C=C) str. 1486, 1573, (C-N) str. 1382; (C-O) str. 1271; ^{1}H-NMR ($CDCl_3$) δ ppm: 3.0 (s, 3H, OCH_3), 7.2-7.6 (m, 8H, J = 10 Hz, Ar-H), 8.5 (s, 1H, Ar-H, H-4), 11.3 (s, 1H, -N=CH-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 54.9, 110.5, 112.6, 116.6, 121.2, 122.4, 123.6, 124.3, 125.1, 126.5, 127.7, 136.6, 144.6, 158.1, 159.7, 160.5, 164.6, 168.3.

* The values established by elemental analysis were within ± 0.4% in comparison to calculated values.

ternating current applied for 0.25 s. The mice were previously administered *i.p.* with the test drug solution in polyethylene glycol at three dose levels (30, 100 and 300 mg/kg), the anticonvulsant activity was assessed after 30 min. and 4 h intervals of administration. The abolition of hind limb tonic extensor spasm was recorded as a measure of anticonvulsant activity.[7]

Neurotoxic effects

Rota-rod test

Minimal motor impairment was measured in mice by the rotorod test.[8] The mice were trained to stay on an accelerating rotorod that rotates at 10 revolutions/min. The rod diameter was 3.2 cm. Trained animals were given *i.p.* injection of the test compounds 30, 100 and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min. in each of the trials.

Histopathological studies

The selected compounds were evaluated for their histopathological study. The Luna's technique[9] was used to access the livers of mice, which were administered with test compounds at the dose level of 30 mg/kg body weight for 15 days; comparison was done with the control group. Microphotographs of section of liver were taken at the magnification of 100X and 400X.

RESULTS AND DISCUSSION

The pharmacological evaluation of the compounds (**1-18**) was initially carried out according to the protocols of antiepileptic drug development programme (ADD), Epilepsy Branch, NIH. The methods employed have been previously described.[7] The compounds were initially screened in the mouse MES test. Minimal motor impairment was measured by rotorod test. Data is represented in (Table 2). All the coumarin incorporated Schiff bases of oxadiazoles were active in MES test at a dose of 300 mg/kg indicative of their ability to protect the seizure spread. At a dose of 30 mg/kg, compounds that showed protection in half or more tested mice were (**1, 5, 9, 10, 11, 12, 14, 17** and **18**) after 0.5 h time interval. These compounds also showed protection after 4 h but at a higher dose of 100 mg/kg. The compounds (**2, 3, 4, 6, 8, 15** and **16**) showed protection at a dose 100 mg/kg after 0.5 h. These compounds also showed protection after 4 h but at a higher dose of 300 mg/kg. Compounds (**7** and **13**) showed protection in MES test at 300 mg/kg both after 0.5 h and 4 h duration.

Table 2. Anticonvulsant profile and rota-rod toxicity of the examined compounds (**1-18**) in mice

Compd.	Intraperitonial injection in mice[a]			
	MES screen		Toxicity screen	
	0.5 h	4 h	0.5 h	4 h
1	30	100	300	-
2	100	300	300	-
3	100	300	300	-
4	100	300	300	300
5	30	100	300	-
6	100	300	300	300
7	300	300	300	300
8	100	300	300	300
9	30	100	300	100
10	30	100	300	100
11	30	100	300	300
12	30	100	300	300
13	300	300	300	300
14	30	100	300	300
15	100	300	300	300
16	100	300	300	300
17	30	100	300	100
18	30	100	300	100
Phenytoin[b]	30	30	100	100
Carbamazepine[b]	30	100	100	300
Phenobarbital[b]	100	30	100	300

[a] Doses of 30, 100 and 300 mg/kg were administered. The figure in the table indicates the minimum dose whereby bioactivity was demonstrated in half or more of the animals (n = 6). The animals were examined 0.5 and 4 h after administration. The (-) indicates an absence of activity at maximum dose administered (300 mg/kg).

[b] Data from references[10-12]

In neurotoxicity screening, the compounds (**1, 2, 3** and **5**) were neurotoxic at a maximal dose of 300 mg/kg after 0.5 h. Compounds (**9, 10, 17** and **18**) showed neurotoxicity at a dose of 100 mg/kg after 4 h. All the compounds showed neurotoxicity at a higher dose of 300 mg/kg after 0.5 h time interval. However all the compounds were less neurotoxic than phenytoin.

In hepatotoxicity studies samples from group **1** (compounds showing protection at 30 mg/kg at 0.5 h) showed normal hepatic parenchyma and were similar to control group (Fig. 1).

CONCLUSIONS

Substitutions of NO_2, Cl and OCH_3 at distal phenyl ring showed potent activity against MES test. The Presence

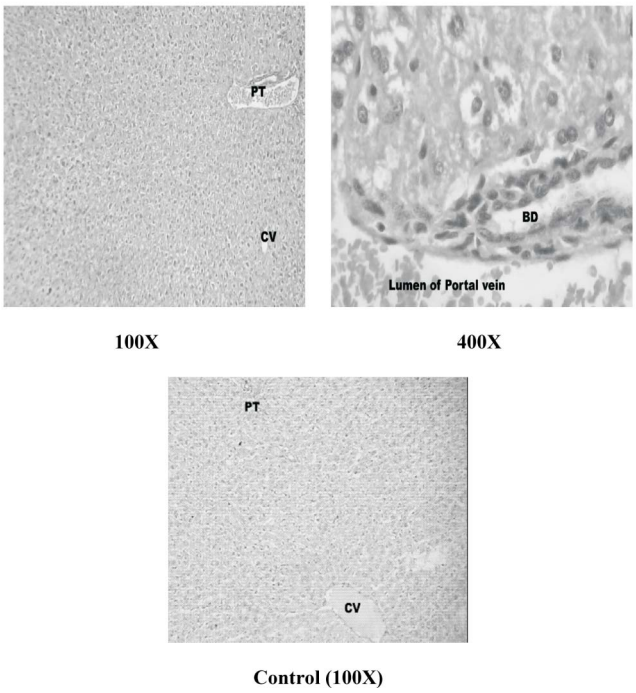


Fig. 1. Low power (HE × 100x) and high power (HE × 400x) photomicrographs of liver from group **1** showing a normal **PT** = portal triad, **BD** = Bile Duct as compared to control.

of OH, F and 3,4-$(OCH_3)_2$ at distal phenyl ring showed moderate activity against MES test. Substitution with $N(CH_3)_2$ showed protection against MES test at higher dose of 300 mg/kg. Introduction of double bond in the structures provides additional feature of rigidity. In conclusion, the majority of the compounds of coumarin incorporated Schiff bases of 1,3,4-oxadiazoles were active in MES test and all the compounds were less neurotoxic than phenytoin.

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