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Synthesis and Evaluation of Antiepileptic Activity of Newly Designed Coumarin-Sulfonamide Hybrids

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The combination of different heterocyclic rings to form a multifunctional compound is a new approach to get the potent and selective compounds, which can act as antiepileptic drugs. In this study we designed and synthesized the hybrid of the coumarin ring with sulfonamide moiety. Coumarin sulfonamide hybrids (**CS1-CS7**) were synthesized by Knoevenagel condensation of methyl anilinosulfonyl acetate with substituted salicyaldehyde in the presence of catalytic base. The synthesized hybrid compounds were characterized by means of mass, ^{1}H & ^{13}C NMR and FTIR spectroscopy, moreover antiepileptic activity was screened through seizure model of epilepsy using pentylenetetrazole and maximal electroshock. According to results, compound **CS-2** remained to be highest potent and presented significant protection at 60 mg/kg in both the seizure models. Furthermore, compound **CS-2** was also evaluated for biochemical and a histopathological study in which no significant results were obtained. In addition to former activities, compound **CS-2** was also examined for liver toxicity.

Keywords: Coumarin, Sulfonamide, Knoevenagel condensation, Salicyaldehyde, Anilinosulfonyl acetate, Antiepileptic activity.

INTRODUCTION

Epilepsy is the common seizure ailment disturbing 40 million people all over the world. It has frequency of at least 0.63% and an annual incidence of 0.05% approximately. It affects males more as compared to females, mostly 80% disorders are found in developing world. Epilepsy characterized as intermittent derangement of nervous system anticipated by excessive and clustered discharge of central nervous tissues functioning muscles. The disease is manifested as abnormal motor, sensory phenomenon often with impaired or loss of consciousness. Seizure is found to be paroxysmal event reflecting abnormal excessive hypersynchronous discharges from a cluster of central nervous system of neurological diseases distinguished by recurrent seizures [1,2]. The development of seizure occurs due to quick changes in ionic constitution, which causes increase in extracellular K^+ concentration and pH in the brain. The increase in extracellular K^+ and pH results in high neuronal excitability which ultimately results in epileptiform activity [3-6].

Normally, alkalosis results in neuronal excitability and multiplies seizure spread, whereas acidosis shows reverse results [7]. The CO_2/HCO_3-buffer mainly potentiate pH buffering equally of extracellular then intracellular spaces therefore these two species are in equilibrium with Zn enzyme with carbonic anhydrase. These additionally assist interconversion of CO_2 and H_2O into H^+ and HCO_3^- [8-10]. Acetazolamide and similar nucleus, from several decades were used as carbonic anhydrase inhibitors (CAIs) and antiepileptics [11,12]. Several sulfonamides were appeared to possess different biological profiles out of which antiepileptic activity was significant and found in compounds like acetazolamide, methazonamide, zonasamide, topiramate which inhibits carbonic anhydrase [13]. Similarly, coumarin nucleus also reported to possess antiepileptic and carbonic anhydrase inhibitory activity [14,15]. Hence considering the above facts we have designed a nucleus which may efficiently lower the epileptic effects in patients and have lesser side effects and toxicity. Therefore, new antiepileptic

agents can be developed by hybrid approach [16,17] development where two or more pharmacophoric nucleus possessing good biological activity can be joined together to attain a single nucleus having better pharmacological action [18].

The above findings suggested that incorporation of sulfonamide nucleus to the coumarin entity can be done to predict antiepileptic activity. All these facts encouraged us to synthesize coumarin fused sulfonamides which can be assumed safe, non-toxic and efficient [19-23]. Designed hybrid for antiepileptic compound (**CS1-CS7**) reflects all the characteristic features necessary for the pharmacological effect such as hydrogen binding domain (HBD), hydrophobic ring (A) and donor moiety (D) as represented in Fig. 1. In this work, the synthesis and antiepileptic activity of coumarin fused sulfonamide derivatives are reported. The biological activities were determined by two established animal convulsive model, a pentylenetetrazole (PTZ) methods and maximal electroshock seizure (MES). The most potent compound was also assessed for histopathological effects as all antiepileptic drugs affect liver functions.

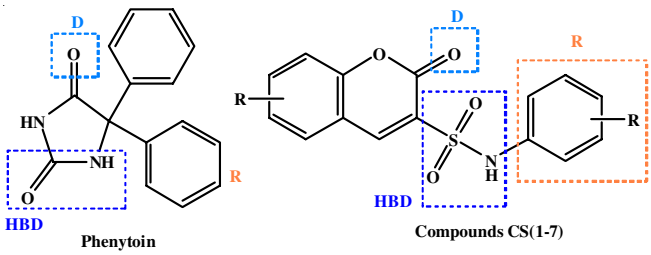


Fig. 1. Antiepileptic agents reflecting necessary pharmacophoric element found in their structure, hydrogen bond donor (HBD), hydrophobic ring (R) and donor moiety (D)

EXPERIMENTAL

Laboratory grade chemicals were used for the synthesis acquired from S.D. Fine Chemicals (Mumbai, India). Open capillary tubes in melting point apparatus (Hicon, India) used to determine melting point and remained uncorrected. Reaction completion was checked by thin-layer chromatography using (9.5:0.5 v/v) chloroform:methanol as moving phase. Shimadzu FTIR spectrophotometer was applied to acquire the FT-IR spectra *via* KBr pellets. ^{13}C NMR and ^{1}H NMR bands were recognized from Bruker AVANC model in DMSO-d_6 using tetramethylsilane (TMS) as internal standard. Mass spectra were documented in Q-T of Micromass, WATERS (LC-MS) by TOF MSEST (8.29e3) and represented as *m/z*.

Synthesis of coumarin sulfonamide hybrids (CS1-CS7): At 10 ºC, substituted aniline (**1**, 10.0 g, 107 mmol) in 150 mL THF was added to trimethylamine (16.3 g, 161 mmol) drop wise with constant stirring of 15 min. The methyl-2-(substituted phenylsulfomyl)acetate (**2**) (22.0 g, 118 mmol) dissolved in THF (25 mL) was added slowly to the above solution at 10 ºC and then the reaction mixture remained stirred for 3 h at room temperature. Water as solvent was added after the reaction completion, by stirring for 15 min in order to isolate the organic layer. The obtained product was desiccated over anhydrous sodium sulfate then under reduced pressure to evaporate crude 2-(substituted phenyl sulfamoyl)acetic acid (**3**) as an oil. Afterword, a solution of 2-substituted hydroxybenzaldehyde (**4**), (10 mmol) was dissolved in absolute ethanol (20 mL) was added methyl-2-(substituted phenylsulfamoyl) acetate (**2**), (11 mmol) and 0.15 mL piperidine. The solution was refluxed for 5 min, then obtained crystalline product after cooling was separated by filtration and washed three times with absolute ethanol in order to obtain hybrid compounds **CS1-CS7**. Thin layer chromatography (TLC) was used for monitoring completion of reaction by using silica gel F_{254} plates (Sigma-Aldrich), using chloroform:methanol (9.5:0.5v/v) as solvent system.

***N*-(3-Amino-4-fluorophenyl)-4-ethoxy-2-oxo-2*H*-chromene-3-sulfonamide (CS-1):** Yield: 84.3%; white solid, m.p.: 313-315 ºC and R_f: 0.56. IR (KBr, ν_{max}, cm^{-1}): 3327.21 (-NH of amine), 1712.79 (-C=O lactone ring), 1325.10 (S-O asymmetric *str.*), 1195.87 (S-O sym. *str.*), 1097.50 (C-F *str.*), 759.96 (-CH of disubstituted ring). ^{1}H NMR (DMSO-d_6, 600 MHz): 7.28-7.02 (m, 4H coumarin-H), 6.67-6.56 (d, 1H, Ar-H), 5.91-5.57 (m, 3H, Ar-H), 4.41 (s, 2H, -NH), 4.04-4.03 (q, 2H, -OC_2H_5), 1.09-1.22 (t, 3H, -OC_2H_5). ^{13}C NMR (DMSO-d_6, 125 MHz): 147.7 (C-F), 81.1 (C-3), 174.0, 61.9, 15.06 (-OC_2H_5), 137.0, 134.1, 117.6, 107.9, 104.3, 150.0, 121.1, 128.8, 125.2, 110.9, 146.6, 162.0 (Ar-C, CN and C=O). EI-MS, *m/z:* 356.30. Elemental analysis of $C_{17}H_{15}N_2O_5SF$ calcd. (found) % (*m.w.* 378): C, 53.96 (53.88); H, 4.00 (3.84); N 7.27 (7.40).

8-Chloro-*N*-(3-hydroxy-4-methoxyphenyl)-2-oxo-2*H*-chromene-3- sulfonamide (CS-2): Yield: 82.3%; white solid, m.p.: 327-329 ºC and R_f: 0.47. IR (KBr, ν_{max}, cm^{-1}): 3395.19 (-NH of amine), 3275.71 (br., OH), 3047.44 (3047.44), 1685.47 (-C=O lactone ring), 1379.18 (S-O asym. doublet), 1163.78 (S-O symm.), 754.70 (-CH of disubstituted ring). ^{1}H NMR (DMSO-d_6, 600 MHz): 7.76 (s, 1H coumarin-H), 7.15-7.12 (m, 3H, coumarin-H), 6.95 (s, 1H, Ar-H), 6.80-6.79 (d, 2H, Ar-H), 4.03-4.04 (s, 2H, -NH & OH), 3.95-3.76 (s, 3H, -OCH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): 56.2, (C-Cl), (C-3) 117.0, (OCH_3) 129.8, 102.1, 109.0, 116.6, 123.0, 124.6, 128.8, 130.5, 131.7, 141.4, 145.3, 146.6, 162.5 (Ar-C, CN and C=O). EI-MS, *m/z:* 366.78. Elemental analysis of $C_{16}H_{12}NO_6SCl$ calcd. (found) % (*m.w.* 381): C, 50.34 (50.04); H, 3.17 (3.03); N, 3.67 (3.32).

8-Chloro-*N*-(4-methoxyphenyl)-2-oxo-2*H*-chromene-3-sulfonamide (CS-3): Yield: 92.7%; creamish white solid, m.p.: 319-323 ºC and R_f: 0.49. IR (KBr, ν_{max}, cm^{-1}): 3271.46 (-NH of amine), 1656.82 (-C=O lactone ring), 789.55(-CH) bending, 1325.22 (S-O asym. *str.*), 1254.84 (S-O sym. *str.*). ^{1}H NMR (DMSO-d_6, 600 MHz): 8.28 (s, 1H, coumarin-H), 6.94 (t, 1H, coumarin-H), 6.51 (d, 2H, coumarin-H), 6.13-6.15 (d, 2H, coumarin-H), 6.35 (d, 2H, Ar-H), 4.41 (s, 1H, -NH), 3.71-3.74 (s, 3H, -OCH_3)^{13}C NMR (DMSO-d_6, 125 MHz): 117.00 (C-3), 55.9 (-OCH_3), 115.1, 115.6, 117.3, 117.8, 123.7, 124.6, 126.4, 128.8, 129.8, 130.7, 145.3, 150.7, 130.1, 162.0 (Ar-C, CN and C=O). EI-MS, *m/z:* 343.7. Elemental analysis of $C_{16}H_{12}N$- O_5SCl calcd. (found) % (*m.w.* 365): C, 52.54 (52.04); H, 3.31 (3.11); N, 3.83 (3.25).

***N*-(3-Methoxyphenyl)-2-oxo-2*H*-chromene-3-sulfonamide (CS-4):** Yield: 91.4%; white solid, m.p.: 312-316 ºC and R_f: 0.46. IR (KBr, ν_{max}, cm^{-1}): 3353.23 (-NH of amine), 3098.13 (-CH of aromatic ring), 1682.52 (-C=O lactone ring), 1330.22 (S-O asym. *str.*), 1153.44 (S-O sym. *str.*), 759.55 (-CH)

bending. ^{1}H NMR (DMSO-d_6, 600 MHz): 7.91 (s, 1H, coumarin-H), 7.12-7.55 (m, 4H, coumarin-H), 5.92-5.94 (d, 2H, Ar-H), 5.09-5.24 (d, 2H, Ar-H), 4.56 (s,1H,-NH), 3.05-3.27 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): (C-3) 117.98, 54.91 (-OCH_3), 161.50, 150.82, 130.07, 130.06, 128.83, 126.61, 125.22, 122.82, 121.26, 108.80, 104.40, 99.54, 138.86, 162.10 (Ar-C, CN and C=O). EI-MS, *m/z:* 315.3. Elemental analysis of $C_{16}H_{13}NO_5S$ calcd. (found) % (*m.w.* 331): C, 58.00 (49.96); H, 3.95 (3.87); N, 4.23 (4.13).

***N*-(4-Methylphenyl)-2-oxo-2*H*-chromene-3-sulfonamide (CS-5):** Yield: 94.3%; white solid, m.p.: 309-311 ºC and R_f: 0.50. IR (KBr, ν_{max}, cm^{-1}): 3311.58 (-NH of *sec*. amine), 3095.79 (-CH of aromatic ring), 1697.38 (-C=O lactone ring), 1366.37 (S-O asym. *str.*), 1170.67 (S-O sym. *str.*), 719.45 (-CH) bending. ^{1}H NMR (DMSO-d_6, 600 MHz): 8.14 (s, 1H, coumarin-H), 7.56-7.95 (m, 4H, coumarin-H), 6.98-6.99 (d, 2H, coumarin-H), 6.78-6.80 (d, 2H, Ar-H), 4.41 (s, 1H, -NH), 2.11-2.15 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): 117.0 (C-3), 26.1 (CH3), 150.8, 130.8, 129.9, 129.8, 128.8, 128.7, 126.8, 125.1, 122.0, 121.2, 116.2, 116.3, 134.8, 162.3 (Ar-C, CN and C=O). EI-MS, *m/z:* 291.3. Elemental analysis of $C_{16}H_{13}NO_4S$ calcd. (found) % (*m.w.* 315): C, 60.94 (60.88); H, 4.16 (3.97); N, 4.44 (4.13).

***N*-(4-Bromophenyl)-2-oxo-2*H*-chromene-3-sulfonamide (CS-6):** Yield: 88.7%; white solid, m.p.: 315-317 ºC and R_f: 0.42. IR (KBr, ν_{max}, cm^{-1}): 3379.58 (-NH of *sec.* amine), 2974.23 (-CH of aromatic ring), 1747.51 (-C=O lactone ring), 1375.25 (S-O *asym. str.*), 1168.56 (S-O *sym. str.*), 883.40 (-C-Br). ^{1}H NMR (DMSO-d_6, 600 MHz): 8.21 (s, 1H, coumarin-H), 7.01-7.21 (m, 4H, coumarin-H), 5.98-6.21 (d, 2H, Ar-H), 5.78-5.80 (d, 2H, Ar-H), 4.01 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 125 MHz): 117.05 (C-3), 150.4, 132.5, 132.2, 130.6, 128.4, 128.2, 126.1, 125.7, 122.3, 121.3, 118.5, 115.9, 136.8, 162.1 (Ar-C, CN and C=O). EI-MS, *m/z:* 356.2. Elemental analysis of $C_{15}H_{10}NO_4SBr$ calcd. (found) % (*m.w.* 378): C, 47.39 (47.14); H, 2.65 (2.27); N, 3.68 (3.46).

***N*-(3-Amino-4-fluorophenyl)-2-oxo-2*H*-chromene-3-sulfonamide (CS-7):** Yield: 89.3%; white solid, m.p.: 320-323 ºC and R_f: 0.43. IR (KBr, ν_{max}, cm^{-1}):3327.21 (-NH of aliphatic *sec*. amine), 3120.82 (-CH of aromatic ring), 1620.21 (-C=O lactone ring), 1367.53 (S-O asym. *str.*), 1209.37 (S-O sym. *str.*), 1136.07 (C-F *str.*). ^{1}H NMR (DMSO-d_6, 600 MHz): 8.39 (s, 1H, coumarin-H), 7.01-7.21 (m, 4H, coumarin-H), 5.98 (s, 1H, Ar-H), 5.78-5.80 (d, 2H, Ar-H), 4.11-4.21 (s, 2H, - NH_2), 4.01 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 125 MHz): 117.31 (C-3), 150.2, 144.5, 130.1, 128.2, 126.3, 125.7, 122.3, 121.0, 117.0, 107.9, 104.3, 134.0, 147.2, 162.1 (Ar-C, CN, CF and C=O). EI-MS, *m/z*308.6. Elemental analysis of $C_{15}H_{11}N_2O_4SF$ calcd. (found) % (*m.w.* 334): C, 53.89 (53.36); H, 3.32 (3.02); N, 8.38 (8.13).

Pharmacology: The albino rats (150-200 g) of either male or female were procured from National Institute of Biologicals, Noida, India. These animals were stayed in animal house of Department of Pharmacy, IEC Group of Institutions, Greater Noida, India for acclimatization to laboratory conditions. All animals were provided with normal laboratory diet, room temperature and environmental conditions. A 12:12 h light/dark cycle were maintained so that animals get acclimatized all over the experimental protocol. All the synthesized compounds were administered orally as a suspension in Tween 80 (0.5%). Pentylenetetrazole (60 mg/kg) dissolved in Tween 80 (0.5%) was given to control group and severity and onset of convulsions was observed. Anti-epileptic activity data wasshownby means of mean ± SEM. By using student's t-test along with one-way ANOVA, statistical variance between control and test groups were recorded and evaluated through Origin software. The Committee for the Purpose of Control and Supervision of Experiments with IAEC approval no. IEC/IAEC/2021/05 has permitted the experimental protocol.

Acute toxicity studies: Acute toxicity experiment were executed as per OECD Guidelines 423 for synthesized hybrid compounds [24]. Female albino rats who were nulliparous or non-pregnant balancing 150-200 g were randomly selected, (n = 3) in each group. According to OECD guidelines 5, 50, 300 and 2000 mg/kg body weight were dose selected for the toxicity studies. In order to carry out limit test at dose equalto 2000 mg/kg body weight was supported with six animals. Animals were perceived separately after treating, at least once for the duration of first 30 min, occasionally through the first 24 h, with distinctcare given for the first 4 h and regularly monitored for complete 14 days. Everyday monitoring of rat cages limit an assessment of signs of harmfulness like sleep, inactivity, tiredness, tread, tremor, convulsions, salivation, lacrimation, perspiration, diarrhea, evacuation and also alterations in skin, eyes, mucus membrane, breathing rate, circulatory rate, *etc*. Incidence of mortality if exist, was observed for the duration of about 14 days.

Maximal electroshock (MES) method: In present study, MES seizure model was used to consider antiepileptic activity of all test compounds in a dose of 15, 30 and 60 mg/kg orally. Albino rats weighing 150-200 g were randomly taken for study by dividing them into groups of (n = 6) animals. Group I work for control by receiving comparablevolume of distilled water while group II is administered with phenytoin sodium, which served as a standard. Similarly other groups treated with hybrid compounds with doses of 15, 30 and 60 mg/kg (orally), respectively. Seizures were generated in rats with the help of electroconvulsometer using ear clip for 0.2 s to deliver electric shock of 150 mA. After 30 and 240 min of administration of test compounds, the animals were observed closely in different phase's convulsive phases (flexion, extension, clonus and stupor) for 2 min. The abolition or disappearance of extensor phase was used as positive response which was recorded in terms of percentage seizure inhibition between test and standard drug [25].

PTZ induced seizures method: Albino rats of weight 150-200 g were designated randomly and distributed into groups, each containing (n = 6) animals. Group I animals were given a comparable volume of purified water orally to assist as control whereas group II was administered through diazepam which served as a standard. Similarly, other groups remained treated with hybrid compounds at measure of 60 mg/kg (orally). pentylenetetrazole (PTZ) was administered (i.p.) to induce seizures in rats at the dose of 60 mg/kg. After 60 min of pre-

treatment with hybrid compounds and standard drug, PTZ was given to all groups and rats were observed for 30 min to invent interruption of onset, which was considered in relation to the control group. After administration of pentylenetetrazole, animals were observed for onset of convulsion for 30 min and then within 48 h were recorded. If any test drug showed delay in onset of convulsion and absence of mortality then that test candidate can be regarded as effective antiepileptic agent.

Enzyme activity and estimation of histopathology of rat liver: In order to determine the hepatic enzyme and overall protein, most potent compound was provided in a quantity of 60 mg/kg/day for two weeks in methylcellulose to one and all animals [26]. On completion of specified time, animals were anesthetized using ether and blood was taken from liver in order to check the biochemical limitations for example SGOT, SGPT, alkaline phosphate (ALT) and inclusive protein. The synthesized hybrid compounds were also examined for liver toxicity in albino rat relative to standard phenytoin, 60 mg/kg. The synthesized compounds (**CS1-CS3**) were injected intraperitoneally (i.p.) in a dose of 60 mg/kg for animal activity. After drug administration, rat was sacrificed and liver was removed and stored in 10% formalin after washing with Krebs solution; then the specimen was observed under microscopic. The studies revealed that animal treated with phenytoin, effects the hepatotoxicity, but the animals administered with test compounds had shown decreased or almost similar necrosis and inflammation as compared to standard.

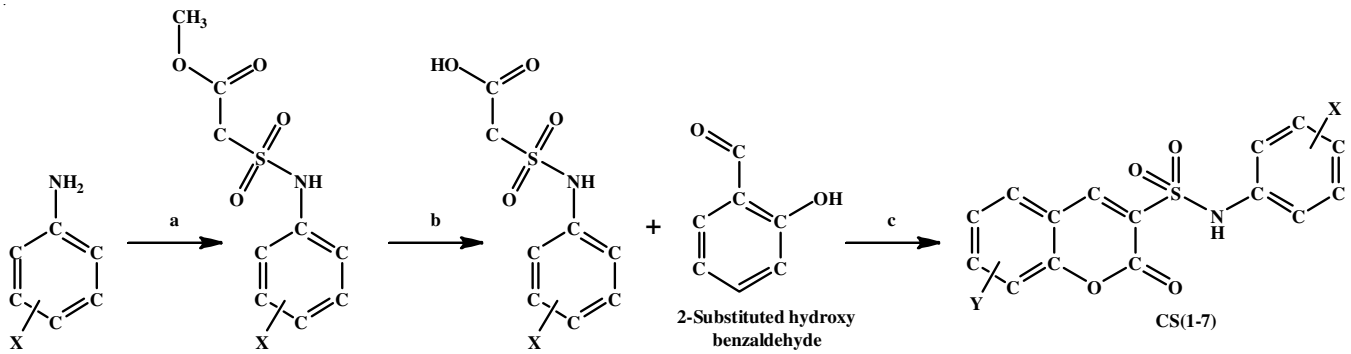
RESULTS AND DISCUSSION

In this work, Knoevenagel condensation method was applied for synthesizing coumarin sulfonamide hybrid is shown in **Scheme-I**. The hybrid compounds synthesized were in good amount and their structural arrangement was established using spectral examinations. Although very less work is reported for the formation of coumarin fused sulfonamides, generally either Knoevenagel condensation method is utilized to obtain coumarin-3-sulfonamide [27,28] or by the condensation of aromatic amines with coumarin-3-sulfonyl chlorides [26]. Methyl-2-(substituted phenyl sulfamoyl)acetate was prepared in a reasonable yield on reacting substituted aniline with methyl(chlorosulfonyl)acetate, which further undergoes hydrolysis to give 2-(substituted phenyl sulfamoyl)acetic acid which on condensation with 2-substituted hydroxyl benzaldehyde produces coumarin-sulfonamides hybrids (**CS1-CS7**). Reaction completion and compound purity was ascer-tained by melting point and TLC by means of chloroform: methanol (9.5:0.5 v/v) as a solvent. IR spectra in the series of 1750-1650 cm^{-1} and 3500-3300 cm^{-1} provide confirmations of –C=O (lactone ring) and N-H spectral bands, respectively. ^{1}H NMR spectra had shown a characteristic peak of methine proton at 7.28-8.25 ppm, which approves the cyclization reaction for the establishment of coumarin fused sulfonamides. ^{13}C NMR spectra confirms presence of (C-3), -CN,–C=O in range of 110, 140 and 160 ppm, respectively.

Pharmacological activity

Acute toxicity studies: The albino male/female rats (150-200 g) were used for preliminary studies to determine actual LD_{50} values. The acute toxicity studies were carried out to determine the safe dose for further studies. Coumarin sulfonamide hybrids for a dose of 300 mg/kg body weight did not revealed any signal of toxicity and mortality but when the dose was increased to 2000 mg/kg mortality as well as toxicity was observed after 14 days of experiment. Hence, it was concluded that the toxic dose for test compounds was 2000 mg/kg of the body weight even though 300 mg/kg stood to be safe dose conferring to OECD 423 guidelines. Hence, 1/10th, 1/5th and half value of 1/10th doses of accepted dose being 15, 30 and 60 mg/kg, respectively remained certain to perform anti-epileptic study.

MES and PTZ models: Synthetic coumarin fused sulfonamides prepared were screened using two models, maximal electroshock (MES) and pentylenetetrazole (PTZ). For MES



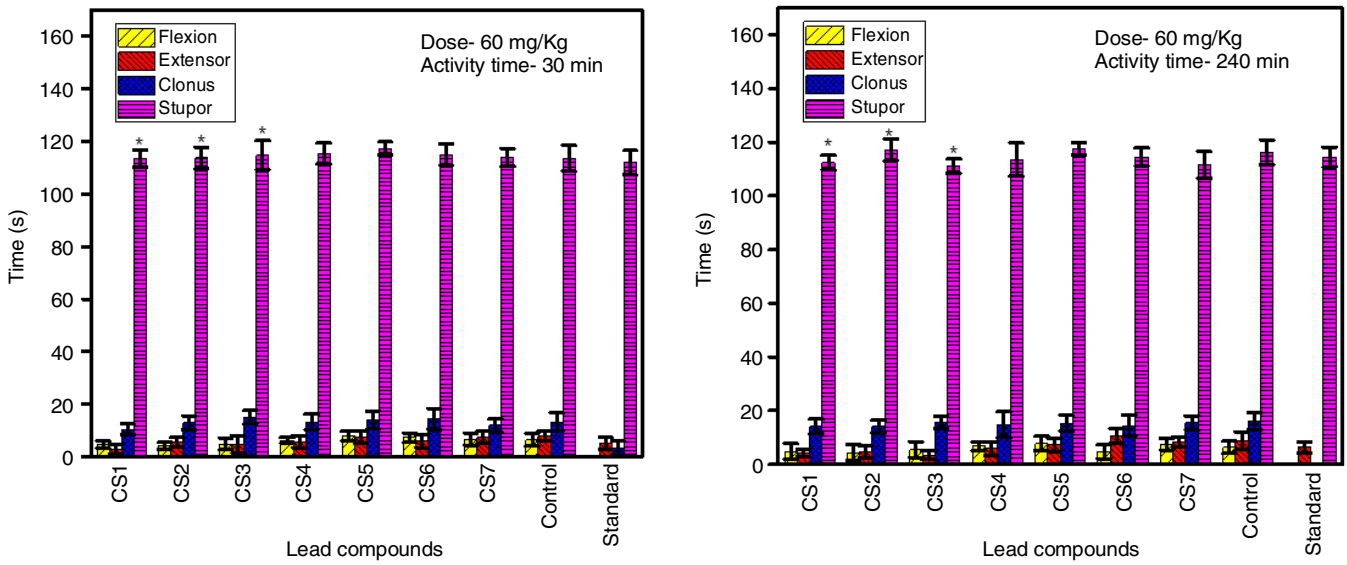
Compound	X	Y
CS-1	4-F, 3-NH_2	8-OC_2H_5
CS-2	4-OCH_3, 3-OH	8-Cl
CS-3	4-OCH_3	8-Cl
CS-4	4-OCH_3	–
CS-5	4-CH_3	–
CS-6	4-Br	–
CS-7	4-F, 3-NH_2	–

Scheme-I: Synthesis Scheme: Reagents and conditions: (a) $ClSO_2CH_2COOCH_3$, triethylamine/THF, N_2, room temperature, 3 h (b) 10% NaOH in H_2O. (c) Piperidine, ethanol, reflux

study, compounds were given orally in a dose of 15, 30 and 60 mg/kg to albino rats and duration of different convulsive phases as flexion, extensor, stupor and death/recovery were observed at two different time intervals *i.e.* 30 and 240 min, respectively. In MES convulsion, all the synthesized compounds showed abolition of extensor phases so we can concludethat all compounds have seizure preventive threshold property. The test compounds (**CS1-CS3**) had shown abolition in extensor phase ranging from 80-100% protection at a dose level of 60 mg/kg as presented in Fig. 2, at time interval of 30 and 120 min indicating fast onset and long duration of action. Compounds (**CS1-CS7**) did not show reduction in extensor phaseat doses 15 & 30 mg/kg hence, there was no significant anti-epileptic activity at these doses. Compound **CS-2** was the most active compound having 100% protection in both the screening models (Table-1). It had shown speedy onset and extensive duration of antiepileptic action as associated through control group. Similarly in PTZ screening model, test groups were treated with dose of 60 mg/kg of the synthesized compounds and after 30 min all the test groups showed protection against seizure threshold but compound **CS-2** showed 100% protection and complete abolition of convulsion as presented in Fig. 3.

It was further confirmed that configuration and effect of substituents on coumarin fused sulfonamides affected the anti-epileptic activity. *in vivo* results in albino rat showed that the presence of both X and Y substituentsin compounds **CS-1**, **CS-2** and **CS-3** demonstrated greater seizure protection to presence of X substituent (**CS-4**, **CS-5**, **CS-6** and **CS-7**). Thus it can be confirmed that hybrid of coumarin moiety to sulfonamide showed significance in antiepilepsy.

It had also been observed that compounds **CS-2** and **CS-3** showed 100% and 97% seizure protection respectively whereas compound **CS-1** showed 88% protection; it can be explained by the chemical nature of Y substituents. Compounds **CS-2** and **CS-3** had electron withdrawing as Y substituent and **CS-1** had electron donating group as Y substituent. Thus, it can be concluded that if Y substituents have electron withdrawing nature they will have more seizure protection ability as compared to Y substituents possessing electron donating groups.



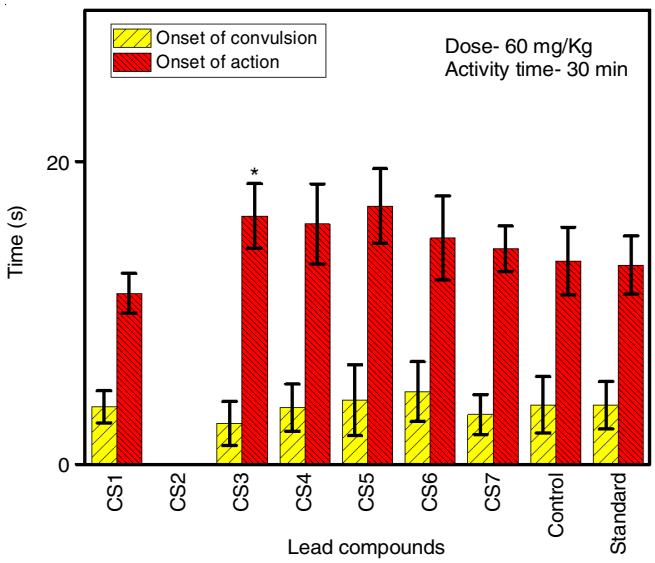
The data were examined by means of one-way ANOVA thenstudents's; t- test; *$p < 0.01$ comparativetoward control group shown in Table-3

Fig. 2. Result of synthesized hybrid compounds for convulsive phase

TABLE-1
MES AND PTZ% PROTECTION OF HYBRID COMPOUNDS

Compound	X	Y	Antiepileptic activity		
			MES[a] sceen (% protection)		PTZ[b] screen (% protection)
			30 min	240 min	
CS-1	4-F, 3-NH_2	8-OC_2H_5	*88.21	*84.52	88
CS-2	4-O CH_3, 3-OH	8-Cl	*100	*100	*100
CS-3	4-OCH_3	8-Cl	*92.74	*91.93	97
CS-4	3-OCH_3	–	64.33	65.78	76
CS-5	4-CH_3	–	77.89	77.89	83
CS-6	4-Br	–	80.45	80.45	68
CS-7	4-F, 3-NH_2	–	63.72	62.89	77
Phenytoin	–	–	100	100	100

[a]Dose of 60 mg/kg administered. The animals were inspected 30 min and 240 min afterward administration.
[b]60 mg/kg dose was administered and animals examined after 30 min.
*$p < 0.01$ relative to control group.



The data were examined by means of one-way ANOVA then students's t- test; *$p < 0.01$ relative to control group shown in Table-3

Fig. 3. Result of pentylenetetrazole for onset of seizure

Serum liver enzyme and histopathological evaluation: Most of the drugs do not show the desired pharmacological action due to its hepatotoxicity. It has been reported in literature that carbamazepine and valporic acid are the drugs showing severe hepatotoxicity, whereas phenytoin also found to possess acute acetaminophen toxicity [29]. From the above findings, comp-ound **CS-2** remained to be maximum dynamic at 60 mg/kg equally in MES and PTZ convulsive models; this hybrid compound was directed chronically to animals for period of 2 weeks and biochemical limits are presented in Table-2. The results displayed SGOT, SGPT, alkaline phosphatase (ALP), overall albumin also the entire protein of compound **CS-2** does not display any important growth or reduction levels as associated with standard.

In animals phenytoin treated liver, was found to be hepatotoxic as well shows acute necrosis and inflammation, these observations were recorded in transverse section of liver. Animals of induced control group treated, Tween 80 (0.5%) showed fatty liver then necrosis, however liver of the animals treated using test compound, **CS-2** showed very low necrosis as well as inflammation, as presented in Fig. 4. Hence, it was confirmed that test compound is less hepatotoxic than standard.

Conclusion

A hybrid of coumarin sulfonamide analogues (**CS1-CS7**) were synthesized and evaluated for their antiepileptic action through maximal electroshock (MES) and pentylenetetrazole (PTZ) models. Hepatic as well as acute toxicity of synthesized molecules were also determined. Compound **CS-2** was found to be most potent with 100% protection at 60 mg/kg, which was comparable to phenytoin for antiepileptic action in both MES and PTZ convulsive models. Furthermore, these compounds did not show any toxicity and hepatotoxicity at tested dose. As perassociation with the standard drug, the synthesized derivatives **CS-1** to **CS-3** were more active andshowed less toxicity. Thus, coumarin fused sulfonamide would lead to the development of ideal pharmacophoreas antiepileptic agents. The test compound validated the pharmacophore model for antiepileptic activity by hydrophobic domain, lipophilic unit, electron withdrawing group and electron donor and acceptor in all the synthesized hybrid compounds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

TABLE-2
COMPOUND CS-2 HEPATIC ENZYME AND TOTAL PROTEIN DETERMINATION

Compound	SGOT ± SEM	SGPT ± SEM	ALP ± SEM	Total albumin (g/100 mL) ± SEM	Total protein (g/100 mL) ± SEM
CS-2	36.35 ± 2.30	28.58 ± 1.84	66.03 ± 2.13	1.69 ± 0.034	4.75 ± 0.38*
Control[b]	39.18 ± 1.82	31.62 ± 1.33	53.17 ± 2.05	2.37 ± 0.056	7.59 ± 0.39

[a]Data were investigated by means of ANOVA then student's t test on behalf of n = 4 at *$p < 0.05$.
[b]For 15 days Control set animals were directed by means of 0.5% methyl cellulose.

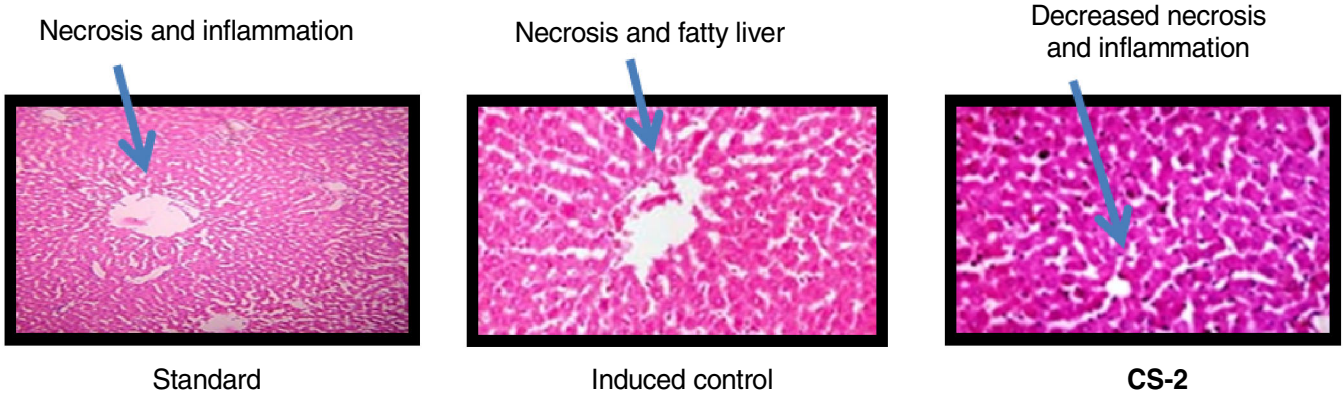


Fig. 4. Transverse section of hepatic liver of **CS-2** comparable with standard phenytoin

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