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Design, Synthesis, *Insilco* Study and Anticonvulsant Activity of 4'-Methoxy-5,7-Dihydroxy Flavone Scaffold Hybrids

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ABSTRACT

Background: *Epilepsy/Convulsion is a prevalent psychological condition. Treating convulsions remains a complex task because some people do not respond to a range of available drugs and have adverse effects.*

Aim and Objective: *Acacetin or the 4'-methoxy-5,7-dihydroxy flavone ring, is a significant and often used precursor in synthesizing bioactive compounds. In this research, a distinct series of 7- [(substituted nitrogen-containing heterocyclic compound) alkoxy] flavone was designed, synthesized and screened for antiepileptic activity in mice.*

Methods: *Acacetin (4) and 1,4-dibromo butane were incorporated with the base using potassium carbonate and solvent using DMF in anhydrous form to create 4'-methoxy-5-hydroxy flavones (6a-6g), which were then further processed with different substituted N'-heterocycles. Elemental and spectral analysis (UV, IR, NMR, Mass) verified the structure assigned to the synthesized acacetin derivative. The binding affinities were assessed using Insilco molecular docking against the GABA A receptor (PDB id: 4COF). Some physical-chemical variables have been determined to predict the toxicity profile and ADME parameters. The antiepileptic properties were examined using a Pentylenetetrazol Induced Seizure. To evaluate the in vivo effects of these compounds on convulsions, adult Swiss albino mice (wt.: 30 g-40 g) were treated with compound 6a-6g, a healthy control and disease control.*

Result: *All compounds exhibit favorable toxicological profiles, docking results (Highest Docking Score: -Kcal/mol) and pharmacokinetic parameters. Furthermore, compared to acacetin (4), most derivatives exhibit noticeably higher anticonvulsive (6c, 6d, 6f, 6g) activity at a dose of 10 mg/kg.*

Conclusion: *According to our investigation, commercially produced 4'-methoxy,5,7-dihydroxy flavone derivatives may be used as a potential drug to treat convulsion.*

Keywords: Flavonoids; Flavone; Acacetin; Anticonvulsant; Pentylenetetrazole (PTZ) induced seizure; 4COF

INTRODUCTION

The most prevalent neurological and non-communicable brain condition affecting people of almost all ages is epilepsy. There are more than 50 million epileptics in the world today. Nearly 80% of epileptics, according to WHO data, reside in low- and middle-income nations. Certain traditional Antiepileptic Drugs (AEDs), such as Carbamazepine (CBZ), Phenobarbital (PB), Phenytoin (PHT) and Valproate (VPA), are used in the treatment of epilepsy. However, the available medicines exhibit unfavorable side effects. Additionally, some patients with epilepsy exhibit resistance to the AEDs that are currently on the market. Therefore, the development of a novel class of active molecules with improved antiseizure efficacy and no side effects is desperately needed. Therefore, since natural source compounds don't have any harmful side effects or drug resistance, they are the best option for finding effective epilepsy drugs. As such, research on natural compounds yields encouraging findings for the discovery of AEDs [1].

The various AEDs' intricate modes of action consist of: (i) altering nerve excitability through voltage-activated sodium channel blocking; (ii) by affecting GABA receptors through direct Positive Allosteric Modulation (PAM) of the GABAA receptor or indirectly by raising GABA levels through GABA transporter or GABA transaminase inhibition; (iii) voltage-gated calcium channel blockage; (iv) Peroxisome proliferator-activated

receptor alpha activation. GABAergic inhibition is believed to be the primary mechanism rebalancing the inhibition of hypersynchronous neuronal discharges and glutamatergic excitation in relation to epilepsy. Additionally, GABA functions by activating either metabotropic (GABAB) or ionotropic (GABAA) receptors. The two most widely used standard medications that work by blocking voltage-gated sodium ion channels (VGSCs) are phenytoin and carbamazepine. These medications are additionally shown to improve GABA-mediated response and reduce seizures [2,3].

According to epidemiological research, eating flavonoids may reduce the risk of mood swings and neurological condition. The majority of fruits and vegetables that are grown in the ground contain flavonoids. Flavonoids represent a significant class of natural products. Fruits, vegetables and coffee all contain this kind of secondary plant metabolite, which has a polyphenolic structure. The flavonoid consists of a heterocyclic ring (C) with an oxygen insertion and two phenyl rings (A and B) that make up its chemical structure. There are fifteen carbons in total. These are benzo-γ-pyrone derivatives that have a pyran ring (1). Flavonoids are thought to have a wide range of health benefits, such as anti-inflammatory, anti-tumor, anticancer, antiviral and anti-HIV properties [4].

Flavone (2) is one of the classes of flavonoids as Apigenin has three hydroxyl groups inserted at the 4',5,7-position of the flavone basic structure, it is known as the 4',5,7-trihydroxy flavone (3). Strong anticancer, anti-diabetic, anti-Alzheimer and anti-inflammatory properties have been reported for apigenin derivatives. Chemically, 4'-methoxy apigenin, also known as 5,7-dihydroxy-4'-methoxyflavone (4), is formed when the methoxy group in apigenin is substituted for its 4'-hydroxy group. It is commonly known as acacetin. It has been linked to broad-spectrum pharmacological traits like *in vitro* cardioprotective, anti-aging, anti-cancer and antibacterial qualities. Strong antidepressant and anticonvulsant properties are exhibited by methoxy apigenin [5,6] (Figure 1).

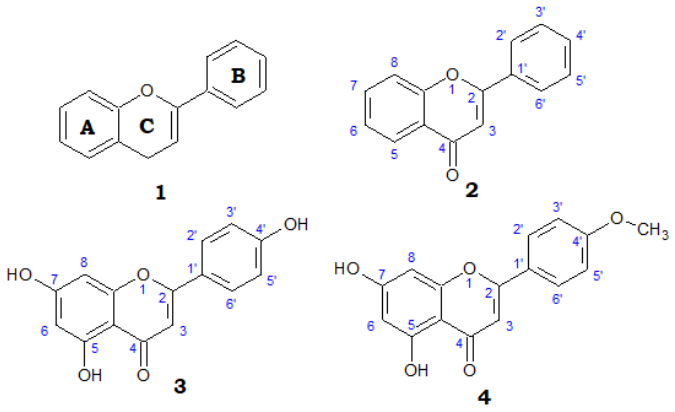

Figure 1: 1-Basic structure of flavonoids; 2-basic structure of flavone; 3-apigenin; 4-acacetin.

Many of the important classes of synthetic and natural chemicals known as nitrogenous heterocycles have advantageous pharmacological characteristics. N'-heterocycles comprising substances like morpholine, nicotinamide, benzimidazole, pyridine, etc. possess anticonvulsant properties [7]. In contrast, if an n-carbon chain spacer is placed in between the parent molecule and different N'-heterocycles, lipophilicity is enhanced [8]. Henceforth, the aim of this research was to deliberate the development of 4'-methoxy-5,7-dihydroxy flavone scaffold hybrids (6a-6g), as anticonvulsant agents.

MATERIALS AND METHODS

Rationale and design

AEDs like phenytoin, carbamazepine, progabide, remacemide and ralitoline are frequently used in clinical studies to treat epileptic patients. A general model of anticonvulsant action was proposed as a result of the structural analysis of these AEDs and it implies that the model should include a distal region with various functional groups that form hydrogen bonds and two aromatic rings or their equivalent arranged in a particular spatial arrangement. In their model, Dimmock, et al., described the different pharmacophoric elements-the distal aryl ring (C), Hydrogen Bonding Domain (HBD), electron donor moiety (D) and lipophilic aryl ring (A)-that are necessary for interaction at the binding site [9]. The designed 4'-methoxy-5,7-dihydroxy flavone scaffold hybrids 6a-6g possess all these pharmacophoric elements as shown in Figure 2.

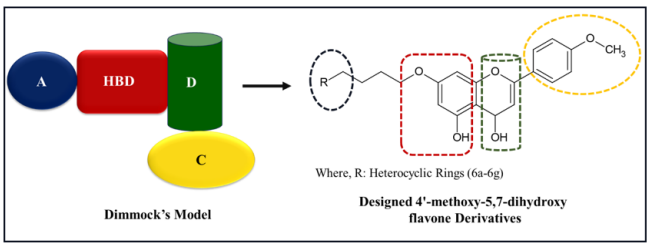

Figure 2: Features of Dimmock's model's pharmacophory and the designed derivatives 6a-6g.

RESULTS AND DISCUSSION

Chemistry

Within this research, 4'-methoxy-5,7-dihydroxy flavone Scaffold hybrids (acacetin derivatives) were effectively synthesized. The general method shown in Scheme 1 was followed to synthesize the derivatives (6a-6g). The target compound's synthesis required Compound 5 as a crucial step. There are two steps involved. The first step involved treating acacetin with dibromoethane for two hours at 120°C in anhydrous N, N-Dimethylformamide (DMF) and potassium carbonate as a base. This resulted in compound 5, which has a carbon chain at acacetin's C7 position. In

the second step, the intermediate ring system (5) was connected to various N-heterocycles in anhydrous DMF at 80°C for 3 hours (Figure 3).

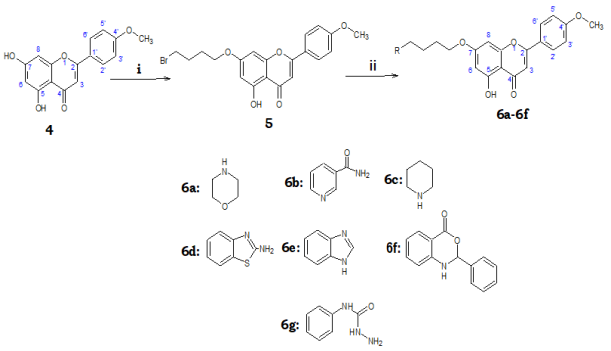

Figure 3: Synthesis of substituted N' heterocycles containing acacetin derivatives (6a-6g). **Note:** i: $Br(CH_2)4Br$, K_2CO_3, DMF,120°C, 2hr; ii: N-heterocyclic moieties, DMF, 80°C, 3hr.

Predicted reaction mechanism

A retrosynthetic investigation of the methoxy analog of Apigenin (III) discloses 2,4-benzoyloxyacetophenone (II), which is readily produced by benzoylation of 2,4,6 trihydroxy acetophenone (I). II cycles in two stages in actuality. First, II is changed into 2,4-dihydroxydibezoylmethane (IV) by a base-catalyzed rearrangement. IV can then be separated and cyclized to produce flavone (1) when acid is present. Add 1,4-dibromo butane to the hydroxyl group in the presence of DMF after these addition reactions. The hydroxyl bromide is eliminated as shown in Figure 4.

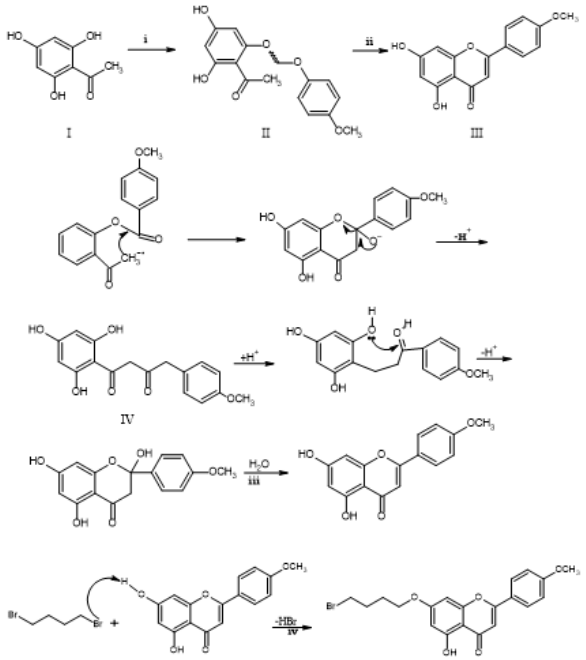

Figure 4: Predicted reaction mechanism.

We created 4'-methoxy-5,7-dihydroxy flavone Scaffold Hybrids (acacetin derivatives), which were more lipophilic due to the acacetin ring system being connected to the N'-heterocycle analog at the C-7 position by a 4-chain carbon spacer, to improve the biological activities of acacetin. These synthetic derivatives were selected to investigate the dimensions of the linked groups and the importance of the substitution. All newly synthesized substances provided satisfactory analytical, physical and spectroscopic data that were fully consistent with the structures they displayed. Melting point analysis and acetic acid, ethyl acetate and toluene in Thin-Layer Chromatography (TLC) as a mobile phase at a ratio of 1:15:34 were used to assess the purity of each newly synthesized derivative.

Spectral analysis

Spectral analysis was used to confirm the structures of synthesized 4'-methoxy-5,7-dihydroxy flavone Scaffold Hybrids (acacetin derivatives).

UV-visible spectroscopy

The UV absorption spectra showed characteristic peaks from 203 nm-260 nm and 290 nm-342 nm demonstrating that the compounds contain the n-π^* and π-π^* transitions because of the presence of hetero atom and aromatic ring system which cause bathochromic shift. Literature have revealed

that flavones and flavanols exhibit two absorption bands at band-I, 320-385 nm and band-II, 250 nm-285 nm. The UV spectral data of all the synthesized compounds showed these two bands associated with the transitions in the A-ring and B-ring systems respectively. These are also showing the presence of C-N transition.

FTIR spectroscopy

Their infrared spectra demonstrated the structures of the synthesized derivatives. Every compound's FTIR spectrum was examined between 4000-600 cm^{-1}. The 6a-6g showed the IR absorptions characteristics of 1170.04-1315.73cm^{-1}(C-O str), 854.74-1019.45cm^{-1}(C=C str aromatic), 772.21-3009.91cm^{-1} (C-H str), 1255.10-3396.60 cn^{-1} (OH bending),1512.20-3399.26 cm-1(N-H str) and for 4' carbon spacer and nitrogen containing heterocyclic derivative attached at 2778.81cm^{-1} (C-H bending) and (C-N str aromatic) indicating that the compounds are successfully synthesized.

NMR spectroscopy

NMR analysis of synthesized derivative indicated signals for -CO-CH=CH- at 6.24-7.01 δ and a multiplet for aromatic protons at 7.05-8.18 δ (Ar-H), singlet for -OCH3 at 3.82 δ and phenolic -OH at 12.21-12.61 δ.

Each synthetic derivative generated data that showed a satisfactory correlation with the chosen structure.

Mass spectroscopy

All of the synthesized derivative, 6a-6g, displayed the anticipated molecular weight, according to the mass spectra. This shows that obtained molecular weights of all the synthesized derivative match the structures that were assigned.

Pharmacology

Anti-convulsant activity: *In-vivo* convulsant activity of all the synthesized derivatives on Adult Swiss albino mice of Either sex (wt. 30-40g) was performed using a Pentylenetetrazol Induced Seizure. As represented in Figures 3 and 4, Compounds 6a, 6b and 6e possess moderate to good anticonvulsant activity and compounds 6c, 6d, 6f and 6g possess strong anticonvulsant activity as compared to that of a control group (10mg/kg p.o). All the synthesized derivatives are found to be more active than Acacetin (4) except 6a.

Statistical analysis: Anticonvulsant activity of synthesized compound (4, 6a-6g) determined by Pentylenetetrazol (PTZ) induced seizure. Mice were divided by three groups (control, standard and teste) consisting three animals each. The latency of first myoclonic jerk, total seizure duration and percentage of mortality were observed. The data was analyzed by one-way ANOVA Bonferroni's multiple comparison teste using Graph Pad Prism Software. A value of $p<0.05$ was considered as statistically significant (Figure 5).

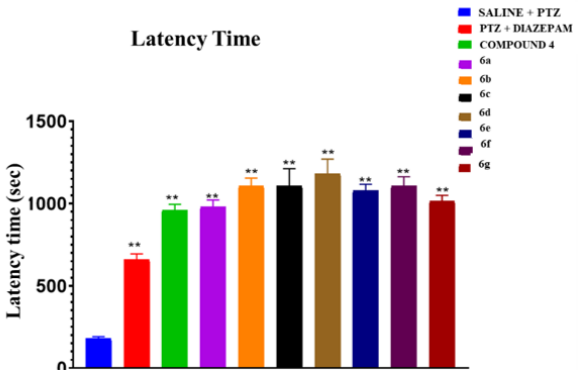

Figure 5: Data expressed as mean ± SEM (n=3) **P<0.01 statistically significant (4, 6a-6g) treated Mice *Vs.* Diazepam+PTZ Mice. # P< 0.05 and ## P< 0.01 Test compound treated mice vs S4 compound Treated Mice (One-way ANOVA followed by post hoc Bonferroni Multiple comparison test).

Figure 4: Data expressed as mean ± SEM (n=3) **P< 0.01 statistically significant (4, 6a-6g) treated Mice vs saline + PTZ Mice. # P<0.05 and ## P<0.01 Test compound treated mice vs 4 compound Treated Mice (One-way ANOVA followed by post hoc Bonferroni Multiple comparison test).
The potency of all compounds as compared to standard from higher to lower is as follows,
6f <6g<6d <6c<6e<6b<4<6a
The mortality of the treated animal by synthesized compound is observed in 33.3% in comparison with control group.

Insilico study

Molecular docking

To learn more about the molecular interactions and binding mechanisms, of Synthesized derivatives, we docked these derivatives with GABA A receptor with PDB id-4COF using a molecular docking software Swiss dock and UCSF Chimera. It has been discovered that synthesized derivatives (6a-6g) with pertinent receptors have docking scores higher than those of the parent drug, acacetin (4).
The molecular docking calculations including docking score, estimated free binding energy values (kcal/mol) and 2D and 3D interaction of compounds (4, 6d and 6g) with key residues of amino acids in the active site of the receptor are shown in Table 1 and Figure 5, 6 and 7.
Every synthesized derivative has the same binding mechanism and is docked at almost identical locations inside the active region of the receptor. The docking mechanism involves all important interactions like van der Walls forces, hydrogen bonding and hydrophobic interaction (Pi-alkyl and

pi-pi T-shaped interactions).

The active synthesized derivative 6d revealed that it binds to the active region of GABA A receptor by forming a conventional hydrogen bond with THR E:256 and THR B:256; carbon hydrogen bond with ILE E:255, Van der Waals interaction with ILE C:255, ALA C:252, THR C:256, ALA B:252, ILE D:255, THR D:256, ILE B:255, THR A:256, THR A:256, THR A:260, THR A:263, LEU B:259; alkyl interactions with ALA A:252, ILE A:255, ALA E:252, ALA D:252, LEU A:259 as shown in Figure 6. Synthesized derivative 6g displayed an important interaction through alkyl and pi-alkyl with HIS E:267, ILE E: 264, LEU A:259; pi-pi T shaped stacked with TYR E:220; Vander Waals interaction with THR E:271, LYS A:274, TYR E:143, PHE E:221, LEU E:268, PRO E:228, THR E:263, THR E:260, LEU E:259, ILE E:255; conventional hydrogen bond through GLU A:270, THR E:225; carbon hydrogen bond with THR E:256, THR A:266, GLN E:224 and pi-sigma interaction with THR A:263 as shown in Figure 6 (Figure 7 and Table 1).

Table 1: Docking score and estimated free binding energy values (kcal/mol) of synthesized derivatives.

Code No.	Docking score	Free binding energy values (kcal/mol)
Parent (4)	-6.92	-2374.14
6a	-8.57	-2338.51
6b	-9.28	-2369.21
6c	-8.7	-2377.32
6d	-9.07	-2413.09
6e	-8.72	-2368.05
6f	-8.5	-2338.41
6g	-9.32	-2426.08

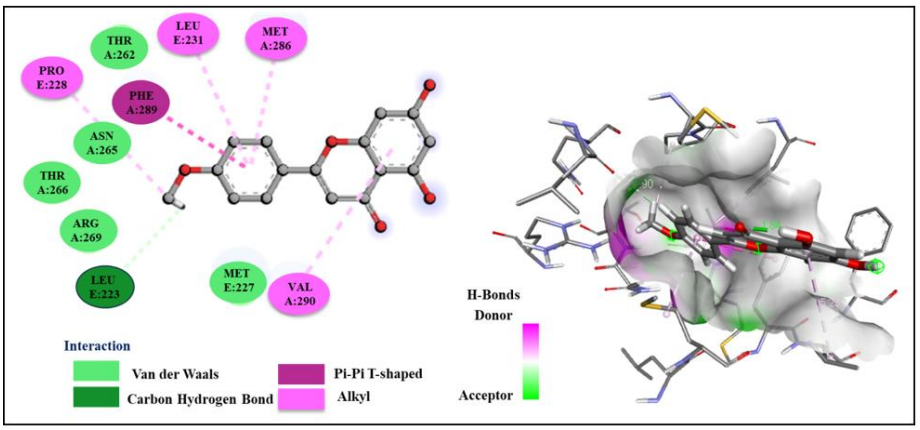

Figure 6: 2D and 3D interaction of parent acacetin.

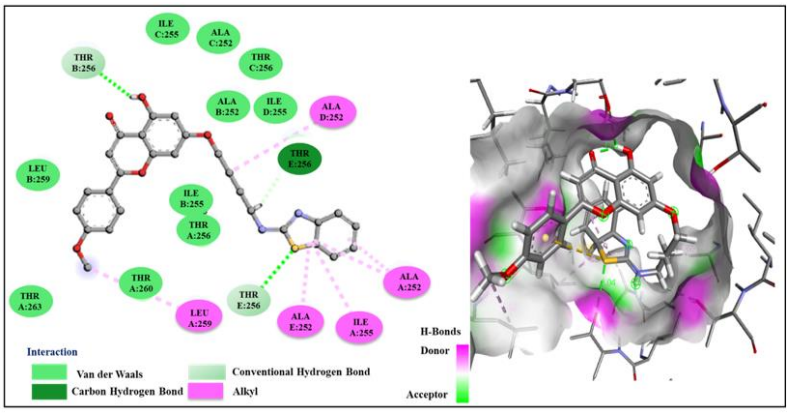

Figure 7: 2D and 3D interaction of synthesized derivative 6d.

In a mechanistically interesting reaction the carbonyl of a delta-alkenyl ketone traps the pi-complex obtained by treatment of the olefin with an electrophile, resulting the corresponding acetal (6). This unique approach to electrophile-induced cyclisations takes advantage of the nucleophilicity of carbonyl oxygen of the ketone (Figure 8).

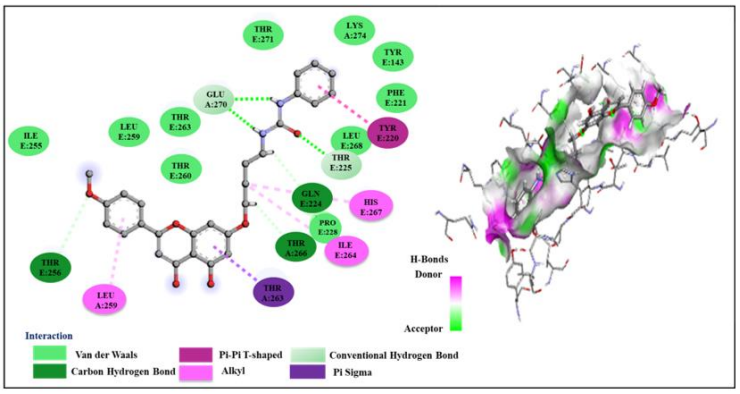

Figure 8: 2D and 3D interaction of synthesized derivative 6 g.

ADME prediction

Pharmacokinetic profile of synthesized derivatives was carried out by using SwissADME software. Lipinski's rule of five states that all synthesized derivatives 6a-6g follow the recommendations, resulting in a maximum of one violation. Except for 6f, the majority of the synthesized derivatives met all of Lipinski's criteria, suggesting that they might represent useful treatments for depression.
Table No. 2 displays the theoretical computations for the following parameters: aqueous solubility (logS), topological polar surface area (TPSA), octanol/water partition coefficient (logP), Molecular Weight (MW), Hydrogen Bond Acceptors (HBA), donors (HBD), acceptors (HBD) and donors (HBD) (Table 2).

Table 2: Some physiochemical parameters of the synthesized compound S1-S7 used in the prediction of ADME profiles.

Code no.	RB	MW	HBD	HBA	LogP	LogS	TPSA	VRF
4	2	284.26	2	5	2.56	-4.14	79.9	0
6a	8	425.47	1	7	4.41	-4.48	81.37	0
6b	10	460.48	2	7	3.99	-5.22	110.89	0
6c	8	423.5	1	6	4.78	-5.64	72.14	0
6d	9	488.55	2	6	4.63	-6.9	122.06	0
6e	8	458.49	1	6	4.24	-5.95	86.72	0
6f	9	578.63	1	7	0	-8.13	104.04	1
6g	12	489.52	4	7	3.75	-5.59	122.06	0

MW: Molecular weight (preferably <500 g/mol); RB: number of rotatable bonds (recommended value: 0-15); HBD: Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (recommended value: 0-6); HBA: Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (recommended value: 2-20); log P: Predicted octanol/water partition coefficient (recommended value: -2-6.5); log S: Predicted aqueous solubility (recommended value: -6.5-0.5 mol dm^{-3}); TPSA: Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms (recommended value: 7-200); VRF: Number of violations of Lipinski's rule of five. 7 <TPSA<200, MW<500, logP<5, HBD ≤ 5, HBA ≤ 10 are the regulations. Compounds that meet these requirements are regarded as druglike. The limitations, which are multiples of five, are referred to by the term "five".

Toxicity analysis

The toxicity profile of every synthetic derivative was determined by using Protox II software. All the synthesized derivatives are classified as belonging to toxicity class V. All synthesized derivatives except 6e and 6g exhibit LD50 values that are greater than or equal to the parent derivative (4). Table 3 Contains the LD_{50} value for each synthesized compound (Table 3).

Code	Toxicity class	LD50 (mg/kg)
4	Class V	4000
6a	Class V	4000
6b	Class V	5000
6c	Class V	4000
6d	Class V	5000
6e	Class V	2570
6f	Class V	5000
6g	Class V	2570

The globally harmonized system of categorization and identification of derivatives (GHS) is used to define toxicity classes. The units of LD50 are [mg/kg]. If ingested, Class I is lethal (LD50 ≤ 5), Class II is deadly (5 < LD50 ≤ 50) and Class III is poisonous (50 < LD50 ≤ 300). Class V: may be dangerous if swallowed (2000 < LD50 ≤ 5000); Class VI: non-toxic (LD50 > 5000); Class IV: harmful if swallowed (300 < LD50 ≤ 2000).

HPLC/AR grade UV and TLC solvents were used and the synthesis material and reagents were purchased from Sigma Aldrich (an American company) and LobaChemie (an Indian laboratory). An aluminum plate coated with silica gel (GF) and employing vanillin hydrochloric acid as a visualizing agent allowed researchers to monitor the reaction's progress. For TLC, ethyl acetate: toluene: acetic acid (15:34:1) served as the developing solvents. Using a Bruker Avance 300 MHz, proton NMR spectra were captured with chemical shift δ, using Tetra methyl silane (TMS) served as the internal standard and DMSO-D6 as the solvent. The mass spectra were scanned in the 110-500 m/z range and observed in Q1MSQ1 mode on Pe-Sciex API 2000. the Fourier-Transform Infrared Spectra (FTIR) were obtained using a KBR disc and between 4000-600 cm-1 was scanned. Using 10 mm cuvettes, the UV spectra were recorded on a Jasco V 630 spectrophotometer after being generated in methanol (10µg/mL). Thermoink precision melting point/boiling point apparatus was used to measure melting points using the open capillary tube method. It was heated at a rate of 1°C/min. Heraus was employed to perform C, H and N's elemental analysis. Swiss dock Software and UCSF chimera were used to carry out the molecular docking based on EADock DSS. SwissADME is a free online tool for assessing the pharmacokinetics and drug-likeness of synthesized compounds. An online lab known as Protox-II is used to forecast toxicity. An antidepressant activity was evaluated using the Pentylenetetrazole (PTZ) induced seizure.

Experimental protocol

The general method shown in Scheme 1 was followed to synthesize the derivatives (6a-6g).

General procedure

Step I: A 5 mmol/100 mL solution of acacetin of anhydrous N-dimethylformamide (DMF) was mixed with 125 mmol of 1,2-dibromobutane and 5 mmol of anhydrous potassium carbonate (K_2CO_3) as a base. Next, the mixture was heated to 120°C for two hours. Following the reaction mixture's cooling and filtering, the concentrated filtrate was used. To create an intermediate that is dark brown (I), the residue was recrystallized using a petroleum ether/ethyl acetate (2:1) ratio.

Step II: A solution of intermediate (1 mmol) in 30 mL of anhydrous DMF was combined with the necessary substituted N' heterocycles (10 mmol) and the mixture was heated at 80°C for three hours. A substituted N-heterocycles derivative of acacetin was obtained by evaporating the solvent and recrystallizing the residue with the help of petroleum ether and ethyl acetate [9-12].

Acacetin is 5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen (4) [86]: Mp: 176-178°C. Rf: 0.91 (acetic acid: ethyl acetate: toluene: 1:15:34). UV (MeOH): λ_{max} 207, 251.8 nm. IR (KBr): ν_{max} 1266 (C-O str), 1577.98 (C=C str), 772.21 (C-H str), 1306.50 (O-H str), 1684.73 (C=O str) cm-1. 1H NMR (DMSO-deuterated, 500 MHz): δ 3.78 (3H, s, OMe-4'), 5.895 (1H, d, J=2.8Hz, H-8), 5.895 (1H, d, J=8.2Hz, H-6), 6.9 (1H, d, J=8.4Hz, H-3), 7.44 (2H, d, J=8.8Hz, H-2', H-6'), 6.98 (2H, d, J=8.4Hz, H-3', H-5'). MS: m/z 286 [M]+ (100). Anal. Calcd. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25; O, 28.14.

7-[(morpholine) 4-butoxyl]-2-(4-methoxyphenyl)-5-hydroxy-4H-chromen (6a): Morpholine (10 mmol) was incorporated into a chemical 5 (1 mmol) solution in 30 ml of DMF (anhydrous). The mixture was then heated to 80°C for three hours. Petroleum ether and ethyl acetate were reacted to recrystallize the residue into derivative 6a after the solvent was removed. Yield (70.25%). Mp: 80°C-84°C. Rf: 0.68 (acetic acid: ethyl acetate: toluene: 1:15:34). UV (MeOH): λ_{max} 207, 250.4 nm. IR (KBr): νmax 1257.12 (C-O str), 854.74 (C=C str), 2771.98 (C-H str), 3400.91 (O-H str), 1652.15 (C=O str), 1512.20 (N-H str). 1H NMR (DMSO-deuterated, 500 MHz): δ 1.60 (2H, tt, J=7.4, 2.7 Hz, NCH2CH2), 1.84(2H, quint, J=7.5Hz, OCH2CH2), 2.40-3.10 (6H, 2.49 (4H, ddd, J-7.7, 6.7, 2.5Hz, CH_2-N-CH_2), 2.92 (2H, t, J=2.7Hz, O(CH2)3-CH2)), 3.91(4H, ddd, J=11.7, 6.7, 2.5Hz, CH2-O-CH2), 4.0 (3H, s, OMe-4'), 4.8 (2H, t, J=7.5Hz, OCH2), 6.29 (1H,d, J=2.2Hz, H-6), 6.31-6.95 (2H, 6.3 (1H, s, H-3), 6.45 (d, J=2.2Hz, H-8)), 7.01 (2H, ddd, J=8.8, 1.0, 0.5Hz, H-3',5'), 7.98 (2H, ddd, J=8.8,1.8, 0.5Hz, H-2',6'). MS m/z calculated: 425.47; found: 426.19[M]+. Anal. Calcd. for $C_{24}H_{27}NO_6$: C, 67.75; H, 6.40; N, 3.29; O, 22.56 and found: C, 68.65; H, 6.48; N, 3.89; O, 22.86.

7-[(pyridine-3-carboxamidLe) 4-butoxyl]-2-(4-methoxyphenyl)-5-hydroxy-4H-chromen (6b): Nicotinamide (10 mmol) was incorporated into a chemical 5 (1 mmol) solution in 30 ml of DMF (anhydrous). The mixture was then heated to 80°C for three hours. Petroleum ether and ethyl acetate were reacted to recrystallize the residue into derivative 6b after the solvent was removed. Yield: (66.32%). MP: 90°C-92°C. Rf: 0.71 (acetic acid: ethyl acetate: toluene: 1:15:34). UV (MeOH): λ_{max} 290.2, 261.8, 256 nm. IR (KBr): ν_{max} 1170.04 (C-O str), 885.49 (C=C str), 1020.98 (C-H str), 1255.10 (O-H str), 1669.46 (C=O str), 3385.59 (N-H str). ^{1}H NMR (DMSO-deuterated, 500 MHz): δ 1.34 (2H, t, J=7.4 Hz, Ar-NCH_2CH_2), 1.85 (2H, quint, J=7.5 Hz, NH-CH_2), 3.34 (2H, t, J=7.4Hz, $NHCH_2$), 3.91 (3H, s, OMe-4'), 4.31 (2H, t, J=7.5Hz, OCH_2), 5.82 (1H, d, J=2.2Hz, H-6), 6.35-6.50 (2H, 6.35 (1H,, s, H-3), 6.65 (d, J=2.2Hz, H-8)), 7.31 (2H, ddd, J=8.8, 1.0, 0.5Hz, H-3',5'), 7.51-7.89 (3H, 7.53 (2H, ddd, J=8.8, 1.8, 0.5 Hz, H-2',6'), 7.91 (1H, ddd, J=8.1, 4.7, 0.5Hz, NCHCH)), 8.10 (ddd, J=8.1, 1.9, 1.5Hz, NCHCHCH), 8.54 (1H, dt, J=4.7, 1.9Hz, NCH), 8.89 (1H, ddd, J=1.9, 1.5, 0.5Hz, NCHCCO). MS m/z calculated: 460.48 and found: 465.40 $[M]^+$. Anal. Calcd. for $C_{26}H_{24}N_2O_6$: C, 67.82; H, 5.25; N, 6.08; O, 20.85 and found C, 68.92; H, 5.29; N, 5.88; O, 20.95.

7-[(piperidine) 4-butoxyl]-2-(4-methoxyphenyl)-5-hydroxy-4H-chromen (6c): Pyridine (10 mmol) was incorporated into a chemical 5 (1 mmol) solution in 30 ml of DMF (anhydrous). The mixture was then heated to 80°C for three hours. Petroleum ether and ethyl acetate were reacted to recrystallize the residue into derivative 6c after the solvent was removed. Yield: (60.88%). Mp: 84°C-86°C. Rf: 0.61 (acetic acid: Ethyl acetate: Toluene: 1:15:34). UV (MeOH): λ_{max} 247, 289.6, 297 nm. IR (KBr): νmax 1315.73 (C-O str), 1606.39 (C=C str), 2767.38 (C-H str), 2958.35 (O-H str), 1650.96 (C=O str), 3413.59 (N-H str). ^{1}H NMR (DMSO-deuterated, 500 MHz) δ 1.45-1.89 (10H, 1.46 (2H, dtt, J=10.8, 6.7, 2.8Hz, $NCH_2CH_2CH_2$), 1.59 (2H, tt, J=7.4, 2.7Hz, $OCH_2CH_2CH_2$), 1.71 (4H, ddddd, J=10.8, 6.7, 6.6, 2.8, 2.7Hz, NCH2CH2)), 1.83 (2H, quint, J=7.5Hz, OCH_2CH_2), 2.40-2.60 (6H, 2.47 (ddd, J=9.0, 6.6, 2.7Hz, NCH_2), 2.52 (2H, t, J=2.7Hz, $O(CH_2)3CH_2$), 3.58 (3H, s, OCH3), 4.21 (2H, t, J=7.5Hz, OCH_2), 6.29 (1H, d, J=1.9Hz, H-6), 6.35-6.49 (2H, 6.40 (1H, s, H-3), 6.44 (1H, d, J=1.9Hz, H-8)), 6.98 (2H, ddd, J=8.8, 1.04, 0.51Hz, H-3',5'), 7.78 (2H, ddd, J=8.8, 1.0, 0.5Hz, H-2',6'). MS m/z calculated: 424.50 and found: 426.19$[M]^+$. Anal. Calcd. for $C_{25}H_{29}NO_5$: C, 70.90; H, 6.90; N, 3.31; O, 18.89 and found C, 68.92; H, 6.29; N, 3.88; O, 20.45.

7-[(1,3-benzthiazole-2-amine) 4-butoxyl]-2-(4-methoxyphenyl)-5-hydroxy-4H-chromen (6d): 2-aminobenzthiazole (10 mmol) was incorporated into a chemical 5 (1 mmol) solution in 30 ml of DMF (anhydrous). The mixture was then heated to 80°C for three hours. Petroleum ether and ethyl acetate were reacted to recrystallize the residue into derivative 6d after the solvent was removed. Yield: (70%). Mp: 110-112°C. Rf: 0.65 (acetic acid: ethyl acetate: toluene: 1:15:34). UV (MeOH): λ_{max} 342.6 nm. IR (KBr): ν_{max} 1256.86 (C-O str), 1019.45 (C=C str), 3009.91 (C-H str), 3396.60 (O-H str), 1607.86 (C=O str), 2777.72 (N-H str), 1170.60 (C=N), 2432.59 (C-S). 1H NMR (DMSO-deuterated, 500 MHz) δ 1.46 (2H, quint, J=7.46Hz, NCH2CH2), 1.59 (2H, quint, J=7.50Hz, OCH2CH2), 2.52 (2H, t, J=7.42Hz, NH-CH2), 3.81 (3H, s, OMe-4'), 4.21 (2H, t, J=7.5Hz, OCH2), 6.29 (1H, d, J= 1.39Hz, H-6), 6.35-6.79 (2H, 6.40 (1H, s, H-3), 6.54 (1H, d, J=1.93, H-8)), 7.01 (2H, ddd, J=8.80, 1.04, 0.51Hz, H-3',5'), 7.54 (1H, ddd, J=7.85, 7.43, 1.26Hz, SCCHCH), 7.64 (1H, ddd, J=8.13, 7.43, 1.56Hz), NCCHCH), 7.36 (1H, ddd, J=8.13, 1.26, 0.51Hz, NCCH), 7.52 (2H, ddd, J=8.80, 1.85, 0.51Hz, H-2',6') 7.7 (1H, ddd, J=7.85, 1.56, 0.51Hz, SCCH). MS m/z calculated: 488.55 and found:

491.31[M]$^+$. Anal. Calcd. for C27H24N2O5S: C, 66.38; H, 4.95; N, 5.73; O, 16.37; S, 6.56 and found: C, 66.48; H, 5.95; N, 5.73; O, 16.37; S, 6.66.

7-[(1H-benzimidazole) 4-butoxyl]-2-(4-methoxyphenyl)-5-hydroxy-4H-chromen (6e): 1H-benzimidazole (10 mmol) was incorporated into a chemical 5 (1 mmol) solution in 30 ml of DMF (anhydrous). The mixture was then heated to 80°C for three hours. Petroleum ether and ethyl acetate were reacted to recrystallize the residue into derivative 6e after the solvent was removed. Yield: (85%). Mp: 62-64°C. Rf: 0.75 (acetic acid: ethyl acetate: toluene: 1:15:34). UV (MeOH): λmax 243, 271.4, 278.2 nm. IR (KBr): νmax 1301.96 (C-O str), 1618.69 (C=C str), 2851.69 (C-H str), 2768.89 (O-H str), 1652 (C=O str), 3389.64 (N-H str), 1652.49 (C=N str). 1H NMR (DMSO-deuterated, 500 MHz) δ 1.81-1.99 (4H, 1.88 (tt, J=7.5, 7.0Hz, 7-OCH2CH2), 1.93 (quint, J=7.0Hz, 7-O(CH2)2CH2), 3.78 (3H, s, OMe-4',), 4.08-4.27 (4H, 4.13 (t, J=7.0Hz, 7-O(CH2)3CH2), 7.29 (1H, d, J=1.9Hz, H-6), 7.35-7.49 (2H, 7.40 (1H, s, H-3), 6.44 (1H, d, J=1.9Hz, H-8), 7.88-8.08 (4H, 7.95 (1H, td, J=7.7, 1.2Hz, Ar-H4), 7.96 (1H, ddd, J=7.9, 7.6, 1.3Hz, Ar-H6), 8.01 (2H, ddd, J=8.8, 1.0, 0.5Hz, H-3',5'), 8.47-8.76 (4H, 8.53 (2H, ddd, J=8.8, 1.8, 0.5Hz, H-2',6'), 8.70 (1H, ddt, J=7.9, 1.2, 0.5Hz, Ar-H7), 8.90 (1H, t, J=0.5Hz, N-CH-.N)). MS m/z calculated: 456.17 and found: 457.5[M]$^+$. Anal. Calcd. for $C_{27}H_{24}N_2O_5$: C, 71.04; H, 5.30; N, 6.14; O, 17.52 and found: C, 71.14; H, 5.30; N, 7.14; O, 16.52.

7-[(2-phenyl-4H-3,1-benzoxazine-4-one) 4-butoxyl]-2-(4-methoxyphenyl)-5-hydroxy-4H-chromen (6f): 2-phenyl-4H-3,1-benzoxazine-4-one (10 mmol) was incorporated into a chemical 5 (1 mmol) solution in 30 ml of DMF (anhydrous). The mixture was then heated to 80°C for three hours. After the solvent was removed, the residue was recrystallized with the help of petroleum ether and ethyl acetate, yielding derivative 6f. Yield: (67%). Mp: 112-114°C. Rf: 0.80 (acetic acid: ethyl acetate: toluene: 1:15:34). UV(MeOH): λ_{max} 247, 289.6, 297 nm. IR (KBr): ν_{max} 1171.34 (C-O str), 884.94(C=C str), 2778.81 (C-H str), 1414.85 (O-H str), 1652.88 (C=O str), 3395.95 (N-H str). 1H NMR (DMSO-deuterated, 500 MHz) δ 1.90-2.84 (4H, 1.90 (2H, tt, J=7.4, 7.1Hz, 7-O(CH2)2CH2), 2.32 (2H, quint, J=7.4Hz, OCH2CH2)), 3.21 (2H, t, J=7.1Hz, 7-O(CH2)3CH2), 3.88 (3H, s, OMe-4'), 3.90 (2H, t, J=7.4Hz, O-CH2), 6.29 (1H, d, J=1.9Hz, H-3), 6.44 (1H, s, h-8), 6.58 (1H, s, Ar-H), 6.94-7.08 (3H, 7.00 (2H, ddd, J=8.3, 1.3, 0.5Hz, Ar-H), 7.01 (1H, ddd, J=8.8, 1.0, 0.5Hz, Ar-H)), 7.28-7.82 (10H, 7.34 (tt, J=7.4, 1.3Hz), 7.36 (ddd, J=7.9, 7.3, 1.3Hz), 7.40 (dddd, J=8.1, 7.4, 1.6, 0.5Hz), 7.53 (ddd, J=8.8, 1.8, 0.5Hz), 7.60 (ddd, J=8.3, 7.3, 1.4Hz), 7.69 (ddd, J=7.9, 1.4, 0.5Hz), 7.75 (dtd, J=8.1, 1.2, 0.5Hz) Ar-H). MS m/z calculated: 562.19 and found: 463.17[M]+. Anal. Calcd. for $C_{34}H_{28}NO_7$: C, 72.59; H, 5.02; N, 2.49; O, 19.91 and found: C, 72.69; H, 5.02; N, 2.49; O, 18.91.

7-[(N-cyclohexyl-1,5-diene-1-yl) hydrazine carboxamide) 4-butoxyl]-2-(4-methoxy phenyl)-5-hydroxy-4H-chromen (6g): Hydrazine carboxamide (10 mmol) was incorporated into a chemical 5 (1 mmol) solution in 30 ml of DMF (anhydrous). The mixture was then heated to 80°C for three hours. After the solvent was removed, the residue was recrystallized with the help of petroleum ether and ethyl acetate, yielding a derivative 6g. Yield: (81.20%). Mp: 76-78°C. Rf: 0.56 (acetic acid: ethyl acetate: toluene: 1:15:34). UV (MeOH): λmax 238, 282.8 nm. IR (KBr): νmax 1252.34 (C-O str), 1632.70 (C=C str), 2785.19 (C-H str), 1355.27 (O-H str), 1632.70 (C=O str), 3399.26 (N-H str). 1H NMR (DMSO-deuterated, 500 MHz): δ 2.0 (2H, quint, J=7.4Hz, 7-O(CH_2)3CH_2), 2.30 (2H, quint, J=7.5Hz, 7-OCH_2), 6.00 (1H, d, J=2.2Hz, H-3), 6.95-7.24 (3H, 7.01 (1H, ddd, J=8.8, 1.0, 0.5Hz, H-6), 7.08 (2H, tt, J=7.8, 1.2Hz, OCH2CH2), 7.20-7.42 (4H, 7.27 (1H, dddd, J=8.2, 7.8, 1.4, 0.5Hz, Ar-H), 7.56 (1H, dddd, J=8.2, 1.5, 1.2, 0.5Hz, Ar-H), 8.68 (2H, ddd, J=8.8, 1.8, 0.5Hz, Ar-H). MS m/z calculated: 489.52 and found: 490.19[M]+. Anal. Calcd. for $C_{27}H_{27}N_3O_6$: C, 66.25; H, 5.56; N, 8.58; O, 19.61 and found: C, 66.45; H, 5.66; N, 8.58; O, 19.63.

Biological screening

Animal: Both sexes of adult Swiss albino mice weighing 30 g-40 g were housed in polypropylene cages with controlled temperatures (25°C-20°C) and light-dark cycles (12-12 hours, with the light on from 07-14 hours). There was plenty of food and drink nearby. However, during the experiment, food was withheld overnight, but not water. The animal ethics committee approved every experiment and they were all conducted in compliance with the guidelines set forth by the CPCESEA for the use and handling of laboratory animals.

Anticonvulsant activity

Pentylenetetrazole (PTZ) induced seizure was used to assess each synthesized derivative *in vivo* anticonvulsant efficacy.

Drugs: Pentylenetetrazole, Diazepam, Acacetin (4), all the synthesized derivatives (4, 6a-6g).

Pentylenetetrazole (PTZ) induced seizure: Three mice per group (control, standard and test) were randomly selected from among the mice. A saline solution was used to suspend the test compound. The animals received injections of diazepam (1 mg/kg) subcutaneously (s.c.) and test compound (4, 6a-6g) p.o. at a dose of 10 mg/kg. All of the animals received a PTZ injection thirty minutes later (70 g/Kg. s.c.); it was found that this dosage caused convulsions in over 95% of control mice and that these mice died within twenty-four hours. The endpoints of the study were the percentage of mice that survived after 24 hours, the latency of the first myoclonic jerk and the total duration of the seizures [13,14].

Insilico study

Molecular docking: Molecular docking was carried out utilizing UCSF Chimera software and Swissdock, which is based on EADock DSS. The Protein Data Bank (PDB id: 4COF) provided 3-dimensional structures of GABA A receptors at a resolution of 2.97 Å. The synthesized ligands' 3D structures were created in ChemDraw and the ligands were adjusted using a molecular mechanical force field. The conjugate gradient method of Polak Ribiere was utilized to optimize the molecular structures and AM1 employed a semi-empirical approach to determine the final conformations. The tools from SwissDock were used and the inputs were optimized structures. The grid box was 60 by 60 by 60 with a grid point spacing of 0.375Å. The reference ligand's binding site was identified in order to ascertain each ligand's ideal posture [15].

ADME prediction: SwissADME is a free online tool for assessing a compound's pharmacokinetics and drug-likeness. A biologically active compound's optimization depends on the assessment of bioavailability parameters in terms of satisfying criteria such as Lipinski's, Veber's, Ghose's or Egan's, which enable the selection of compounds that will, in all likelihood, make good drugs when taken orally [16].

Toxicity analysis: A virtual laboratory called Protox-II is used for predicting toxicity. Determining a compound's toxicities is a crucial step in designing a medication. Toxic dosage limits are commonly represented as mg/kg body weight for the LD_{50} values. The median lethal dose or LD_{50}, is the dosage at which 50% of test subjects die following exposure to a substance [17].

CONCLUSION

To summarize, we designed and synthesized an innovative range of N-heterocycles derivatives of 4'-methoxy-5,7-dihydroxy flavone Scaffold Hybrids (acacetin derivatives). We evaluated their anticonvulsant *in vivo* by using a pentylenetetrazol induced seizures. To ascertain the binding affinity of derivatives with specific biological targets, a insilico analysis of synthesized derivatives (6a-6g) was conducted. Protox-II was used to predict the toxicity profile, while SwissADME was utilized to assess the ADMET characteristics. When 10 mg/kg p.o. of the parent drug acacetin is used as a comparison, most of the synthetic derivatives were shown to have strong antidepressant (6c, 6d, 6f, 6g) properties. Our choice to move forward with the testing and synthesis of the substituted N-heterocycle derivative of the 4'-methoxy-5,7-dihydroxy flavone Scaffold Hybrids (acacetin) has been encouraged by these results. The findings imply that they are a great starting point for creating and refining compounds with anticonvulsive properties. Further research is necessary to elucidate their mode of action and strategies.

DECLARATION OF COMPETING INTEREST

There are no known conflicting financial interests or interpersonal ties that could have influenced the research described in this paper.

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