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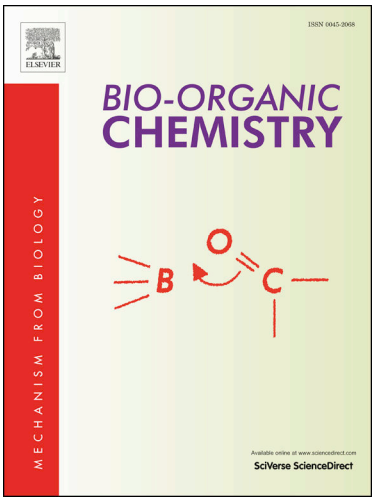

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Design, Synthesis and docking studies of novel benzopyrone derivatives as anticonvulsants

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Abstract

A series of coumarin derivatives **6-8, 9a-h, 11** and **13a, b -16a, b** was synthesized and screened for their anticonvulsant profile. Screening of these analogues using the 'gold standard methods' revealed variable anticonvulsant potential with remarkable effects observed particularly in chemically-induced seizure test. Compounds **6**, **7**, **13b** disclosed the highest potency among the series with 100% protection against scPTZ. Quantification study confirmed that compound **6** (ED_{50} 0.238 mmol/kg) was the most active congener in the scPTZ model and was approximately 1.5 folds more potent than ethosuximide as reference drug Meanwhile, in the MES test, candidate drugs exhibited mild to moderate anticonvulsant efficacy, the highest of which was compound **14a,** imparting 50% protection at 2.1 mmol/kg, followed by other compounds with activity ranging from 14-33%, as compared to diphenylhydantoin. Additionally, all candidate compounds were screened for acute neurotoxicity using the rotarod method to identify motor impairment, where almost all compounds passed the test. Further neurochemical investigation was performed to unravel the effect of the most active compound **(6)** on GABA level in mouse brain, where a significant elevation was evident by 4 and 1.4 folds with respect to that of the

control and reference groups at $p<0.05$. Molecular modeling study using Discovery Studio program was performed, where compound **6** exhibited good binding interaction with γ-aminobutyric acid aminotransferase (GABA-AT) enzyme and this was consistent with the attained experimental results.

Key words:

Synthesis; coumarin; anticonvulsants; GABA; Molecular Modeling

1. Introduction

Epilepsy is defined as disorders of neuronal excitability characterized by periodic and unpredictable occurrences of seizures and transient changes of behavior as a result of synchronous disordered rhythmic firing of neural populations of the central nervous system [1-3]. It is known to be one of the most common serious neurological disorders that affect 1-2% people worldwide. Seizures could be attributed to an abnormality in the major neurotransmitters of the brain; such as over-activity of the excitatory transmitters or impaired activity of the inhibitory transmitters or possibly through a combination of both. However, it is quite difficult to pinpoint a singular biochemical disorder that results in epileptogenesis due to the marked heterogeneity of epilepsy syndromes and emergence of therapy-resistant epilepsy as well [4]. Despite the presence of several novel antiepileptic drugs (AEDs), the control of seizures is attained in only 50% of patients and the decrease in incidence is found in only 75% of patients [2, 5]. Additionally, they have side effects such as ataxia, hepatotoxicity, and megaloblastic anemia [6, 7] .Consequently, search for new antiepileptics with lower toxicity and better efficacy is still a paramount demand. Antiepileptic drugs (AEDs) comprise a diverse range of molecules acting mostly through: i) modulation of voltage-gated ion channels (sodium or calcium), ii) reduction of excitatory, particularly glutamate-mediated neurotransmitter or iii) promotion of GABA receptors directly through positive allosteric modulation of $GABA_A$ receptors or indirectly through enhancing GABA level *via* GABA transaminase inhibition [8]. GABA is considered the main inhibitory neurotransmitter in the brain of mammals and plays a considerable role in the pathogenesis of epilepsy [9]. GABAergic inhibition is considered the main mechanism balancing glutamatergic excitation and hypersynchronous neuronal discharges inhibition.

Coumarins are the privileged structure in medicinal chemistry for the discovery of therapeutic agents [10-12]. They were reported to exhibit various therapeutic profiles among which is antiepileptic activity as represented in compounds **A** [13], **B** [13]**, C** [14]**, D** [15] and **E** [16]**.**

Interestingly, incorporation of dioxopyrrole, thiazoline, imino thiazolidinone and imidazolidinetrione moieties to the scaffold of coumarin entity could improve their anti-convulsant potential [17].

Using target-based studies in the discovery of new antiepileptic agents is uncommon due to lack of information regarding the molecular pathway of epilepsy and complex mode of action for most of the known antiepileptic drugs. Therefore, the development of novel anticonvulsants may be achieved through screening investigations or using hybrid approach [18, 19]. The latter depends on the use of two or more pharmacophoric scaffolds, each having a remarkable pharmacological effect, to be joined in one single structure to attain new components having better biological profile [20].

The designed hybrid of the target compounds **6-8, 9a-h, 11, 13a, b -16a, b** possesses all pharmacophoric elements essential anticonvulsant activity such as lipophilic aryl ring (A), hydrogen bonding domain (HBD) and electron donor moiety (D) [5, 8, 21, 22] (fig 2). The anticonvulsant evaluation for these benzopyran derivatives was performed through two standard animal seizure models; subcutaneous pentylenetetrazole (scPTZ) and a maximal electric shock (MES) seizure tests. Additionally, the effect of the synthesized entities on motor function was assessed *via* rotarod test. The most potent compounds which had remarkable efficacy in scPTZ were further investigated for its effect on GABA brain level. To calculate physicochemical properties of these benzopyran derivatives, computational studies were carried out using swissADME a free online web tool. In addition, docking study was accomplished on the active site of GABA-AT enzyme to confirm GABAergic activity.

Insert Fig.1

Insert Fig.2

2. Results and discussions

2.1. Chemistry

The synthetic route of the target compounds **6-8, 9a-h, 11, 13a, b -16a, b** is illustrated in scheme 1-3. The compounds were produced in a reasonable yield and their structure was confirmed with spectral and elemental analysis. The key intermediate **5** [23]was prepared through hydrazinolysis of the corresponding ester **4**.

Reacting of the acid hydrazide **5** with phthalic anhydride in presence of glacial acetic acid producing pyrrolidin-2, 5-dione **6** (Scheme 2). The IR spectra illustrated bands at 1626-1720 cm^{-1} for 4C=O groups. ^{1}H NMR spectra displayed the disappearance of NH_2 and NH signals of parent acid hydrazide **5** and presence of one signal at 10.98 ppm for NH. ^{13}C NMR spectrum showed presence of 4C=O groups.

Reacting of acid hydrazide **5** with acetylacetone in ethanol afforded compound **7** [23]. The IR spectra illustrated bands at 1712 and 1720 cm^{-1} for 2C=O groups. ^{1}H NMR spectrum showed presence of three peaks at 1.76, 2.00 and 2.39 ppm corresponding to protons of $3CH_3$. ^{13}C NMR spectrum showed 3 peaks at 16.3, 18.6 and 26.2 for carbons of $3CH_3$.

Condensation of acid hydrazide **5** with ethyl acetoacetate in presence of absolute ethanol achieved the corresponding pyrazol-5-one derivative **8** (Scheme 2). IR spectra showed a band for NH at 3446 cm^{-1} and 3C=O bands at 1680-1730 cm^{-1}. ^{1}H NMR spectra showed a broad singlet at 2.09 ppm corresponding to NH and exchangeable with D_2O and another singlet signals at 2.40 ppm assigned to CH_3 at C3 pyrazolone, 4.97 ppm regarding CH pyrazolone.

Refluxing of the hydrazide **5** with appropriate aromatic aldehyde in presence of glacial acetic acid yielded the benzylidene acetohydrazide derivatives **9a-h** (Scheme 2). IR spectra showed bands at 3275–3446 cm^{-1} regarding NH group. ^{1}H NMR showed one signal at 8.04–8.38 ppm regarding benzylidene proton and one signal at 11.67–11.96 ppm regarding NH. Additionally, ^{13}CNMR showed peak at 142.8-147.4 ppm corresponding to (N=C).

4-Benzyl-1-acylsemicarbazide **10** was attained through refluxing of the corresponding hydrazide **5** with benzyl isocyanate in presence of dichloroethane (Scheme 2). IR spectra displayed bands at 3305– 3385 cm^{-1} for 3NH. The structure was confirmed in ^{1}H NMR by presence of one single

signal at 4.25 ppm and a multiplet signal at 7.27–7.36 ppm for CH_2 and phenyl protons of the benzyl moiety, respectively. Furthermore, there are three a singlet signals at 8.04, 9.28 and 9.82 ppm conforming 3NH protons.

Cyclization of acylsemicarbazide **10** using oxalyl chloride achieved imidazolidin-2,4,5-trione **11** (Scheme 2). IR spectra illustrated a band at 3329 cm^{-1} for NH and bands at 1643–1720cm^{-1} for 5C=O groups. ^{1}H NMR exposed a singlet signal at 11.20 ppm regarding one NH as well as ^{13}C NMR showed peaks at 155.9, 158.4 and 158.8 corresponding to 3C=O imidazolidin-2,4,5-trione.

Thiosemicarbazides **12a**[23]**, b** were attained through the reaction of the respective hydrazide **5** with the appropriate isothiocyanate as illustrated in scheme 3. IR spectra showed three bands at 3169–3458 cm^{-1} corresponding to 3NH. ^{1}H NMR spectrum revealed a triplet signal at 1.25 ppm as well as quartet signal at 4.05 ppm related to the ethyl protons in **12a** spectrum. Furthermore, compound **12b** was confirmed *via* presence of singlet signal at 4.77 and a multiplet at 7.22-7.27 ppm corresponding to the phenyl protons. ^{13}C NMR exhibited a single peak at 183.2 corresponding to C=S. MS spectrum illustrated their molecular ion peaks.

13a, b congeners were achieved through cyclization of thiosemicarbazides **12a, b** with a 2 N NaOH yielded (Scheme 3). IR spectra revealed bands at 3124 and 3419 cm^{-1} corresponding to NH. ^{1}H NMR of **13a** [23] congener revealed a broad singlet signal at 13.90 ppm referring NH proton while compound **13b** exhibited a singlet signal at 10.63 ppm regarding one NH proton. Also, ^{13}CNMR for compound **13b** showed peak at 180.1ppm corresponding to C=S. MS spectrum illustrated their molecular ion peaks.

Compounds **14a,b** were elucidated through their IR spectra showing disappearance of a band at 1282–1251 cm^{-1} corresponding to C=S and only appearance one band at 3419 and 3477 cm^{-1} corresponding to one NH. Additionally, their ^{1}H NMR showed a singlet signal at 6.97 and 6.99 ppm assigned to CH thiazoline. ^{13}C NMR for compound **14a** showed peak at 108.3 regarding CH-thiazoline and another peak at 158.0 for C=N.

1,3,4-thiadiazole derivatives **15a, b** were achieved through cyclization of thiosemicarbazides **12a,b** with concentrated sulfuric acid on cold (Scheme 3). Their structures were confirmed through IR spectra *via* disappearance of C=S band at 1282- 1251 cm^{-1} and appearance of band at

3157 and 3277 cm^{-1}corresponding to NH. ^{1}H NMR revealed one singlet signal at 7.82 and 8.36 ppm referred to NH proton.

Iminothiazolidin-4-ones **16a,b** were produced through reaction of the respective thiosemicarbazides **12a,b** with chloroacetic acid and anhydrous sodium acetate in presence of glacial acetic acid (Scheme 3). Their structures were confirmed through IR spectra showing band at 3348 and 3500 cm^{-1} related NH and three bands at 1732–1699 cm^{-1} assigned to three C=O of respective congener. ^{1}H NMR shown two singlet signals at 4.15 ppm, 4.07 ppm corresponding to CH_2 group of the thiazolidinone as well as 10.56 and 10.57 ppm exchanged with D_2O assigned to NH as well as ^{13}C NMR for compound **16a** showed peak at 33.2 corresponding to CH_2-thiazolidinone.

Insert Sheme1

Insert Scheme 2

Insert Scheme 3

2.2. Pharmacology

2.2.1. Anticonvulsant evaluation

The anticonvulsant evaluation was performed according to an anticonvulsant drug development (ADD) protocol [24], where submitted potential anticonvulsants undergo a list of screening tests in various animal seizure models in the discovery phase of drug development. The preliminary screening in phase I for all the compounds in the series was adopted to detect the most potent candidate drug using the gold standards scPTZ and MES models in normal male albino mice, in addition to evaluation of the potential acute neurotoxic effects by the rotarod test. Phase II followed phase I for the quantitative determination of the median effective dose ED_{50} values of the compounds that conferred 100 % protection against one or both tests. Finally, neurochemical estimation of GABA inhibitory neurotransmitter level for the most active compound was carried out in whole mouse brain, isolated post-administration of the most potent congener possessing the least ED_{50} value, using gabapentin as reference standard drug, in order to predict the mechanism of action.

2.2.2. Phase I: Preliminary screening for anticonvulsant activity:

Compounds **6-8, 9a-h, 11** and **13a, b -16a, b** were pharmacologically screened by intraperitoneal administration of a fixed dose; 100 mg/kg to male albino mice in order to evaluate anticonvulsant activity after 45 min post-drug treatment. This acute dose was selected according to a pilot study performed prior to the main study. The results of the primary (phase-I) screening are summarized in Table 1. Candidate drugs showed variable activity against scPTZ–induced absence epilepsy ranging from 17–100% protection at the tested dose level equivalent to 100 mg/kg, where compounds **6**, **7** and **13b** exhibited the highest protection against PTZ screen (100%) at 0.238, 0.239 and 0.283 mmol/kg which is almost 1.49, 1.48, 1.25 folds more potent than reference drug ethosuximide, respectively. The remaining candidates revealed anticonvulsant activity in the following descending order; **9h (83%, 0.243mmol) =15a (83%, 0.279mmol) >16a (67%, 0.24mmol) = 8 (67%, 0.282mmol) >14a (40%, 0.218mmol) > 11 (33%, 0.210mmol)=9b (33%, 0.237mmol) = 9d (33%, 0.246) = 9g (33%, 0.253mmol) = 9a (33%, 0.256mmol) = 9c (33%, 0.256mmol) =13a (33%, 0.279) >9f (29%, 0.214mmol)>14b (17%, 0.186)=16b (17%, 0.209mmol), > 9e (13%, 0.224mmol)**. On the other hand, all compounds failed to fully protect animals against MES-induced grand mal seizures, which imply that they lack the ability to completely abolish generalized tonic-clonic seizures and are unable to prevent seizure spreading in all animal groups [25, 26]. In this test, candidate drugs exhibited mild to moderate anticonvulsant efficacy, the highest of which was compound **14a** imparting 50% protection at 2.1 mmol/kg, followed by weaker compounds with activity ranging from 14-33% in the following descending order; **14b (33%, 0.186mmol) = 9d (33%, 0.246 mmol) >9b (20%, 0.237mmol) = 9a (20%, 0.256mmol) = 8 (20%, 0.282mmol)> 6 (17%, 0.25mmol) = 9f (17%, 0.214mmol)= 9c (17%, 0.256mmol) =9g (17%, 0.253mmol)=13a (17%, 0.279mmol)=15a (17%, 0.279mmol)> 9e (14%, 0.224mmol)** whereas compounds, **7**, **9h**, **11**, **13b**,**16a**, and **16b** at doses ranging from 2.09 to 0.283 mmol/kg had absolutely no effect on the electrically-induced seizures. Noteworthy, compound **15b** showed no anticonvulsant potential in both chemical and electro-convulsive tests. Additionally, all candidate compounds underwent an acute neurotoxicological study using the rotarod apparatus to identify motor impairment. All compounds passed the test as the motor coordination in animals was not affected by any

unexpected toxicities, except for compound **9a**, where 2 out of 6 animals were unable to maintain equilibrium during 60 s. (c.f. Table 1).

2.2.3. Phase II: Quantitative estimation of median effective dose ED_{50} :

Target compounds **6**, **7** and **13b** that raised seizure threshold and conferred 100% protection in the scPTZ screening test, were further analyzed in order to quantitate their median effective doses (ED_{50}) at 95% confidence interval. The candidate with the least ED_{50} will be selected for the final step to predict mechanism of action c.f Table 2. Compound **6** (ED_{50} 54.86 mg/kg, 0.131 mmol/kg) possessed the highest potency and the least ED_{50} with respect to **13b** (77.11 mg/kg, 0.183 mmol/kg), and **7** (69.93 mg/kg, 0.198 mmol/kg), with confidence limits of (57.51-65.34), (97.20-61.18) and (55.83-73.20), respectively. Regarding reference standards in the scPTZ test for median effective dose, compound **6** protected 50 % of animals at almost half the dose of ethosuximide; 130.55 mg/kg (177.87–98.78), which confirmed its superior activity.

2.2.4. GABA estimation in whole mouse brain

Further neurochemical assessment was carried out to predict the possible mechanism of anticonvulsant action for compound **6,** which possessed highest potency with the least median effective dose as previously shown in phases I and II (c.f. tables 1, 2). The target candidate and gabapentin were given to a new set of animal groups (n=6) at dose of 100 mg/kg, i.p., which were then sacrificed 60 min post-treatment. Quantitative estimation of GABA concentration in whole brain homogenate of the target compound was compared against control and gabapentin as reference standard in the current study. As graphically represented in figure (3), GABA level in whole brain of control animals recorded 2.09±0.07µg/g tissue, whereas compound **6** significantly elevated brain GABA level to 4 folds more than that of the normal control group at $p<0.05$. Interestingly, the candidate drug further exhibited 36% higher GABA level than that of gabapentin (6.28±0.25 µg/ g tissue) at $p<0.05$. Hence, it could be proposed that compound **6** exerted its anticonvulsant activity *via* a GABA-mediated mechanism, the principal inhibitory neurotransmitter that exerts a hyperpolarizing action in all forebrain neurons. One possible mechanism may be through non-vesicular release of GABA, which may be mediated in part by the GABA transporters; hence increases inhibitory tone according to During *et al.* (1995) [27]. Other mechanisms may involve $GABA_A$ receptor activation or $GABA_B$ receptor inhibition, or

modulation of enzymes involved in metabolic pathways, thus promoting GABA synthesis or release. In the current investigation, we proposed the possible elevation in GABA brain level to be mediated *via* the latter mechanism, thus we carried out docking studies using the γ-aminobutyric acid aminotransferase enzyme as will be explained below (fig 4).

Table 1

Phase I anticonvulsant activity and minimal motor impairment of the synthesized compounds **6-8, 9a-h, 10-11, 13a, b -16a, b**

Compound	Dose		% Protection		Neurotoxicity
	mg/kg	mmol/kg	scPTZ	MES	
6	100	0. 239	100	17	0/6
7	100	0.283	100	0	0/6
8	100	0.282	67	20	0/6
9a	100	0. 256	33	20	2/6
9b	100	0. 237	33	20	0/6
9c	100	0. 256	33	17	0/6
9d	100	0.246	33	33	0/6
9e	100	0. 224	13	14	0/6
9f	100	0.214	29	17	0/6
9g	100	0.253	33	17	0/6
9h	100	0. 243	83	0	0/6
11	100	0.210	33	0	0/6
13a	100	0.279	33	17	0/6
13b	100	0. 238	100	0	0/6
14a	100	0. 218	40	50	0/6
14b	100	0. 186	17	33	0/6
15a	100	0. 279	83	17	0/6
15b	100	0. 238	0	0	0/6
16a	100	0. 24	67	0	0/6
16b	100	0. 209	17	0	0/6
Phenobarbital	30	0.129	100	-	-
Ethosuximide	50	0.354	100	-	-
Diphenyl Hydantoin	45	0.178	-	100	-

Table 2

The quantification study for determination of ED_{50} of the most active compounds in scPTZ screening test

Compound	ED_{50} (mg/kg)	ED_{50} (mmol/kg)
6	54.86 (57.51-65.34)	0.131(0.137-0.156)
7	69.93 (55.83-73.20)	0.166 (0.133-0.174)
13b	77.11 (97.20-61.18)	0.183 (0.231-0.145)
Ethosuximide	130.55 (177.87–98.78)	0.92 (1.26-0.699)
Phenobarbital	13.2 (15.90–6.80)	0.056 (0.068-0.029)

ED_{50}: median effective dose

Insert Fig.3

2.3. Structure activity relationship

The synthesized benzopyran-2-one hybrids **6-8, 9a-h, 11** and **13a, b -16a, b** were found as a lead for anticonvulsant drug development. According to the above mentioned results we can deduce a conclusion illustrating the relation between the structure and their activity. The protection obtained by these compounds was indicating their ability as anticonvulsants and this property is due to incorporation of various heterocyclic moieties and acylhydrazone scaffold to the coumarin ring.

Compounds **6**, **7** and **13b** hybrid containing pyrrolidin-2, 5-dione, pyrazoles and triazole moieties, respectively produced the most potent activity in the preliminary *in vivo* screening against sc-PTZ seizure.

Incorporation of pyrroilidin- 2, 5-dione and pyrazoles entities (compounds **6** and **7**) to the coumarin ring increase the activity against sc-PTZ seizure. Meanwhile, incorporation of pyrazole-5-one scaffold (compound **8**) decreased the activity.

Regarding acylhydrazone derivatives **9a-h**, compound **9h** bearing 4-Cl substitution exhibited the most potent activity against sc-PTZ seizure.

Concerning **14a, b -16a, b** hybrid, substitution of iminothiazole, thiadiazole and oxathiazolidine with ethyl moiety increased the activity more than the substitution with benzyl moiety. Meanwhile, in the triazole scaffold **13a,b** hybrid the substitution with ethyl moiety deceased the activity.

2.4. Molecular modeling study

Computer-aided drug design is considered an important tool in designing selective and potent inhibitors [5, 8, 28]. Docking study can be used to illustrate the molecular interaction of the novel candidates at protein– ligand interface. GABA-AT (γ-aminobutyric acid aminotransferase) is a validated target for AEDs and being a catabolic in nature, its selective inhibition raises GABA concentrations in brain [8, 21]. The enzyme was obtained from PDB, code: 1OHW [29]. The binding interaction for the inhibitor (viagabatrin) and compound **6** were clarified in Figure 4.

In this work, the amino acids coordinates corresponding to chain A were extracted. The residues containing in the active site of chain A consisting of His44A, Tyr69A, Ile72A, Gly136A, Ser137A, Phe189A, Arg192A, Lys203A, Ile205A, His206A, Glu265A, Ser269A, Glu270A, Glu298A, Val300A, Lys329A, Arg422A, Asn423A, Ile426A, Gly438A, Gly440A [8, 22]. Concerning the viagabatrin, it showed two hydrogen bond interactions with Arg 192 and Lys 329. Additionally, the GABA-AT inhibitor showed Van der Waals interaction with Ile 72, Phe 189, Val 300, Hist 206, Arg445 and Gln301 residues. Furthermore, compound **6** showed good interaction with GABA-AT enzyme, it formed seven hydrogen bond interactions, two hydrogen bonds with Arg 192 which is a gate-keeper residue in the $GABA_A$ active site, two hydrogen bonds with Gly136, one hydrogen bond with His206, one hydrogen bond with Ser 328 and one hydrogen bond with Lys329 and shown Van der Waals interaction with Arg 445, Gln 301 and Val 300. The results illustrated the good interaction of the tested compounds with GABA-AT binding site which may justify the increased GABA level in the brain.

Insert Fig.4

2.5. Pharmacophore distance mapping

The distance between the essential features [presence of one aryl (R) hydrophobic domain, one electron donor (D), and a hydrogen bond acceptor/donor unit (HBD)] for anticonvulsant activity was calculated using Discovery Studio. The result of pharmacophore distance mapping of the most potent compound **6** was illustrated on the generated hypothetical model, which was in good agreement with the clinically available AEDs [5, 8].

Insert Fig.5

2.6. In silico studies

CNS bioavailability of the drug is greatly affected by physicochemical parameters such as log P (partition coefficient), molecular weight (MW), hydrogen bond acceptors and donor. The synthesized molecules were analyzed for physicochemical parameters using swissadme online tool ref (Table 3).

The lipophilicity of all target candidates were l enough (>2) and having the ability to cross the BBB and being potent anticonvulsants. TPSA (topological polar surface area) define molecules ability to penetrate blood brain barrier and absorption through the intestine. TPSA value < 140 is essential for absorption *via* intestine and <90 for the blood brain barrier penetration [8, 30]. Conformational flexibility and binding of the molecules to the receptors are affected by number of rotatable bonds. Generally, all the compounds possess high number of rotatable bonds (7–11). The other physiochemical properties such as hydrogen bond donors and hydrogen bond acceptors were complemented with Lipniski rule of five ref and these parameters strongly show the suitability of the new compounds as drug like candidates. None of the synthesized entities violated Lipinski parameters (rule of 5) which may induce their potential anticonvulsant activity.

Table 3

Physicochemical parameters of the title compounds (**6-8, 9a-h, 10-11, 13a, b -16a, b**).

Compounds	TPSA[a] (A^2)	n-ROTB[b]	Mol. Wt.[c]	LogP[d]	n-HB donor[e]	n-HB acceptor[f]	Lipinski's violation[h]
6	105.92	7	418.40	2.41	1	6	0
7	74.33	6	352.38	3.54	0	5	0
8	94.30	6	354.36	2.24	1	5	0
9a	80.90	8	376.41	2.59	1	5	0
9b	126.72	9	421.40	2.26	1	7	0
9c	80.90	8	390.43	2.86	1	5	0
9d	90.13	9	406.43	2.92	1	6	0
9e	84.14	10	433.50	3.03	1	5	0
9f	108.59	11	466.48	3.52	1	8	0
9g	80.90	8	394.40	2.64	1	6	0
9h	80.90	8	410.85	2.85	1	5	0
11	126.23	9	475.45	2.64	1	7	0
13a	105.14	6	357.43	3.10	1	4	0
13b	105.14	7	419.50	3.10	1	4	0
14a	114.07	9	475.56	3.60	1	5	0
14b	114.07	11	551.66	3.74	1	5	2
15a	105.49	7	357.43	3.26	1	5	0
15b	105.49	8	419.50	3.64	1	5	0
16a	126.51	8	415.46	3	1	6	0
16b	126.51	9	477.53	3.40	1	6	0

All values were calculated using online software swissadme.com, [a] Topological polar surface area, [b] Total number of free rotated bounds, [c] Molecular weight [d] Theoretical $\log_P$, , [e] Number of hydrogen bond donors, [f] Number of hydrogen bond acceptors

3. Conclusion

A series of benzopyrone derivatives **6-8, 9a-h, 10-11, 13a, b -16a, b** were synthesized and evaluated for their anticonvulsant activity and possible side effects (motor dysfunction) through MES, scPTZ, rotarod methods, respectively. Compound **6** was the most potent congener among the tested compounds in scPTZ test and exhibited the highest safety margin as compared to ethosuximide as reference drug. Additionally, compound **6** was devoid of neurotoxicity. *In silico* pharmacophoric pattern as well as drug likeness study illustrated that compound **6** fulfilled all the requirements which were crucial for anticonvulsant drugs. Compound **6** significantly elevated brain GABA level to 4 folds that of the control group and showed good binding interaction in molecular modeling studies, which indicate that the anticonvulsant action might be through GABA-mediated.

4. Experimental

4.1. Chemistry

All chemicals were commercially available. Infrared (IR) spectra were tested as KBr pellets (for solids) using JASCO FT/IR-6100 spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (125 MHz) spectra were performed through Jeol ECA 500 MHz spectrometer and the values of chemical shift were represented as ppm on d scale. Mass spectral data were attained using electron impact (EI) ionization technique at 70 eV. Elemental analyses were carried out in Microanalytical Units at National Research Centre and Cairo University. Melting points were measured on a Walden Precision Electro thermal 9300 apparatus (Stone, Staffordshire, England) and are uncorrected.

*Synthesis of 7-hydroxy-4-methyl-2H-chromen-2-one (**1**)*

Yield: 95%, m.p. 185 °C as reported [31].

*Synthesis of 7-(allyloxy)-4-methyl-2H-chromen-2-one (**2**)*

Yield: 90%, m.p. 105 °C as reported[32].

*Synthesis of 8-allyl-7-hydroxy-4-methyl-2H-chromen-2-one (**3**)*

Yield: 65%, m.p. 197 °C as reported[32].

*Synthesis of ethyl 2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetate (**4**)*

Yield: 85%, m.p. 103 °C as reported [33].

Synthesis of 2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5**)

Yield: 80%, m.p. 165 °C as reported [33]; IR (KBr, cm^{-1}) exhibited bands at 3356, 3317 (NH_2), 3215 (NH), 3007, 3059, 3082, 3107 (CH Ar), 2856, 2924, 2968 (CH aliphatic), 1668, 1712 (2C=O); ^{1}HNMR (DMSO d_6, δ, ppm): 2.40 (s, 3H, CH_3 at C4), 3.58 (d, 2H, *J=8* Hz, C$\underline{H}_2$-CH), 4.75 (s, 2H, OCH_2), 4.77 (d, 1H, *J=8* Hz, C$\underline{H}$=CH), 5.02 (d, 1H, *J*=2 Hz, C$\underline{H}$=CH,), 5.89-5.99 (m, 1H, C$\underline{H}$-CH_2), 6.24 (s, 1H, CH at C3), 7.03 (d, 1H, *J*=8 Hz, CH at C6), 7.59 (d, 1H, *J*=8 Hz, CH at C5), 8.56 (br.s, 2H, NH_2), 10.09 (br.s, 1H, NH, D_2O-exchangeable).

Synthesis of 2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N-(1,3 dioxoisoindolin-2-yl)acetamide ***(6)***

A mixture of compound **5** (10 mmol, 2.7gm) and phthalic anhydride (10 mmol, 1.48gm,) were dissolved in dioxane (20 mL) containing glacial acetic acid (0.14 mL) and heated under reflux for 7h. Subsequently, the reaction mixture was concentrated under reduced pressure then the deposited solid was filtered, dried and recrystallized from ethanol.

Yield: 96%, m.p. 260 °C [23]; IR (KBr, cm^{-1}) exhibited bands at 3379 (NH), 3001, 3076, 3116 (CH Ar), 2922, 2954, 2976 (CH aliphatic), 1626, 1672, 1712, 1720 (4C=O), 1608 (NH); ^{1}HNMR (DMSO d_6, δ, ppm): 2.43 (s, 3H, CH_3 at C4), 3.58 (d, *J=4* Hz, 2H, C$\underline{H}_2$ -CH), 4.81 (s, 2H. OC$\underline{H}_2$), 4.98 (d, 1H, *J=8* Hz, CH=C$\underline{H}$), 5.04 (d, 1H, *J*=12 Hz, C$\underline{H}$=CH), 5.93-5.97 (m, 1H, C$\underline{H}$-CH_2) , 6.26 (s, 1H, CH at C3) , 7.07(d, 1H, *J=12* Hz, H6-Ar,) , 7.67 (d, 1H, *J=4* Hz, H5-Ar) , 7.94 (s, 4H, $H_{ar.}$), 10.35 (br.s, 1H, NH, exchanged with D_2O); ^{13}CNMR (DMSO d_6, δ, ppm): 19.7 (CH_3 at C4), 27.1 ($\underline{C}H_2$-CH), 66.8 (O-CH_2), 109.4 (C6), 111.3 (C10), 112.08 (C3), 114.6 ($\underline{C}H_2$=CH), 115.9 (C8), 116.0 (C5), 124.3 ($2CH_{ar}$), 129.3 (2CH Ar), 135.7 ($2C_{ar.}$), 135.8 ($\underline{C}H$=CH_2), 152.2 (C9), 154.04 (C4), 158.5 (C2, coumarin), 160.5, 165.2, 167.8 (3C=O); MS (EI) m/z (%):418.3 ([M+, 58.4); Anal. Calcd. for $C_{23}H_{18}N_2O_6$: C, 66.03; H, 4.34; N, 6.70, Found: C, 66.32; H, 4.65; N, 6.96.

Synthesis of 8-allyl-7-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethoxy)-4-methyl-2H-chromen-2-one ***(7)***

Acetylacetone (10 mmol, 1mL) was added to a solution of compound **5** (10 mmol, 2.7 gm) in absolute ethanol (20 ml) containing few drops of triethylamine and was refluxed for 12h. The

reaction mixture was concentrated and poured into ice-HCL and the resultant solid was filtered, washed with diluted ethanol, dried and recrystallized from ethanol to give **7** as white solid [23].

Yield: 98%, m.p. 178 °C; IR (KBr, cm^{-1}) exhibited bands at 3001, 3076, 3116 (CH Ar), 2922, 2954, 2976 (CH aliphatic), 1712, 1720 (2C=O), 1625 (C=N), ^{1}HNMR (DMSO d_6, δ, ppm): 1.76 (s, 3H, N=C-C<u>H</u>$_3$) , 2.00 (s, 3H, CH_3 at C4), 2.39 (s, 3H, N=C-C<u>H</u>$_3$), 3.55 (d, 2H, *J*=6 Hz, C<u>H</u>$_2$-CH),4.96 (d, 1H, *J=1.6* Hz, C<u>H</u>=CH), 4.99 (d, 1H, *J*=1.6 Hz, C<u>H</u>=CH), 5.09 (s, 2H, OC<u>H</u>$_2$), 5.93-6.03 (m, 1H, CH_2-C<u>H</u>), 6.22 (s, 1H, CH at C3) , 6.50 (s, 1H, CH-pyrazole), 6.91 (d, 1H, *J*=8.8 Hz, H6-Ar) , 7.58 (d, 1H, *J*=8.8 Hz, H5-Ar); ^{13}CNMR (DMSO d_6, δ, ppm): 16.3, 18.6 (2CH_3- pyrazole), 26.2 (<u>C</u>H_3-at C4), 27.1 (<u>C</u>H_2-CH), 66.9 (O<u>C</u>H_2), 109.1 (C6), 111.6 (C10), 113.9 (C3), 115.2 (<u>C</u>H_2=CH), 118.8 (C8), 124.8 (C5), 135.7 (<u>C</u>H=CH_2), 152.3 (C9), 154.0 (C4), 159.3 (C7), 160.5 (C2-coumarin), 164.7 (N-C=O); (EI) *m/z* (%):, 419 ([M^++1, 39.66), 84.3 (100), Anal.calcd. for $C_{23}H_{18}N_2O_6$: C, 68.17; H, 5.72; N, 7.95, Found: C, 68.25; H, 5.61; N, 7.85.

Synthesis of 2-(2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)-5-methyl-1,2-dihydro-3H-pyrazol-3-one ***(8)***

A mixture of compound **5** (10 mmol, 2.7 gm) and ethyl acetoacetate (10 mmol, 1.3 gm) in absolute ethanol (20 mL) were refluxed for 4h. The reaction mixture was concentrated; the formed precipitate was filtered, washed with water and recrystallized from ethanol to give **8** as yellowish white solid.

Yield: 90%, m.p. 200 °C; IR (KBr, cm^{-1}) exhibited bands at 3446 (NH), 1680, 1708, 1730 (3C=O), 1604 (NH); ^{1}HNMR (DMSO d_6, δ, ppm): 2.09 (br.s, 1H, NH), 2.40 (s, 3H, CH_3-pyrazolone) , 2.50 (s , 3H, CH_3-at C4), 3.53 (d, 2H, *J*=5.8 Hz, C<u>H</u>$_2$-CH) , 4.15-4.20 (m, 2H, OC<u>H</u>$_2$) , 4.97 (s, 2H, C<u>H</u>=CH, CH-pyrazolone), 5.01 (s, 1H, CH=C<u>H</u>) , 5.91-5.93 (m, 1H, CH_2-C<u>H</u>), 6.24 (s, 1H, CH at C3), 6.96 (d, 1H, *J=8* Hz, H6-Ar), 7.63 (d, *J=8* Hz, 1H, H5-Ar); MS (EI) *m/z* (%):$_6$, 354.36 ([M^++1, 70); Anal.calcd. for $C_{19}H_{18}N_2O_4$: C, 64.40; H, 5.12; N, 7.91, Found: C, 64.63; H, 5.33; N, 8.20.

General method for synthesis of (E)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl) oxy)-N'-benzylideneacetohydrazide ***(9a-h)***

A solution of the acid hydrazide **5** (0.01 mol, 2.88 gm) and the appropriate aromatic aldehyde (0.01mol) in glacial acetic acid (20 mL) was refluxed for 6h. The crude mixture was evaporated under pressure and recrystallized from ethanol.

(E)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-benzylideneacetohydrazide ***(9a)***

Yield: 77%, m.p. 245 °C [23]; IR (KBr, cm^{-1}) exhibited bands at 3336 (NH), 3030, 3064, 3078 (CH Ar), 2862, 2922, 2978 (CH aliphatic), 1685, 1716 (2C=O), 1604 (C=N); ^{1}HNMR (DMSO d_6, δ, ppm): 2.39 (s, 3H, CH_3 at C4) , 3.35 (d, 2H, *J*=4 Hz, $C\underline{H}_2$-CH) , 4.85 (s, 2H, OCH_2) , 4.96 (d, 1H, *J*=1.6 Hz, C<u>H</u>=CH) , 5.01 (d, 1H, *J=1.6* Hz, C<u>H</u>=CH), 5.94-5.99 (m, 1H, CH_2-C<u>H</u>,) 6.24 (s, 1H, CH at C3) , 7.02 (d, 1H, *J*=8 Hz, H6-Ar), 7.61 (d, 1H, *J*=8 Hz, H5-Ar) , 7.72-7.73 (m, 5H, H_{ar}), 8.28 (s, 1H, N=C<u>H</u>), 11.67 (br.s, 1H, NH, exchanged with D2O); ^{13}CNMR (DMSO d_6, δ, ppm): 19.5 (CH_3 at C4), 27.1 ($\underline{C}H_2$-CH), 66.1 (OCH_2), 109.1 (C6), 111.6 (C10), 112.5 (C3), 115.4 (CH=$\underline{C}H_2$), 121.6 (C8), 112.4 (C5), 127.5, 129.2, 130.4 ($3CH_{ar}$), 134.4 (C_{ar}), 135.7 ($\underline{C}H=CH_2$), 144.4 (N=<u>C</u>H), 152.3 (C9), 154.01 (C4), 159.2 (C7), 160.7 (C2), 169.07 (CO-NH); MS (EI) *m/z* (%):$_6$, 376.41 ([M^++1, 39.66), 84.3 (100); Anal.calcd. for $C_{22}H_{20}N_2O_4$ C, 70.21; H, 5.36; N, 7.44, Found: C, 70.45; H, 5.61; N, 7.53.

(E)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(4-nitrobenzylidene)acetohydrazide ***(9b)***

Yield: 77%, m.p. 250 °C; IR (KBr, cm^{-1}) exhibited bands at 3275 (NH), 3080, 3196 (CH Ar), 2922, 2943, 2970 (CH aliphatic), 1685, 1705 (2C=O), 1600 (NH), 1521, 1344 (NO_2). ^{1}HNMR (DMSO d_6, δ, ppm): 2.56 (s, 3H, CH_3 at C4) , 3.56 (d, 2H, *J*=6 Hz, $C\underline{H}_2$-CH) , 4.90 (s, 2H, OCH_2), 4.97 (d, 1H, *J*=10.4 Hz, C<u>H</u>=CH), 5.04 (s, 1H, C<u>H</u>=CH), 5.93-5.95 (m, 1H, CH_2-C<u>H</u>), 6.24 (s, 1H, H3-Ar) 7.04 (d , 1H, *J*=8 Hz, H6-Ar), 7.61 (d, 2H, *J*=12 Hz, H5-Ar) ,7.97 (d, 2H, *J*=8 Hz, H_{ar}) , 8.27 (d, 2H, *J*=8 Hz, H_{ar}), 8.38 (s, 1H, N=C<u>H</u>), 11.96 (br.s,1H, NH, exchanged with D_2O); ^{13}CNMR (DMSO d_6, δ, ppm): 18.6 (CH_3 at C4), 27.28 ($\underline{C}H_2$-CH), 68.2 (O-CH_2), 109.2 (C6), 115.9 (CH=$\underline{C}H_2$), 120.3 (C9), 124.4 (CH_{ar}), 124.5 (C5), 128.43 (CH_{ar}), 130.0 (C_{ar}), 135.6 ($\underline{C}H=CH_2$), 140.7 (N=CH-<u>C</u>), 142.8 (N=CH), 150.9 (C=NO_2), 152.3 (C4), 159.2 (C=O), 169.5 (CO-NH); MS (EI) *m/z* (%):$_6$, 421.41 ([M^+, 25), Anal.calcd. for $C_{22}H_{19}N_3O_6$ C, 62.70; H, 4.54; N, 9.97, Found: C, 62.95; H, 4.67; N, 710.16.

(E)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(4-methylbenzylidene)acetohydrazide ***(9c)***

Yield: 78%, m.p. 250 °C; IR (KBr, cm^{-1}) exhibited bands at 3336 (NH), 3001, 3089, 3194 (CH Ar), 2872, 2933, 2976 (CH aliphatic), 1685, 1720 (2C=O), 1639 (C=N), 1604 (NH), ^{1}HNMR

(DMSO d_6, δ, ppm): 2.39 (s, 3H, CH_3-C_6H_5), 2.51 (s, 3H, CH_3 at C4) , 3.57 (d , 2H, *J*=8 Hz, C$\underline{H}_2$-CH) , 4.83 (s, 2H, OC$\underline{H}_2$) , 4.97 (t, 1H, *J*=8 Hz, C$\underline{H}$=CH), 5.03 (t, 2H, *J*=8 Hz, C$\underline{H}$=CH), 5.92-6.00 (m, 1H, C$\underline{H}$-CH_2) , 6.24 (s ,1H, CH at C3), 6.95 (d, 2H, *J=8* Hz, H_{ar}), 7.01 (d, 1H, *J= 8* Hz, H6- Ar) , 7.61 (d, 2H, *J=8* Hz, $H_{ar.}$), 7.65 (d, 1H, *J*=8 Hz, H5- Ar), 7.9 (s, 2H, $H_{ar.}$), 8.21 (s, 1H, N=CH) , 11.5 (br.s, 1H, NH, exchanged with D2O); ^{13}CNMR (DMSO d_6, δ, ppm): 19.9 (CH_3-C_6H_5), 20.1 (CH_3 at C4), 27.1 (C$\underline{H}_2$-CH), 66.1 (OCH_2), 109.2 (C6), 111.6 (C10), 112.9 (C3), 115.9 (CH=C$\underline{H}_2$), 119.9 (C8), 124.5 (C5), 127.04, 129.04 (CH_{ar}), 129.7 ($C_{ar.}$), 134.5 (C$\underline{H}$=CH_2), 144.5 (N=C$\underline{H}$), 150.9 (C-NO_2), 152.3 (C9), 154.06 (C4), 159.3 (C7), 160.5 (C2), 168.8 (CO-NH); MS (EI) *m/z* (%):$_6$, 390.44 ([M^+, 40),; Anal.calcd. for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.18, Found: C, 70.92; H, 5.77; N, 7.26.

(E)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(4-methoxybenzylidene)acetohydrazide ***(9d)***

Yield: 77%, m.p. 250 °C;IR (KBr, cm^{-1}) exhibited bands at 3446 (NH), 3082, 3032, 3001 (CH Ar), 2978, 2966, 2933 (CH aliphatic), 1722, 1685 (2C=O), 1604 (C=N); ^{1}HNMR (DMSO d_6, δ, ppm): 2.39 (s, 3H, CH_3 at C4) , 3.59 (d, 2H, *J*=6 Hz, C$\underline{H}_2$-CH), 3.80 (s, 3H, OCH_3) , 4.83 (s, 2H, OCH_2) , 4.98 (d, 1H, *J*=1.6 Hz, C$\underline{H}$=CH), 5.01 (d, 1H, *J*=1.6 Hz, C$\underline{H}$=CH), 5.92-6.02 (m, 1H, CH_2-C$\underline{H}$), 6.24 (s, 1H, CH at C3), 7.00 (d, 1H, *J*=6 Hz, H6-Ar) , 7.03 (d, 2H, *J*=9 Hz, $H_{ar.}$), 7.63 (d, 2H, *J*=8.8 Hz, H_{ar}), 7.65(d, 1H, *J*=8.8 Hz, H5-Ar) , 8.21 (s, 1H, N=C$\underline{H}$), 11.4 (br.s,1H, NH, exchanged with D2O); ^{13}CNMR (DMSO d_6, δ, ppm): 19.5 (C$\underline{H}_3$ at C4), 27.1 (C$\underline{H}_2$-CH), 55.7 (OCH_3), 66.1 (OCH_2), 109.1 (C6), 111.6 (C10), 112.2 (C3), 115.9 (CH=C$\underline{H}$), 120.2 (C8), 122.4 (C5), 127.1 ($C_{ar.}$), 129.1 (2C$\underline{H}_{ar}$), 135.7 (C$\underline{H}$=CH_2), 144.5 (N=C$\underline{H}$), 152.2 (C9), 154.05 (C4), 159.3 (C7), 160.4 (C2), 161.2 ($C_{ar.}$), 168.2 (CO-NH); MS (EI) *m/z* (%):$_6$, 406.44 ([M^+, 30); Anal.calcd. for $C_{23}H_{22}N_2O_5$: C, 67.97; H, 5.46; N, 6.89, Found: C, 67.83; H, 5.09; N, 6.56.

2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-((1E)-3-(4-(dimethylamino)phenyl)allylidene)acetohydrazide ***(9e)***

Yield: 70%, m.p. 260 °C; IR (KBr, cm^{-1}) exhibited bands 3446 (NH), 3062, 3035, 3012 (CH Ar), 2976, 2960, 2926 (CH aliphatic), 1714, 1627 (2C=O); ^{1}HNMR (DMSO d_6, δ, ppm): 2.41 (s, 3H, CH_3 at C4) , 2.86 (s, 6H, $N(CH_3)_2$) , 3.57 (d, 2H, *J*=6 Hz, C$\underline{H}_2$-CH), 4.81 (s, 2H, OCH_2), 4.97 (d, 1H, *J*=8.6 Hz, C$\underline{H}$=CH) , 5.02 (s, 1H, CH=C$\underline{H}$), 5.90-6.00 (m, 1H, CH_2-C$\underline{H}$), 5.23 (br.s, 1H, NH, exchangeable with D_2O), 6.25 (s, 1H, H3-Ar), 6.65 (d, 2H, *J*=8.8 Hz, $H_{ar.}$) 6.99 (d, 2H,

J=8.5 Hz, H6-Ar) , 7.05 (d, 2H, *J*=8.8 Hz, $H_{ar.}$), 8.04 (s, 1H, N=CH), 7.64 (d, 1H, *J*=8.8 Hz, H5-Ar), 10.26 (br.s, 1H, NH); MS (EI) *m/z* (%):$_6$, 445.52 ([M^+, 23); Anal.calcd. for $C_{26}H_{27}N_3O_4$: C, 68.72; H, 6.01; N, 10.02, Found: C, 68.88; H, 6.22; N, 10.30.

*(E)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(3,4,5-trimethoxybenzylidene)acetohydrazide (**9f**)*

Yield: 76%, m.p. 250 °C;IR (KBr, cm^{-1}) exhibited bands at 3213 (NH), 3076 (CH Ar), 2935, 2954, 2974 (CH aliphatic), 1712,1720 (2C=O), 1624 (C=N), 1604 (NH); ^{1}HNMR (DMSO d_6, δ, ppm): 2.40 (s, 3H, CH_3 at C4), 3.55 (d, 2H, *J=4* Hz, C$\underline{H}_2$-CH), 3.71 (s, 3H, OCH_3), 3.81 (m, 6H, OCH_3), 4.85 (s, 2H, O-CH_2), 4.99 (d, 1H, *J=6.8* Hz, C$\underline{H}$=CH), 5.03 (d, 1H, *J=6* Hz, C$\underline{H}$=CH), 5.91-5.95 (m, 1H, CH=C$\underline{H}_2$), 6.23 (s, 1H, CH at C3), 7.00 (d, 1H, *J=7.4* Hz, H6-Ar), 7.22 (s, 2H, CH-Ar), 7.64 (d, 1H, *J=8* Hz, H5-Ar), 7.93 (s, 1H, N=CH), 11.56 (br.s, 1H, NH, exchanged with D_2O); ^{13}CNMR (DMSO d_6, δ, ppm): 18.22 (CH_3 at C4), 26.71 ($\underline{C}H_2$-CH), 55.94 (OCH_3), 60.12 (2 OCH_3), 65.5 (O-CH_2), 104 (CH_{ar}), 108.6 (C6), 111.1 (C10), 113.4 (C3), 115.4 (CH=$\underline{C}H_2$), 124.09 (C8), 124.3 (C5), 129.4 (C_{ar}), 135.2 ($\underline{C}$H=CH_2), 140.2 (C_{ar}), 147.7 (N=CH), 151.8 (C9), 153.16 (C_{ar}), 158.8 (C-O), 160 (C2), 168.6 (CO-NH); MS (EI) *m/z* (%):, 466.49 ([M^+, 5.04); Anal.calcd. for $C_{25}H_{26}N_2O_7$: C, 64.37; H, 5.36; N, 6.01, Found: C, 64.53; H5.39; N, 6.24.

*(E)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(4-fluorobenzylidene)acetohydrazide **(9g)***

Yield: 77%, m.p. 240 °C;IR (KBr, cm^{-1}) exhibited bands at 3446 (NH), 3086, 3176 (CH Ar), 2924, 2943, 2976 (CH aliphatic), 1687, 1716 (2C=O), 1639 (C=N), 1602 (NH); ^{1}HNMR (DMSO d_6, δ, ppm): 2.39 (s, 3H, CH_3 at C4), 3.56 (d, 2H, *J=8* Hz, C$\underline{H}_2$-CH), 4.85 (s, 2H, OCH_2), 4.97 (d, 1H, *J=8* Hz, C$\underline{H}$=CH), 5.03 (s, 1H, *J=8* Hz, C$\underline{H}$=CH), 5.92-6.04 (m, 1H, CH_2-C$\underline{H}$), 6.23 (s, 1H, H3-Ar), 7.00 (d, 1H, *J=8* Hz, H6 Ar), 7.27 (d, 2H, *J=8* Hz, $H_{ar.}$), 7.29 (d, 2H, *J=8* Hz ,$H_{ar.}$), 7.66 (d, 1H, *J=8* Hz, H5- Ar), 8.28 (s, 1H, N=C$\underline{H}$), 11.6 (br.s, 1H, NH, exchanged with D_2O); ^{13}CNMR (DMSO d_6, δ, ppm): 18.22 (CH_3 at C4), 26.71 ($\underline{C}H_2$-CH), 66.93 (OCH_2), 108.7 (C6), 111.45 (C9), 113.53 (C3), 115.7 ($CH_{ar.}$), 115.93 (CH=$\underline{C}H_2$), 124.1 (C8), 124.3 (C5), 129.3, 130.5 (2 CH_{ar}), 135.2 ($\underline{C}$H=CH_2), 146.6 (N=CH), 151.8 (C9), 153.5 (C4), 158.8 (C7), 160 (C2), 164.2 (C_{ar}), 168.6 (CO-NH); MS (EI) *m/z* (%):, 394.40 ([M^+, 15); Anal.calcd. for $C_{22}H_{19}FN_2O_4$: C, 67.00; H, 4.86; N, 7.10, Found: C, 67.27; H, 5.04; N, 7.32.

(E)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(4-chlorobenzylidene)acetohydrazide ***(9h)***

Yield: 77%, m.p. 250 °C;IR (KBr, cm^{-1}) exhibited bands at 3481 (NH), 3066, 3082, 3130 (CH Ar), 2945, 2962, 2978 (CH aliphatic), 1685, 1716 (2C=O), 1602 (C=N), 1570 (NH); ^{1}HNMR (DMSO d_6, δ, ppm): 2.51 (s, 3H, CH3 at C4), 3.55 (d, 2H, *J*=6 Hz, C<u>H</u>$_2$-CH), 4.78 (s, 2H, OCH_2), 4.97 (t, 1H, *J* =8 Hz, CH=CH), 5.03 (d, 1H, *J*=7 Hz, CH=CH), 5.91-6.02 (m, 1H, CH_2-C<u>H</u>), 6.23 (s, 1H, CH at C3), 7.01 (d, 1H, *J*=4 Hz, H6-Ar), 7.5 (d, 2H, *J*=8 Hz, $H_{ar.}$), 7.73 (d, 1H, *J*=4 Hz, H5 Ar), 7.91 (d, 2H, *J*=8 Hz, $H_{ar.}$), 8.27 (s, 1H, N=CH), 11.67 (br.s, 1H, NH, exchanged with D2O); MS (EI) m/z (%):, 410.85 ([M+, 45.11); Anal.calcd. for $C_{22}H_{19}ClN_2O_4$: C,64.32; H, 4.66; N, 6.82, Found: C, 64.35; H, 4.92; N, 6.96.

Synthesis of 2-(2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)-N-benzylhydrazine-1-carboxamide ***(10)***

A mixture of compound **5** (0.01 mol, 2.88 gm) and benzyl isocyanate (0.01 mol, 1.59 gm) in dichloroethane was stirred and refluxed for 3h. The formed precipitate was filtered, washed with diethyl ether and dried. The crude mixture was crystallized from isopropanol to give **10** as a white solid.

Yield: 95%, m.p. 278 °C; IR (KBr, cm^{-1}) exhibited bands at 3305, 3331, 3385 (3NH) 3089, 3064, 3034 (CH Ar), 2956, 2926 (CH aliphatic), 1747, 1710, 1693 (3C=O); ^{1}HNMR (DMSO d_6, δ, ppm): 2.39 (s, 3H, CH_3 at C4), 3.57 (d, 2H, *J*= 4Hz, C<u>H</u>$_2$-CH), 4.25 (s, 2H, N-CH_2), 4.76 (s, 2H, OCH_2), 4.97 (d, 1H, *J=4* Hz, C<u>H</u>=CH), 5.02 (d, 1H, *J=4* Hz, C<u>H</u>=CH), 5.90-6.00 (m, 1H, CH_2-C<u>H</u>), 6.23 (s, 1H, CH at C3), 7.01 (d, 1H, *J=12* Hz, CH at C6), 7.27-7.36 (m, 5H, CH-Ar), 7.58 (d, 1H, *J=8* Hz, CH at C5), 8.04 (br.s, 1H, O=NH, exchanged with D_2O), 9.28 (br.s, 1H, O=C-NH, exchanged with D_2O), 9.82 (br.s, 1H, NH, exchanged with D_2O); ^{13}CNMR (DMSO d_6, δ, ppm): 18.2 (CH_3 at C4), 26.6 (<u>C</u>H_2-CH), 45.11 (NH-CH_2), 66.6 (O-CH_2), 108.7 (C6), 111.4 (C10), 113.8 (C3), 115.4 (CH=<u>C</u>H_2), 123.9 (C8), 124.1 (C5), 126.8, 128.1 ($CH_{ar.}$), 135.3 (<u>C</u>H=CH_2), 140.4 (NH-CH_2-<u>C</u>), 151.7 (C9), 153.5 (C4), 158.7 (NH-C=O), 160.01 (C7), 160.08 (C2), 167.2 (NH-CO); MS (EI) *m/z* (%):, 421.45 ([M^+, 42); Anal.calcd. for $C_{23}H_{23}N_3O_5$: C,65.55; H, 5.55; N, 9.97, Found: C, 65.82; H, 5.75; N, 10.08.

Synthesis of 2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl) oxy)-N-(3-benzyl-2,4,5-trioxoimidazolidin-1-yl) acetamide ***(11)***

To a solution of compound **10** (0.001 mol, 0.47 gm) in dry benzene (5 mL), oxalyl chloride (0.002 mol, 0.25 gm) was added dropwise while stirring, then refluxed at 60-65℃ for 2h. The solvent was distilled under reduced pressure and the obtained residue was dried. The crude product was crystallized from ethanol to obtain **11** as a white solid.

General procedures for the synthesis of 2-(2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)-N-aralkylhydrazine-1-carbothioamide ***(12a, b)***

To a solution of compound **5** (0.01 mol, 2.88 gm) in ethanol (30 mL), an appropriate substituted isothiocyanate (0.01 mol) was added, and then the reaction mixture was refluxed under stirring for 12h. The separated solid was filtered, washed with diethyl ether and dried. The crude product was crystallized from isopropanol.

2-(2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)-N-ethylhydrazine-1-carbothioamide ***(12a)***

Yield: 76%, m.p. 225 °C ; IR (KBr, cm^{-1}) exhibited bands at 3169, 3329, 3379 (3NH), 3078 (CH Ar), 2976, 2931 (CH aliphatic), 1720, 1710 (2C=O), 1122 (C=S); ^{1}HNMR (DMSO d_6, δ, ppm): 1.25 (t, 3H, *J=6.8* Hz, CH_2-C$\underline{H}_3$), 2.41 (s, 3H, C$\underline{H}_3$ at C4), 3.47 (d, 2H, *J=8* Hz, C$\underline{H}_2$-CH), 4.05 (q, 2H, *J=6.8* Hz, C$\underline{H}_2$-CH_3), 4.77 (s, 2H, OCH_2), 4.94 (t, 1H, *J*=9.6 Hz, C$\underline{H}$=CH), 5.02 (t, 1H, *J=1.2* Hz, C$\underline{H}$=CH), 5.87-5.91 (m, 1H, C$\underline{H}$-CH_2), 6.26 (s, 1H, C$\underline{H}$ at C3), 7.04 (d, 1H, *J=8* Hz, H6-Ar), 7.28 (s, 1H, -CH_2), 7.71 (d, 1H, *J=8* Hz, H5-Ar), 8,0 (br.s, 1H, NH=O, exchanged with D_2O), 9.3 (br.s, 1H, NH=S, exchanged with D_2O); ^{13}CNMR (DMSO d_6, δ, ppm): 15.32 (CH_2-C$\underline{H}_3$), 19.8 (CH_3 at C4), 27.1 (C$\underline{H}_2$-CH), 47.4 (NH-CH_2), 67.8 (OCH_2), 109.2 (C6), 111.9 (C10), 114.3 (C3), 115.4 (CH=C$\underline{H}_2$), 124.6 (C8), 125.4 (C5), 128.5 (C$\underline{H}_{ar}$), 152.2 (C9), 152.9 (C4), 158.7 (C7), 160.4 (C2), 166.5(C=O), 183.2 (C=S); MS (EI) *m/z* (%):, 375.44 ([M^+, 34); Anal.calcd. for $C_{18}H_{21}N_3O_4S$: C, 57.58, H, 5.64, N, 11.19, Found: C, 57.81, H, 5.70, N, 11.33.

2-(2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)-N-benzylhydrazine-1-carbothioamide ***(12b)***

Yield: 80%, m.p. 230 °C; IR (KBr, cm^{-1}) exhibited bands at 3344, 3444, 3458 (3NH), 3005, 3034, 3070, 3099 (CH Ar), 2916, 2937, 2947, 2980 (CH aliphatic), 1716, 1705 (2C=O); ^{1}HNMR (DMSO d_6, δ, ppm): 2.50 (s, 3H, CH_3 at C4) , 3.58 (d, 2H, *J=6* Hz, C$\underline{H}_2$-CH), 4.75(s, 2H, OCH_2), 4.77 (t, 1H, *J=6* Hz, C$\underline{H}$=CH), 4.78 (s, 2H, C$\underline{H}_2$-C_6H_5), 4.98 (t, 1H, *J*=10.4 Hz,

CH=C<u>H</u>), 5.0 (d, 2H, *J=2* Hz, CH=C<u>H</u>) , 5.90-5.98 (m , 1H, CH_2-C<u>H</u>,) , 6.24 (s, 1H, CH at C3) , 7.03 (d, 1H , *J=8* Hz, H6-Ar), 7.22-7.27 (m, 5H, $H_{ar.}$), 7.29 (d, 1H, *J=4* Hz, H5-Ar), 8.5 (br.s, 1H, N<u>H</u>-CH, exchanged with D_2O), 10.09 (br.s,1H, N<u>H</u>-C=S, exchanged with D_2O); MS (EI) *m/z* (%):, 437.51 ([M^+, 14); Anal.calcd. for $C_{25}H_{21}N_3O_7S$: C,63.14; H, 5.30; N, 9.60, Found: C, 63.35; H, 5.39; N, 9.71.

General procedures for the synthesis 8-allyl-7-((4- aralkyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) methoxy)-4-methyl-2H-chromen-2-one ***(13a,b)***

A solution of 2 N sodium hydroxide (12 mL) was added to a solution of compounds **12a,b** (0.04 mol) then refluxed with stirring for 12h. The reaction mixture was cooled and acidified with 10% hydrochloric acid to PH 5. The produced precipitate was filtered, washed and crystallized from methanol.

8-allyl-7-((4-ethyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one ***(13a)***[23]

Yield: 70%, m.p. 200 °C 3419 (NH), 3003, 3043, 3157 (CH Ar), 2939, 2953, 2976 (CH aliphatic), 1705 (C=O), 1608 (C=N), 1568 (NH); ^{1}HNMR (DMSO d_6, δ, ppm): 1.25 (t, 3H, *J=4* Hz, CH_2-C<u>H</u>$_3$), 2.43 (s, 3H, CH_3 at C4), 3.44 (d, 2H, *J=4* Hz, C<u>H</u>$_2$-CH), 4.05 (d, 2H, *J=4* Hz, C<u>H</u>$_2$-CH_3), 4.83 (d, 1H, *J=16* Hz, C<u>H</u>=CH), 4.92 (d, 1H, *J=12* Hz, C<u>H</u>=CH), 5.40 (s, 2H, OCH_2), 5.82-5.91 (m, 1H, C<u>H</u>-CH_2), 6.22 (s, 1H, CH at C3), 7.28 (d, 1H, *J=8* Hz, CH at C6), 7.68 (d, 1H, *J=8* Hz, CH at C5), 13.90 (br.s, 1H, NH, exchanged with D_2O); MS (EI) *m/z* (%):, 357.43 ([M^+, 100); Anal.calcd. for $C_{18}H_{19}N_3O_3S$: C,60.49; H, 5.36; N, 11.76, Found: C, 60.69; H, 5.531; N, 11.95.

8-allyl-7-((4-benzyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one ***(13b)***

Yield: 70%, m.p. 200 °C 3124 (NH), 3043, 3103 (CH Ar), 2904, 2935, 2983 (CH aliphatic), 1705 (C=O), 1602 (C=N); ^{1}HNMR (DMSO d_6, δ, ppm): 2.5 (s, 3H, CH_3 at C4), 3.07 (d, 2H, *J=8* Hz, C<u>H</u>$_2$-CH), 4.73 (s, 2H, N-CH_2), 4.75 (d, 1H, *J=8* Hz, C<u>H</u>=CH_2), 4.87 (d, 1H, *J=8* Hz , C<u>H</u>=CH_2), 5.24 (s, 2H, C<u>H</u>$_2$-C_6H_5), 5.53 (s, 2H, OCH_2), 5.91-5.98 (m, 1H, C<u>H</u>-CH_2), 6.23 (s, 1H, CH at C3), 7.14 (d, 1H, *J=8* Hz, H6-Ar), 7.18-7.28 (m, 5H, $H_{ar.}$), 7.64 (d, 1H, *J=8.8* Hz, H5-Ar), 10.63 (br.s, 1H, NH, exchanged with D_2O); ^{13}CNMR (DMSO d_6, δ, ppm): 18.4 (CH_3 at

C4), 26.5 (CH_2-CH), 48.6 (CH_2-C_6H_5), 61.6 (O-CH_2), 109.06 (C6), 112.09 (C10), 114.5 (C3), 115.6 (CH=CH_2), 124.7 (C8), 125.3 (C5), 126.1, 128.07, 129.23 (3CH_{ar}), 135.6 (CH=CH_2), 135.8 (C_{ar}), 148.2 (C9), 152.1 (C4), 153.9 (N=C), 157.9 (C7), 160.3 (C=O), 180.1 (C=S); MS (EI) *m/z* (%):, 419.50 ([M^+, 45.11); Anal.calcd. for $C_{23}H_{21}N_3O_3S$: C,65.85; H5.05; N, 10.02, Found: C, 66.03; H, 5.15; N, 10.32.

General procedures for the synthesis of (Z)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N-(2-(ethylimino)-4-phenylthiazol- or N-(4-phenyl-2-(phenylimino)thiazol- 3(2H)-yl)acetamide ***(14a, b)***

A mixture of compound **12a**,**b** (0.002 mol) and the appropriate phenacyl bromide (0.002 mol, 0.39gm) in ethanol/chloroform 1:3 mixture (100 mL) were refluxed for 3h, concentrated, left to cool, filtered and dried.

(Z)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N-(2-(ethylimino)-4-phenylthiazol-3(2H)-yl)acetamide ***(14a)***

Yield: 75%, m.p. 200 °C 3477 (NH), 3078,3061 (CH Ar), 2974, 2933 (CH aliphatic), 1712, 1600 (2C=O), 702 (C-S); ^{1}HNMR (DMSO d_6, δ, ppm): 0.94 (s, 3H, CH_2-CH_3), 1.11 (q, 2H, *J=8* Hz, CH_2-CH_3), 2.42 (s, 3H, CH_3 at C4), 3.61 (d, 2H, *J=8* Hz, CH_2-CH), 3.91 (q, 2H, *J=12* Hz, CH_2-CH_3), 4.97 (s, 2H, O-CH_2), 4.99 (t, 1H, *J=8* Hz, CH=CH), 5.02 (t, 1H, *J=4* Hz, CH=CH), 5.91-5.98 (m, 1H, CH_2-CH), 6.26 (s, 1H, CH at C3), 6.97 (s, 1H, CH-thiazoline), 7.09 (d, 1H, *J=12* Hz,H6-Ar), 7.49-7.58 (m, 5H, $H_{ar.}$), 7.7 (d, 1H, *J=8* Hz, H5-Ar); ^{13}CNMR (DMSO d_6, δ, ppm):12.6 (CH_2-CH_3), 18.2 (CH_3 at C4), 26.6 (CH_2-CH), 42.2 (CH_2-CH_3), 66.6 (OCH_2), 108.3 (CH-thiazoline), 111.5 (C6), 114.1 (C3), 115.0 (C3), 115.49 (CH=CH_2), 124.3 (C8), 128.7 (C5), 129.01, 129.46, 130.26 (3 CH_{ar}), 135.2 ($C_{ar.}$), 141.66 (CH_2=CH), 151.83 (C9), 153.5 (C4), 158.0 (C=N-), 159.98 (C7), 160.7 (C2), 161.5 (C-C_6H_5, thiazoline), 170.4 (CO-NH); MS (EI) *m/z* (%):, 475.56 ([M^+, 50.2); Anal.calcd. for $C_{26}H_{25}N_3O_4S$: C,65.67; H,5.30; N, 8.84, Found: C, 65.81; H, 5.39; N, 8.91.

(Z)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N-(2-(benzylimino)-4-phenylthiazol-3(2H)-yl)acetamide ***(14b)***

Yield: 70%, m.p. 236 °C 3419 (NH), 3030, 3061, 3107 (CH Ar), 2926, 2978 (CH aliphatic), 1724, 1618 (2C=O), 1602 (C=N); ^{1}HNMR (DMSO d_6, δ, ppm): 2.40 (s, 3H, CH_3), 2.42 (s, 2H,

CH_2-C_6H_5), 3.53 (d, 2H, *J=4* Hz, C<u>H</u>$_2$-CH), 4.87 (s, 2H, OCH_2), 4.98 (s, 1H, CH=C<u>H</u>), 5.14 (s, 1H, CH=C<u>H</u>), 5.94-5.97 (m, 1H, C<u>H</u>-CH_2), 6.25 (s, 1H, H3-Ar.), 6.99 (s, 1H, CH-thiazoline), 7.08 (d, 1H, *J=8* Hz,H6-Ar.), 7.25-7.44 (m, 10H, $H_{ar.}$), 7.63 (d, 1H, *J=8.8* Hz, H5-Ar.); MS (EI) *m/z* (%):, 537.63 ([M^+, 42.5); Anal.calcd. for $C_{31}H_{27}N_3O_4S$: C,69.26; H,5.06; N, 7.82, Found: C, 69.36; H, 5.25; N, 8.05.

General procedures for the synthesis of 8-allyl-7-((5-(aralkyl amino)-1,3,4-thiadiazol-2-yl) methoxy)-4-methyl-2H-chromen-2-one ***(15a,b)***

Conc.H_2SO_4 (10 mL) was added to compound **12a,b**. and the mixture allowed to stand overnight. The reaction mixture was cooled then poured onto ice water and neutralized with ammonium hydroxide solution to PH 7. The produced precipitate was filtered off, washed and crystallized from glacial acetic acid.

8-allyl-7-((5-(ethylamino)-1,3,4-thiadiazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one ***(15a)***

Yield: 90%, m.p. 248 °C 3277 (NH), 3061,3041 (CH Ar), 2964, 2931 (CH aliphatic), 1735 (C=O), 617 (C-S); ^{1}HNMR (DMSO *d_6*, δ, ppm): 1.16 (t, 3H, *J=8* Hz, CH_2-C<u>H</u>$_3$), 2.39 (s, 3H, CH_3 at C4), 3.29 (d, 2H, *J=8* Hz, C<u>H</u>$_2$-CH), 3.40 (s, 2H, C<u>H</u>$_2$-CH_3), 4.92 (s, 1H, CH=C<u>H</u>), 5.03 (s, 1H, CH=C<u>H</u>), 5.83-5.90 (m, 1H, CH_2-C<u>H</u>), 5.45 (s, 2H, OCH_2), 6.22 (s, 1H, CH at C3), 7.20 (d, 1H, *8* Hz, H6-Ar), 7.61 (d, 1H, *8* Hz, H6-Ar), 7.82 (br.s, 1H, NH, exchanged with D_2O); MS (EI) *m/z* (%):, 357.34 ([M^+, 45.11); Anal.calcd. for $C_{18}H_{19}N_3O_3S$: C,60.49; H,5.36; N, 11.76, Found: C, 60.2; H, 5.31; N, 11.65.

8-allyl-7-((5-(benzylamino)-1,3,4-thiadiazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one ***(15b)***

Yield: 90%, m.p. 250 °C 3157 (NH), 1705 (C=O), 1600 (C=N), 1566 (NH).; ^{1}HNMR (DMSO *d_6*, δ, ppm): 2.40 (s, 3H, CH_3 at C4), 2.92 (d, 2H, *J*=8 Hz, C<u>H</u>$_2$-CH), 4.49 (s, 2H, NH-C<u>H</u>$_2$), 4.55 (d, 1H, *J*=4 Hz, C<u>H</u>=CH), 5.18 (s, 1H, C<u>H</u>=CH), 5.37 (s, 2H, OCH_2), 5.89-5.92 (m, 1H, CH_2-C<u>H</u>), 6.24 (s, 1H, CH at C3), 7.09 (d, 1H, *J=8* Hz, CH at C6), 7.64 (d, 1H, *J=12* Hz, CH at C5), 8.36 (br.s, 1H, NH, exchanged with D_2O); MS (EI) *m/z* (%):, 419.50 ([M^+, 45.11); Anal.calcd. for $C_{23}H_{21}N_3O_3S$: C, 65.85; H,5.05; N, 10.02, Found: C, 65.81; H, 5.39; N, 8.91.

General procedures for the synthesis of 2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl) oxy)-N'-(3-aralkyl -4-oxothiazolidin-2-ylidene) acetohydrazide ***(16a, b)***

To a suspension of substituted thiosemicarbazide derivatives **12a,b** (0.01 mol) in glacial acetic acid (5 mL), anhydrous sodium acetate (0.02 mol, 1.64gm) and chloroacetic acid (0.02mol, 1.89gm) were added. The reaction mixture was refluxed for 4h then cooled, diluted with water and allowed to stand overnight. The product was filtered off, washed and dried.

(Z)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(3-ethyl-4-oxothiazolidin-2-ylidene)acetohydrazide ***(16a)***

Yield: 65%, m.p. 166 °C 3500 (NH), 3016, 3188 (CH Ar), 2877, 2935, 2980 (CH aliphatic), 1699, 1710, 1728 (3C=O), 1602 (C=N); ^{1}HNMR (DMSO d_6, δ, ppm): 0.87 (t, 3H, *J*=4Hz, CH_2-C$\underline{H}_3$), 1.10 (q, 2H, *J*=4Hz, C$\underline{H}_2$-CH_3), 2.40 (s, 3H, CH_3 at C4), 3.46 (d, 2H, *J= 8* Hz, C$\underline{H}_2$-CH), 4.07 (s, 2H, CH_2-thiazolidinone), 4.83 (s, 2H, OCH_2), 4.86 (t, 1H, *J=4* Hz, C$\underline{H}$=CH), 4.95 (q, 2H, *J=8* Hz, C$\underline{H}_2$-CH_3), 5.02 (t, 1H, *J=4* Hz, C$\underline{H}$=CH), 5.91-5.98 (m, 1H, CH_2-C$\underline{H}$), 6.25 (s, 1H, H3-Ar.), 7.06 (d, 1H, *J=8* Hz, H6-Ar.), 7.67 (d, 1H, *J=8* Hz, CH at C5), 10.57 (br.s, 1H, NH, exchanged with D_2O); ^{13}CNMR (DMSO d_6, δ, ppm): 12.5 (CH_2-$\underline{C}H_3$), 18.7 (CH_3 at C4), 27.1 ($\underline{C}H_2$-CH), 33.2 (CH_2-thiazolidinone), 37.9 ($\underline{C}H_2$-CH_3), 67.2 (OCH_2), 109.5 (C6), 111.8 (C10), 114.5 (C3), 115.9 (CH=$\underline{C}H_2$), 124.7 (C8), 124.9 (C5), 135.7 ($\underline{C}$H=CH_2), 151.4 (C9), 154 (C4), 158.8 (C-O), 160.5 (C2), 169.7 (CO-NH), 171.6 (CO-thiazolidinone); MS (EI) *m/z* (%):, 415.46 ([M^+, 25); Anal.calcd. for $C_{20}H_{21}N_3O_5S$: C, 57.82; H, 5.1; N, 10.11; Found: C, 57.62; H, 5.22; N, 10.31.

(Z)-2-((8-allyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(3-benzyl-4-oxothiazolidin-2-ylidene)acetohydrazide ***(16b)***

Yield: 65%, m.p. 170 °C 3348 (NH), 3008, 3034, 3064 (CH Ar), 2931, 2958, 2985 (CH aliphatic), 1732, 1708, 1604 (2C=O), 1604 (C=N), 1570 (NH); ^{1}HNMR (DMSO d_6, δ, ppm): 2.40 (s, 3H, CH_3 at C4), 3.87 (d, 2H, *J=12* Hz, C$\underline{H}_2$-CH), 4.15 (s, 2H, CH_2-thiazolidinone), 4.81 (s, 2H, OCH_2), 4.96 (t, 1H, *J*=8 Hz, C$\underline{H}$=CH), 5.01 (s, 1H, C$\underline{H}$=CH), 5.40 (s, 2H, CH_2-C_6H_5), 5.93-5.97 (m, 1H, C$\underline{H}$-CH_2), 6.23 (s, 1H, C$\underline{H}$ at C3), 7.03 (d, 1H, *J=8* Hz, H6-Ar), 7.29-7.35 (m, 5H, H_{ar}), 7.64 (d, 1H, *J=4* Hz, H5-Ar), 10.56 (br.s, 1H, NH, exchanged with D_2O); MS (EI) *m/z* (%):, 477.54 ([M^+, 45.11); Anal.calcd. for $C_{25}H_{23}N_3O_5S$: C, 62.88; H,4.85; N, 8.80, Found: C, 63.01; H, 4.95; N, 9.02.

4.2. Pharmacology

4.2.1. Materials and Methods

Materials

4.2.1.1. Animals

In the current study, 200 adult male Swiss albino mice (18–25 g) were purchased from European Reef Animal House Colony (Egypt). They were kept under standardized conditions; 12 h-light/dark cycle with room temperature adjusted to 23 + 2 ^{0}C and relative humidity at 55 + 5%. Free access to tap water and standard rat chow was provided daily during the entire experimental period. Animal care and handling were performed after receiving approval of the ethical committee of Misr University for Science & Technology (approval no ID MC3). Procedures followed herein were in accordance with the recommendations for the proper care and use of laboratory animals "Canadian Council on Animal Care Guidelines, 1984" and "ARRIVE guidelines, 2010". To minimize animal suffering, extensive efforts were made with the least number of animals (n=6) in order to obtain reliable data.

4.2.1.2. Drugs and Chemicals

Ethosuximide (Pfizer Co., Giza, Egypt), diphenylhydantoin (Nasr Co., Giza, Egypt) and gabapentin (Pfizer Co., Egypt) were used as standard drugs for the study. Tween-80 and pentylenetetrazole were purchased from Sigma (St. Louis, MO, USA). Animals were given candidate drugs or standards (0.1 mL/10 g body weight, i.p) as a suspension in tween-80 (7%).

Methods

One week following adaptation to laboratory conditions, animals were randomly assigned to series and reference standard groups consisting of six mice each in preparation for phase I study. Another set of animals were allocated for phases II and neurochemical assay to determine ED_{50} as well as mechanistic action of the most active congener, respectively.

4.2.1.3. Phase I: Preliminary screening for anticonvulsant activity

4.2.1.3.1. Subcutaneous Pentylenetetrazole (scPTZ) Screen

This test, which simulates human absence/petit mal epilepsy or myoclonic seizures, produces threshold or minimal (clonic) seizures [34, 35]. An aqueous solution of PTZ (85 mg/kg) was administered subcutaneously (sc) in the loose fold of skin of the mouse nape. This

dose induces seizures in more than 97% of animals, which is known as the convulsive dose 97 (CD97) according to Löscher *et al*., 1991 [36]. Compounds of the series (at a dose level equivalent to 100 mg/kg) were injected intraperitoneal (i.p), 45 min prior to scPTZ. Animals were closely monitored for seizure episodes for 30 min. An episode of clonic convulsions that persisted for at least 5 s, with loss of righting reflex was considered as a threshold convulsion. Absence of a single 5 s episode of clonic spasms during the 30 min period of observation is taken as the end point in this test and considered positive [34, 37].

4.2.1.3.2. Maximal Electroshock Seizure (MES) Screen

The test was performed according to standard protocol followed by the National Institute of Health NIH [38] and Krall *et al.* [34]**.** Electro-convulsions were induced using a Rodent Shocker generator (constant-current stimulator Type 221, Hugo Sachs Elektronik, Freiburg, Germany) adjusted to a fixed 25 mA current intensity and 0.2 s stimulus duration, delivered via ear clip electrodes, forty-five min post-injection of the test compound (i.p). Occurrence of tonic hind limb extension, i.e. the hind limbs of animals outstretched 180 degree to the plane of the body axis, was considered as the criterion for positive seizure activity [39].

4.2.1.3.3. Neurotoxicity

Neurotoxicity of test compounds was assessed by adopting the rotarod method to determine any signs of motor impairment [40]**.** In this test, the animals were pre-trained to maintain balance on a rotating 1 inch diameter knurled plastic rod (rotarod, UGO Basile, 47600, Varese, Italy) at a fixed speed of 10 rpm for 60 s. Animals that fulfill this criterion and pass the test after three trials were included in the experiment. This test was carried out forty-five min post- injection (i.p) of each of the candidate drugs of the series. The mice were re-located on the rotating rod and the motor performance was closely monitored for up to 60 s. Neurotoxicity was indicated by the loss of motor coordination and inability of the animal to maintain equilibrium on the rod for at least one minute.

4.2.1.4. Phase II: Quantitative estimation of median effective dose (ED_{50}):

For the determination of median effective dose ED_{50}, the most potent surrogates were administered to 5 groups of 6 mice at variable selected doses that cover 10-90% protection range against PTZ-induced seizure. The median effective dose (at 95% confidence interval) was

calculated as the dose of the candidate drug that protects 50% of animals in the group against scPTZ [41].

4.2.1.5. Quantitative Estimation of GABA in whole mouse Brain

Free γ-aminobutyric acid (GABA) content in the brain tissue homogenate was determined by High Performance Liquid Chromatography (HPLC) according to the method described by Heinrikson and Meredith [42]**.** The HPLC system of Agilent (Germany) consisted of quaternary pump; a column oven, Rheodine injector, 20µl loop and UV detector.

The technique involved a two-step process; sample preparation then derivatization. The first step was sample preparation, where whole mouse brain was weighed and homogenized in 1/10 weight/volume of 75% aqueous HPLC grade methanol. The second step involved derivatization procedure which involved a series of processing, whereby derivatizing agent consisted of 7:1:1:1 mixture (by volume) of methanol:, triethylamine (TEA): double distilled deionized water: phenylisothiocyanate (PITC)**.** Derivatized samples were re-dried by vacuum (70millitore), diluted by a sample diluent then mixed with acetoniltrile. 20µl derivatized amino acid standards and derivatized samples were injected into the column at flow rate 2ml/min for separation by PICO- TAG column (Waters, Milford, USA) for free-amino acid analysis (3.9 x 30 cm). The assay conditions were adjusted at 46 °C, and the resulting chromatogram (at 250 nm) identified each amino acid position and concentration from the sample as compared to that of the amino acids standard. The report and chromatogram were obtained from chemstation program. The content of GABA in brain homogenate was calculated as µg per gram tissue.

4.2.2. Statistical Analysis

Statistical analysis of the obtained data regarding GABA level in mouse brain was accomplished by means of one-way analysis of variance (one-way ANOVA), followed by Tukey's post hoc test. Results were expressed as mean ± SEM. The level of statistical significance was set at $p < 0.05$.

4.3. Docking study

Molecular modeling study was accomplished using Discovery Studio 2016. The crystal structure of γ-aminobutyric acid aminotransferase GABA-AT) was retrieved from RCSB protein

data bank (PDB: 1OWH). In general, the protein structure was prepared then hydrogen atoms were added. All the prepared molecules were minimized and docked into the protein structure using CDOCKER protocol.

4.4. In-Silico Study

All physicochemical descriptors and pharmacokinetic properties were calculated using swissADME a fee online web tool.

Conflict of interest

The authors have declared no conflict of interest.

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Figure legends

Fig.1. Structures of certain anticonvulsants bearing coumarin moiety

Fig.2. Pharmacophoric features for anticonvulsants in marketed drugs and the representative compound **6**.

Fig.3. Effect of treatment of target compound **6** (100 mg/kg) on GABA concentration in whole mouse brain (µg/ g tissue). * Significantly different from control group at $p<0.05$. # significantly different from reference standard GBP (100 mg/kg) at $p<0.05$. Each value represents mean ± SEM (n=6).

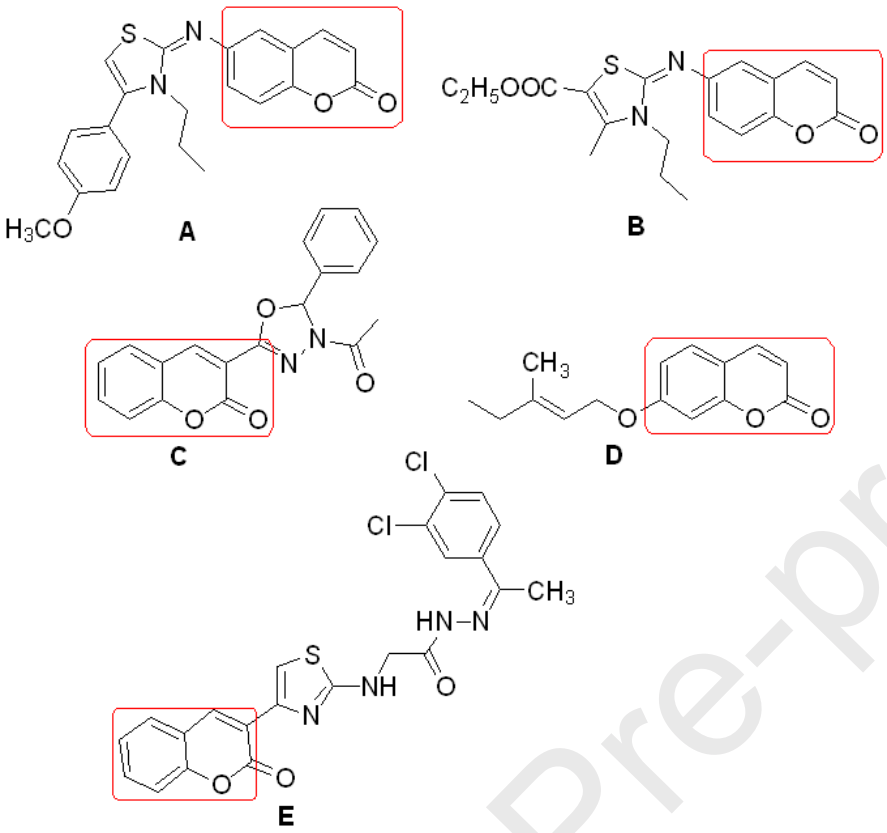
Fig.4. A) 2D-representation for the binding mode of co-crystallized ligand (viagabatrin) in the active site of GABA-AT enzyme (PDB ID: 1OHW). B) Predicted binding pose of compound **6** into the active site of GABA-AT enzyme (PDB ID: 1OHW).

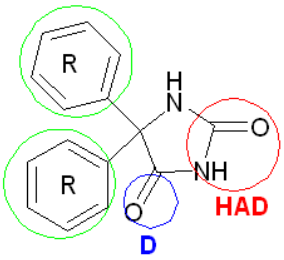
Fig.5. Distance between the essential structural elements, The chemical features colored green, and orange and light blue represent hydrogen bonding acceptor (HBA), ring aromatic (RA) and hydrophobic features (HY), respectively.

Scheme 1. Synthesis of compounds 1-5

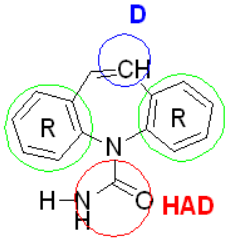
Scheme 2. Synthesis of compounds 6-11

Scheme 3. Synthesis of compounds 12a, b -16a, b

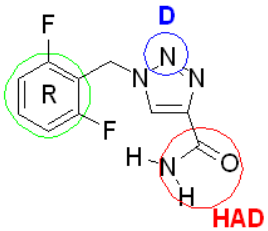




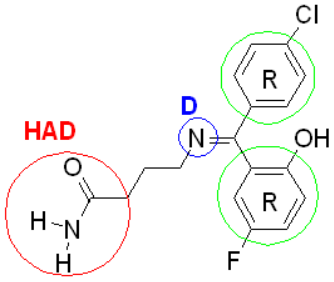
Phenytoin



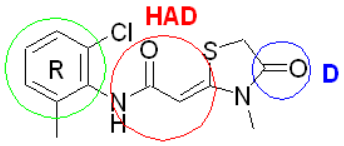
Carbamazepine



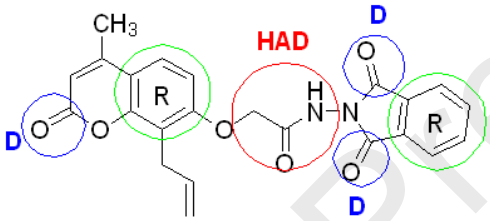
Rufinamide



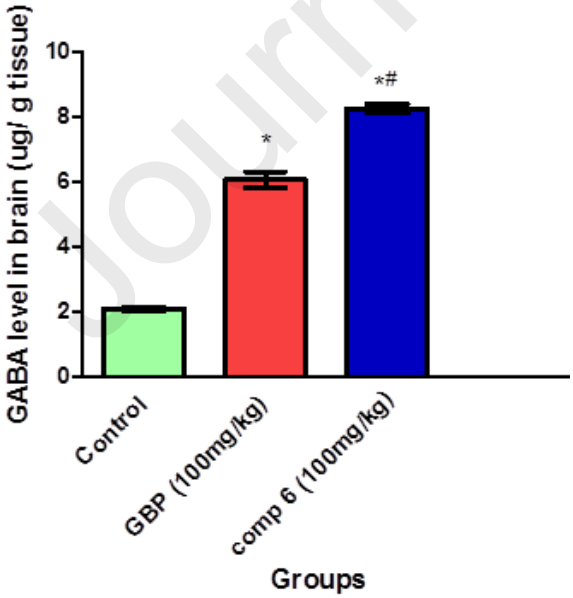
Progabide

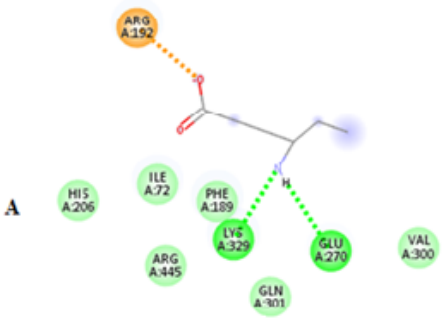


Ralitoline

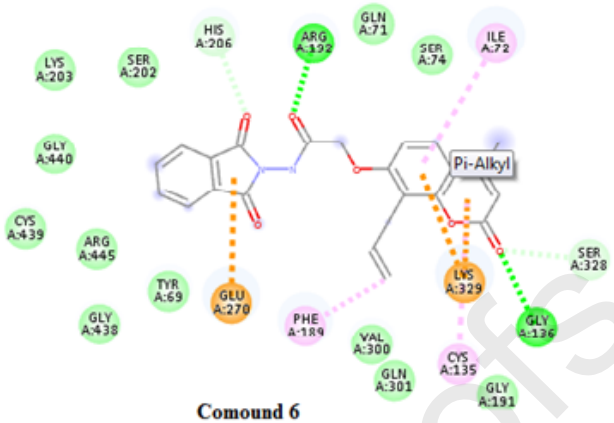


representitive compound

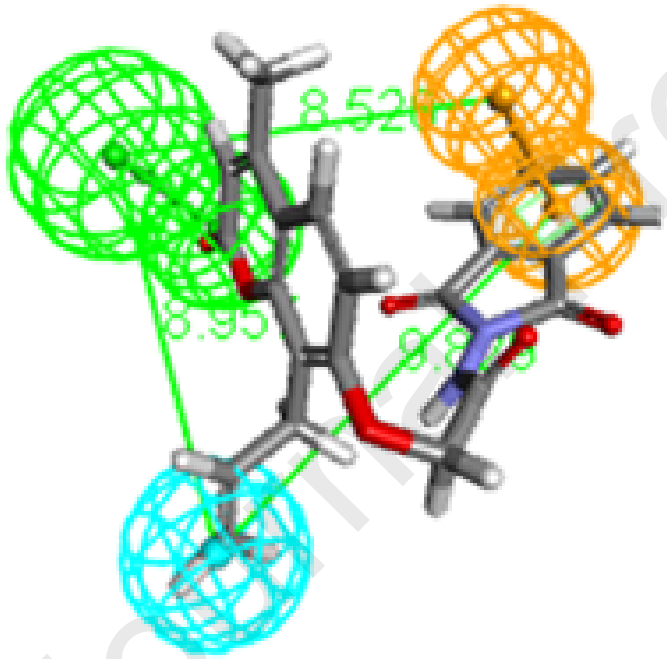


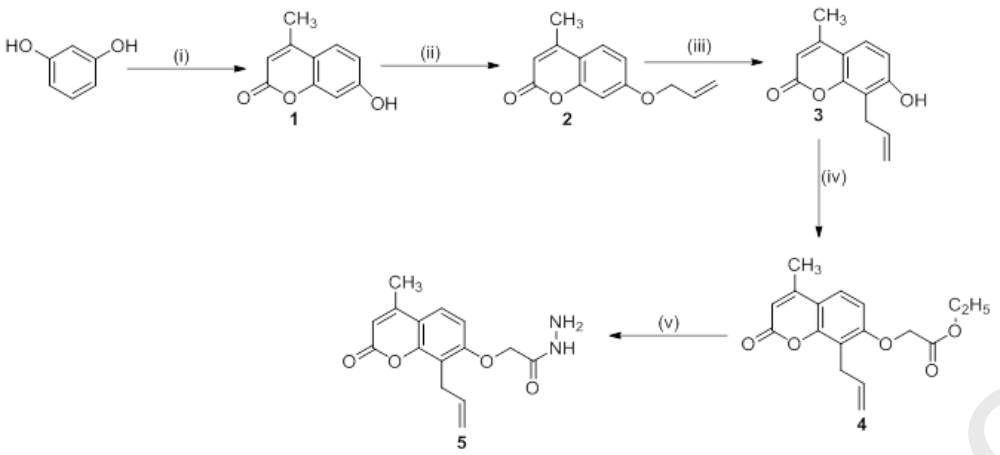


Ligand

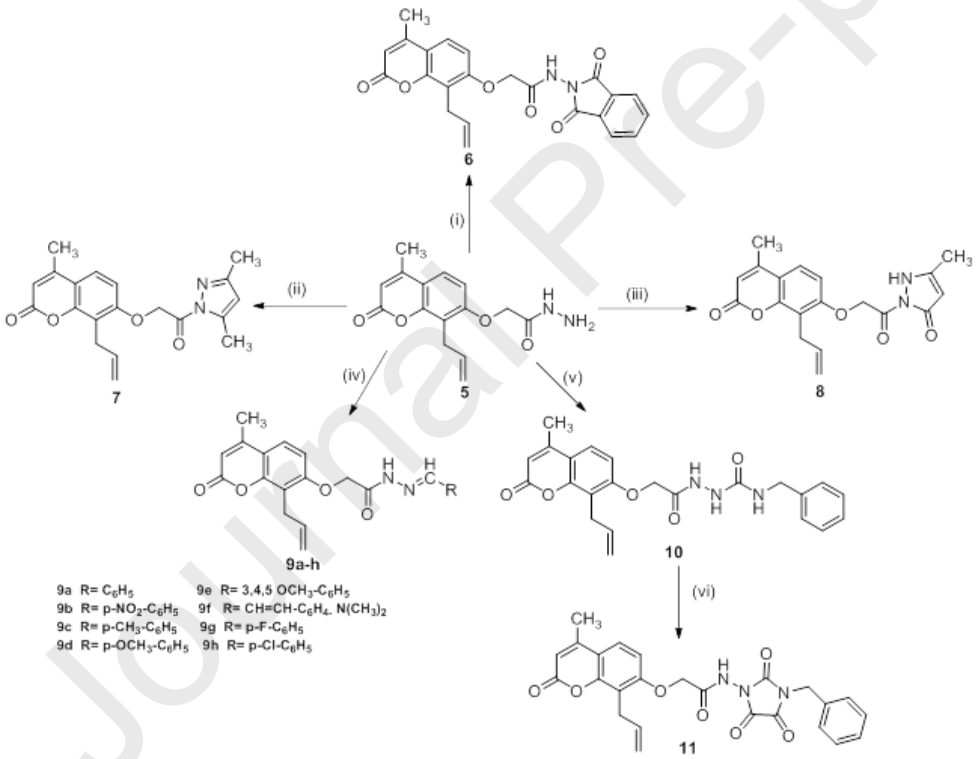


Comound 6



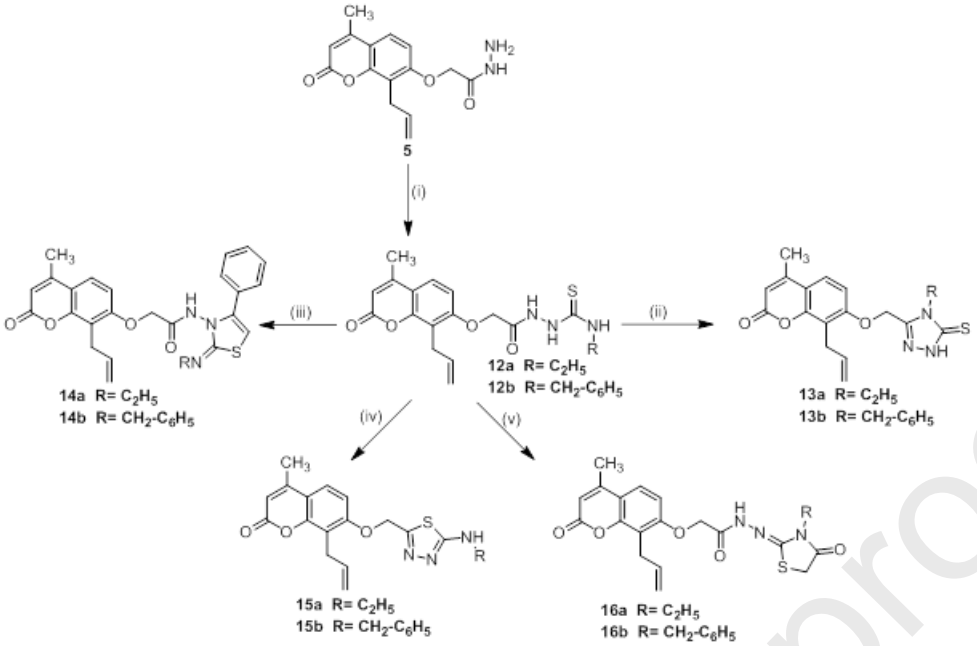


Reagents and conditions: (i) Ethyl acetoacetate, conc.H_2SO_4, reflux, 18h. (ii) Allyl bromide, anhydrous K_2CO_3, acetone, reflux, 16h. (iii) Diethyl aniline, reflux, 2h. (iv) Ethyl chloroacetate, anhydrous K_2CO_3, acetone, reflux, 2h. (v) Hydrazine hydrate, ethanol, reflux, 4h.



9a R= C_6H_5 9e R= 3,4,5 OCH_3-C_6H_5
9b R= p-NO_2-C_6H_5 9f R= CH=CH-C_6H_4-$N(CH_3)_2$
9c R= p-CH_3-C_6H_5 9g R= p-F-C_6H_5
9d R= p-OCH_3-C_6H_5 9h R= p-Cl-C_6H_5

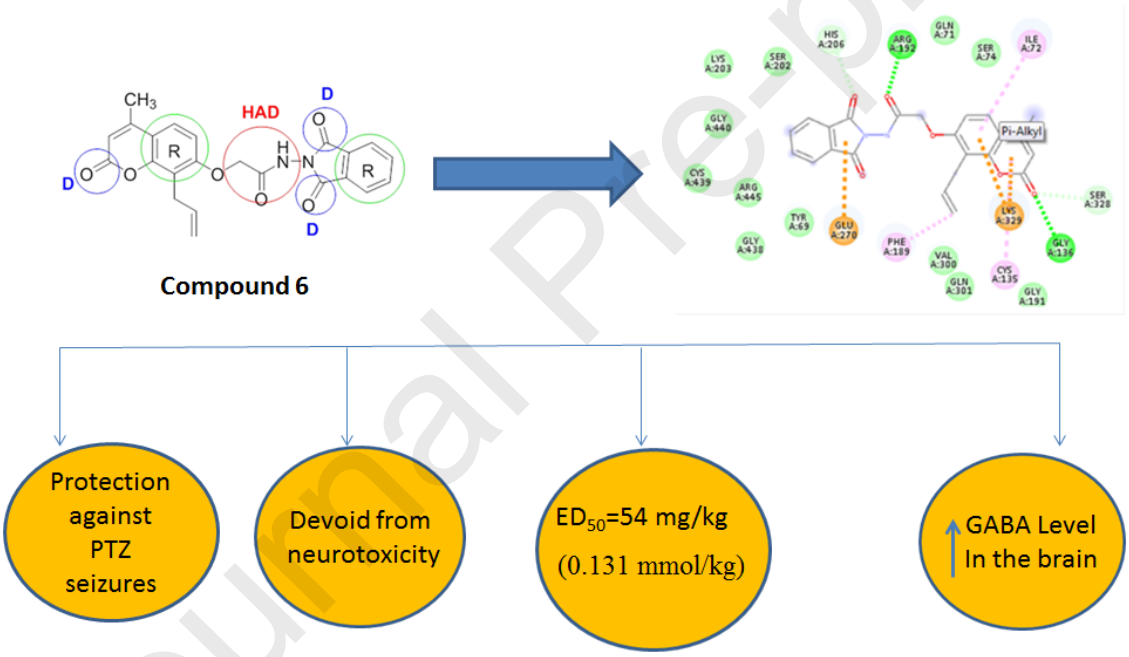
Reagents and conditions: (i) Phthalic anhydride, dioxane, acetic acid, reflux, 7h (ii) Acetyl acetone, ethanol, tri ethylamine, reflux, 12h (iii) Ethyl acetoacetate, ethanol, reflux, 4h (iv) appropriate aromatic aldehyde, glacial acetic acid, reflux, 6h (v) benzyl isocyanate, dichloroethane, reflux, 3h (vi) oxalyl chloride, benzene, reflux, 2h.



Reagents and conditions: (i) appropriate aryl/alkyl isothiocyanate, ethanol, 12h, (ii) 2N NaOH, reflux, 2h, (iii) appropriate phenacyl bromide, ethanol/chloroform 1:3, reflux, 3h, (iv) conc.H_2SO_4, r.t. overnight, v) chloroacetic acid, anhydrous sodium acetate, glacial acetic acid, reflux, 4h.

Highlights

1. Synthesis of novel benzopyrone derivatives as anticonvulsants.
2. In preliminary phase I screening, compound 6 exhibited the most potent anticonvulsant activity against scPTZ test with the lowest ED_{50}=54.86 mg/kg (0.131mmol/Kg) and no signs of neurotoxicity.
3. Compound **6** significantly elevated the brain GABA level to 4 folds that of the control group.
4. Molecular docking Study for compound **6** into γ-aminobutyric acid aminotransferase (GABA-AT) enzyme was performed.
5. An *in silico* pharmacophoric pattern and drug likeness studies showed that compound **6** fulfills all the requirements crucial for the anticonvulsant drugs



Conflict of interest

The authors have declared no conflict of interest.