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Synthesis and preliminary evaluation of some substituted coumarins as anticonvulsant agents

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Abstract—Some new substituted coumarinylthiazolines, coumarinylthiazolidin-4-ones, and substituted chromenothiazoles were synthesized and evaluated for anticonvulsant activity. Some selected compounds were assayed against seizures induced by pentylenetetrazole (PTZ) and strychnine in mice. Compounds **3b**, **6b**, and **7b** were the most active of the series against PTZ induced seizures. Compound **7b** provided anticonvulsant activity (PD_{50} = 95 mg/kg, ip) at a dose 200 mg/kg compared to phenobarbital (PD_{50} = 16 mg/kg, ip) at a dose 30 mg/kg (90% protection). No clear correlation was observed between the antiepileptic activity and molecular lipophilicity descriptors of the tested compounds.

1. Introduction

Epilepsy is the most frequent neurologic affection characterized by excessive temporary neuronal discharge.[1] The overall prevalence of the disease is 0.5–1.0% of the population and up to 50 million people world wide.[2] Many patients with epilepsy do not respond well to currently available antiepileptic drugs (AED) such as phenytoin, carbamazepine, diazepam, phenobarbital, ethusoximide, valproate, valrocemide, vigabatrin, gabapentin, zonisamide, topiramate, tiagabine, felbamate, retigabine, lamotrigine, and levetiracetam which are effective towards only 50–80% of the patients and present some undesirable side effects such as vertigo, ataxia, headache, hirsutism, hepato toxicity, gastrointestinal, and cardiovascular side effects.[2–7]

Consequently, a real need exists to develop new anticonvulsant compounds to cover seizures which are so far resistant to presently available drugs. A strategy along this line is to search for compounds with new modes of action.

A novel class of potential antiepileptics emerged from studies in the field of plant coumarins. Calophyllolide and scoparone were found to possess moderate sedative and anticonvulsant properties.[8,9] Recently, it was shown that both selective MAO-A and MAO-B inhibitors exert anticonvulsant activity in seizure models. Esuprone, selective MAO-A inhibitor, exhibited antiepileptic activity and may be an interesting new approach for treatment of epilepsy.[10] Tonabersat, a member of a family of novel benzoylamino–benzopyran compounds, had potent anticonvulsant activity in a number of seizure models with potential for a good therapeutic ratio compared to other anticonvulsants.[11]

In addition many thiourea,[12–14] thiazolidinone,[15–23] thiazoline,[21] and benzothiazole[24–27] derivatives exhibit anticonvulsant activity. In this study, we report the synthesis and the pharmacologic evaluation of new series of novel synthetic coumarins fitted with functional moieties believed to enhance antiepileptic activity to assess their in vivo activity for future development of AED. The newly synthesized compounds belong to the following series, *N*-substituted-3-(2-oxo-2*H*-chromen-6-yl)thioureas **3a**–**f**, 3-substituted-2-(2-oxo-2*H*-chromen-6-ylimino)thiazolidin-4-ones **4a**–**f**, 3-substituted-5-(4-substituted arylidene)-2-(2-oxo-2*H*-chromen-6-ylimino)thiazolidin-4-ones **5a**–**e**, 3-substituted-4-(4-substituted phenyl)-2-(2-oxo-2*H*-chromen-6-ylimino)thiazolines **6a**–**k**, 3-substituted-4-methyl-2-(2-oxo-2*H*-chromen-6-ylimino)thiazoline-5-carboxylic acid ethyl esters **7a**–**e**, 2-substituted amino-7*H*-

Keywords: Coumarinylthioureas; Coumarinylthiazolidin-4-ones; Coumarinylthiazolines; Chromeno[6,5-*d*]thiazoles; Anticonvulsant.
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chromeno[6,5-*d*]thiazol-7-ones **8a**–**c**, and 2-(4-oxothiazolidin-2-ylideneamino)-7*H*-chromeno[6,5-*d*]thiazol-7-one **11**. Some of the newly synthesized compounds were tested for anticonvulsant activity against chemically induced convulsion using PTZ and strychnine.

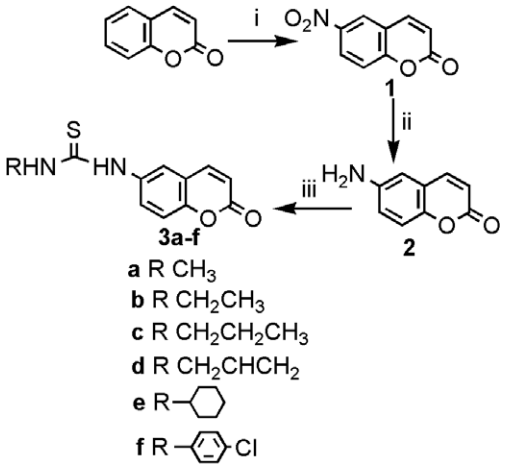
2. Results and discussion

2.1. Chemistry

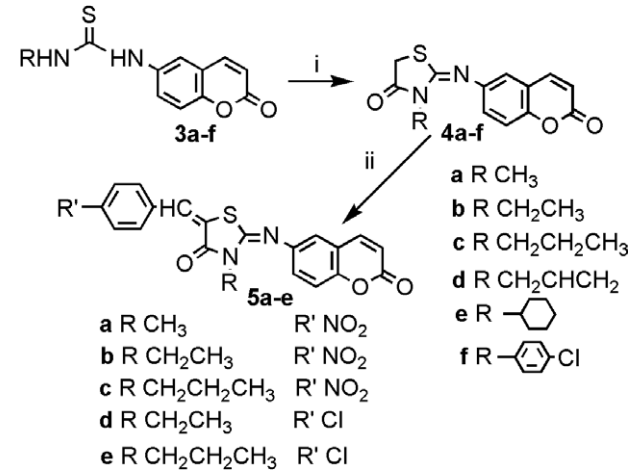
6-Aminocoumarin **2**, was prepared in two steps from coumarin following the reported procedures (Scheme 1).[28–30]

The amine compound **2** was reacted with alkyl/aryl isothiocyanate and gave some new *N*-substituted-3-(2-oxo-2*H*-chromen-6-yl)thioureas **3a**–**f**,Scheme 1. The proposed structures of compounds **3a**–**f** were confirmed on the basis of elemental analysis and spectroscopic data (IR, ^{1}H NMR). The IR spectra showed NH and C=S stretching bands at 3350–3128 and 1285–1260 cm^{-1}, respectively and the lactone carbonyl at 1720–1707 cm^{-1}. The ^{1}H NMR spectra of the compounds **3a**–**f** showed the NHs of the thiourea functionality at the region δ = 7.74–7.85 ppm for the (S=CNHR) and at δ = 9.43–10.01 ppm for the (S=CNHcoumarin). The remaining protons appeared at the expected chemical shifts.

Cyclization of the thiourea derivatives **3a**–**f** with monochloroacetic acid led to formation of 3-substituted-2-(2-oxo-2*H*-chromen-6-ylimino)thiazolidin-4-ones **4a**–**f**, Scheme 2. The cyclization reaction to 2-iminothiazolidinones, possibly proceeds via the non isolated intermediate B following ene-thiolization.[31] (Fig. 1) The corresponding yields of the reaction indicated that compounds **3a**–**d** are more reactive than compounds **3e**, **f**. This is maybe due to the steric effect of cyclohexyl and phenyl that reduces the ability of compounds **3e**, **f** to undergo nucleophilic cyclization. The formation of compounds **4a**–**f** was confirmed by the disappearance of thiourea NHs and C=S absorption bands and appearance of the characteristic lactam CO bands at 1738–1706 cm^{-1} in the IR spectra. Appearance of a singlet assigned to methylene thiazolidinone protons at δ = 3.67–4.07 ppm in the ^{1}H NMR spectra of compounds **4a**–**f** proved ring closure and confirmed their formation.



Scheme 1. Reagents and conditions: (i) HNO_3, H_2SO_4, glacial acetic acid (ii) $SnCl_2$, HCl (iii) RNCS, C_2H_5OH, reflux, 8 h.



Scheme 2. Reagents and conditions: (i) $ClCH_2COOH$, CH_3COONa, CH_3COOH, reflux, 24–48 h (ii) ArCHO, CH_3COONa, CH_3COOH, reflux, 48 h.

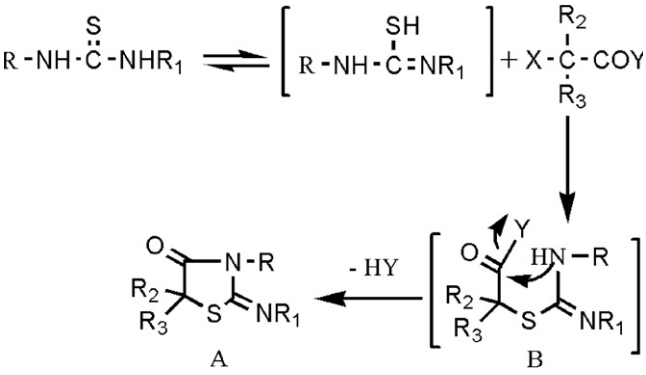


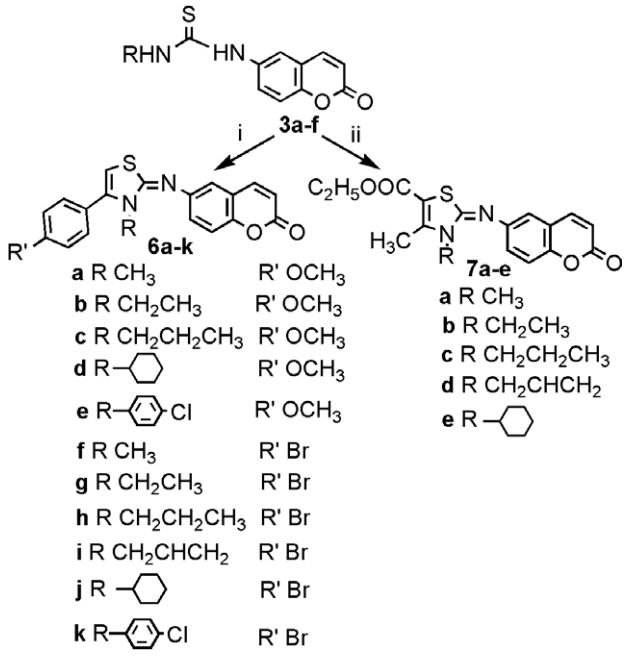
Figure 1. The proposed mechanism of 2-iminothiazolidin-4-one formation.

Condensation of thiazolidinones **4a**–**c** with appropriate aldehydes in glacial acetic acid buffered with sodium acetate afforded 5-arylidene-2-imino-4-thiazolidinones **5a**–**e**, Scheme 2. It was noted that the yield of the reaction of 4-nitro or 4-chlorobenzaldehydes with **4a**–**c** was good and benzaldehyde or its 2-nitro and 4-dimethylamino failed to react with **4a**–**c**. The proposed structures of **5a**–**e** were confirmed by micro analysis and spectroscopic data. Compounds **5a**–**e** exist as potential E and Z geometrical isomers; the Z conformation of the 5 exocyclic C=C double bond was assigned on the basis of ^{1}H NMR spectra and on the basis of literature data for analogs 4-thiazolidinones and 2,4-thiazolidindiones.[32,33] For example, the ^{1}H NMR spectrum of compound **5a** showed only one kind of the methine proton that deshielded by the adjacent C=O and was detected at δ = 7.41 ppm. Also in ^{1}H NMR spectrum of **5b**, the absence of the signal of ($COCH_2$) protons of the starting compounds **4b** at δ = 4.07 ppm together with the appearance of methine proton CH at δ = 6.49–8.22 ppm agreed with the proposed structures.

The synthetic steps adopted for the preparation of 3-substituted-4-(4-substituted phenyl)-2-(2-oxo-2*H*-chromen-6-ylimino)thiazolines **6a**–**k** and 3-substituted-4-methyl-2-(2-oxo-2*H*-chromen-6-ylimino)thiazoline-5-carboxylic acid ethyl esters **7a**–**e** were illustrated in Scheme 3.

3-Substituted-4-(4-substituted phenyl)-2-(2-oxo-2*H*-chromen-6-ylimino)thiazolines **6a**–**k** were prepared by refluxing thiourea derivatives **3a**–**f** with 4-bromophenacyl bromide or 4-methoxyphenacyl bromide in isopropanol where the products were obtained as hydrobromide salts. The obtained hydrobromide salts were neutralized with sodium acetate to get compounds **6a**–**k**. The key intermediate in the cyclization, enethiol form of the thioureas, is responsible for the formation of the pertinent thiazolines**6a**–**k** according to the following mechanism[34] depicted in Figure 2. The structure assignment for the prepared **6a**–**k** was deduced by elemental and spectral analysis. The IR spectra of**6a**–**k** revealed the disappearance of bands at 3350–3128 cm^{-1} (NHs) and at 1285–1260 (C=S) and appearance of strong bands at 1610–1587 (C=N). Furthermore, the ^1H NMR spectra of compounds**6a**–**k** lacked the NH signals and showed new signal at $\delta = 5.59$–6.47 ppm attributed to C-5 H of thiazoline ring.

Treatment of unsymmetrical thioureas **3a**–**e** with ethyl 2-chloroacetoacetate in absolute ethanol yielded the 4,5-disubstituted thiazoline derivatives **7a**–**e** via loss of H_2O and HCl on neutralization with ammonia. The structures of compounds **7a**–**e** were confirmed by elemental analysis and spectroscopic methods. The IR spectra of these compounds showed disappearance of NH bands at 3350–3128 cm^{-1} and appearance of a strong absorption band at 1735–1715 cm^{-1} (C=O of ester) in addition to bands at 1698–1690 cm^{-1} (C=O of the pyrone ring). The ^1H NMR spectra lacked the NH signals and showed a singlet signal at $\delta = 2.53$–2.75 ppm corresponding to CH_3 at 4-position of thiazoline ring, quartet signal at $\delta = 4.11$–4.23 ppm corresponding to CH_2 of ester and triplet signal at $\delta = 1.12$–1.31 ppm corresponding to CH_3 of ester.



Scheme 3. Reagents and conditions: (i) Substituted phenacyl bromide, isopropanol, reflux, 4 h (ii) Ethyl 2-chloroacetoacetate, C_2H_5OH, reflux, 24 h.

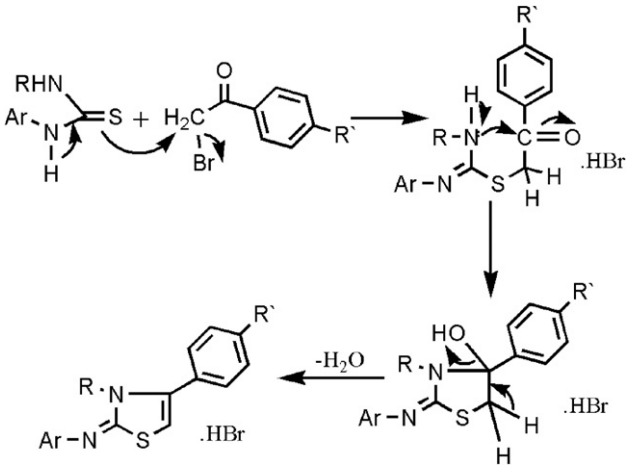
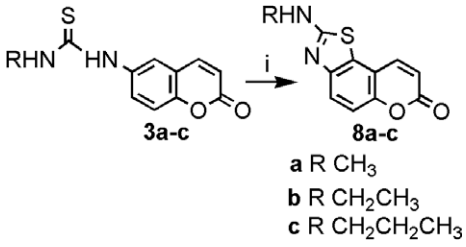


Figure 2. The proposed mechanism for 2-iminothiazolines formation.

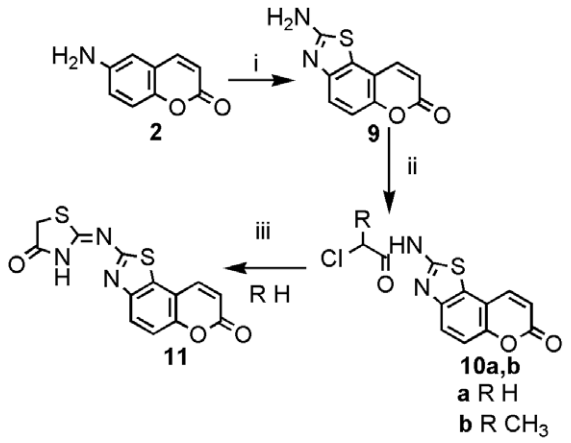


Scheme 4. Reagents and conditions: (i) Br_2, $CHCl_3$, reflux, 30 min.

The synthesis of 2-(substituted amino)-7*H*-chromeno[6,5-*d*]thiazol-7-ones **8a**–**c** was accomplished as shown in Scheme 4. Thus when the thioureas **3a**–**c** were treated with bromine in chloroform, the expected cyclized aminothiazoles **8a**–**c** were obtained. In the IR spectra of **8a**–**c**, the absence of absorption bands at 1285–1260 cm^{-1} of C=S and appearance of bands 3302–3217 cm^{-1} (RNH at C-2 thiazole) together with the absence of signal of C-5H and RNHCS protons in the ^1H NMR spectra of starting compounds **3a** at $\delta = 7.74$ ppm agreed with proposed structures.

2-Amino-7*H*-chromeno[6,5-*d*]thiazol-7-one **9** was synthesized from 6-aminocoumarin **2** via one pot reaction as reported by Merchant et al.[35] and Mulwad and Shirodkar[36] as in Scheme 5. Thus reaction of amine **2** with potassium thiocyanate and bromine in acetic acid at 0–5 °C afforded **9**.

2-Chloro-*N*-(7-oxo-7*H*-chromeno[6,5-*d*]thiazol-2- yl)acetamide/propionamide **10a**, **b** were prepared by refluxing amine **9** with chloroacetyl chloride or α-chloropropionyl chloride, respectively, in dry benzene and anhydrous potassium carbonate as catalyst, Scheme 5. ^1H NMR



Scheme 5. Reagents and conditions: (i) Br_2, CH_3COOH, KSCN (ii) chloroacylchloride, K_2CO_3, Benzene, reflux, 24–36 h (iii) NH_4SCN, C_2H_5OH, reflux, 10 h.

spectrum of compound **10b** (R=CH_3) showed doublet signal at $\delta = 1.69$ for CH_3, quartet signal at $\delta = 4.86$ for CH–Cl and singlet signal at $\delta = 12.94$ ppm for NH (D_2O exchangeable). Furthermore, the IR spectrum of compound **10b** revealed the lacking of typical absorption bands of NH_2 and the presence of NH stretching absorption at 3191 cm^{-1}. In addition carbonyl absorption band of the pyrone ring at 1729 cm^{-1} and that of amide group at 1697 cm^{-1} were detected.

Heterocyclization of acetamide **10a** with potassium thiocyanate in refluxing ethanol, efficiently produced 2-imino-4-thiazolidinone **11** as illustrated in Scheme 5. The 2-thiazolylimino-4-thiazolidinone **11** displays amino–imino tautomerism. The possibility of the tautomerism involving a hydroxylic group was excluded by the evident absence of typical signals of the OH group in IR and ^1H NMR spectra. The IR absorption bands of the lactam NH group at 3105 cm^{-1} together with strong bands at 1720 cm^{-1} (C=O) confirms the **11C** tautomeric form.[33] (Fig. 3) ^1H NMR for compound **11** revealed singlet signal at $\delta = 4.06$ ppm corresponding to (CH_2) and singlet signal exchangeable with D_2O at $\delta = 12.32$ ppm corresponding to NH.

2.2. Anticonvulsant activity

The brain uptake of drugs is generally related to the drug lipophilicity, which can be expressed as the log *P* and drug ionization constant, pK_a.[14] The log *P* of compounds **3b**, **3e**, **3f**, **4b**, **5b**, **6b**, **6d**, **6e**, **6g**, **7a**, **7b**, **7e**, **8a**, **10a**, and **11** was calculated (Table 11) and the pK_a value of compounds **3b**, **6b**, and **7b**, was calculated. The resulted pK_a values of compounds **3b**, **6b**, and **7b** are 5.62±0.42, 7.63±0.33, and 7.44±0.37, respectively.

The selected compounds were screened for anticonvulsant activity using PTZ and strychnine induced seizures.

In PTZ test, thioureas **3b**, **e**, and **f** have shown varying degrees of activity. It was observed that *N*-ethyl thiourea **3b** showed maximum protection 60% in comparison to *N*-cyclohexyl thiourea **3e** 30% and *N*-phenyl thiourea

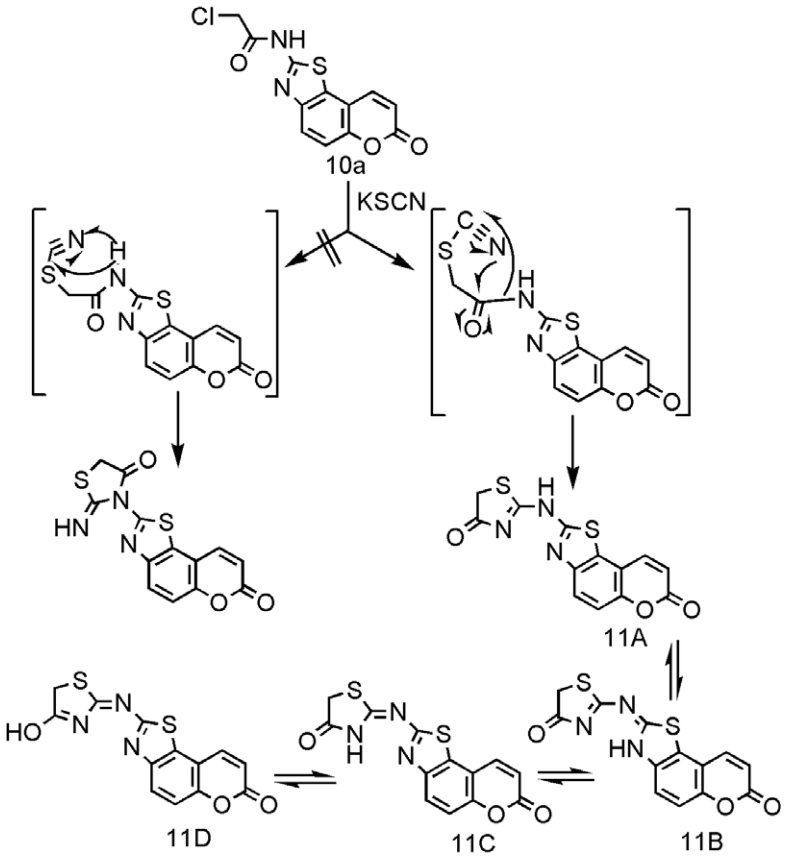


Figure 3. The proposed mechanism of formation and tautomeric structures of **11**.

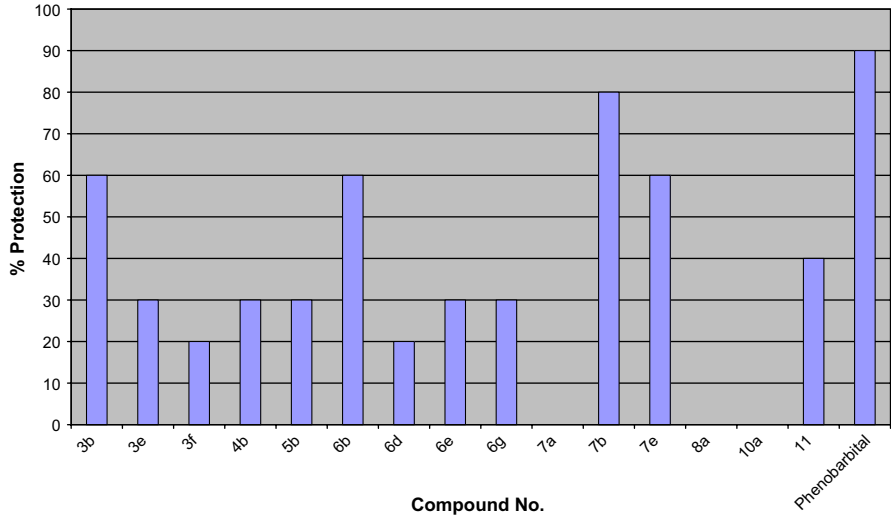


Figure 4. Bar diagram showing anticonvulsant activity (% protection) of tested compounds and Phenobarbital in PTZ test.

3f 20% derivatives (Table 11, Fig. 4). The lipophilicity and pK_a of the most active one **3b** (log $P = 2.12$ and p$K_a = 5.62$) were found close to that of the reference drug phenobarbital (log $P = 1.80$ and p$K_a = 7.4$).

The thiazolidinone derivative **4b** and its 5-(*p*-nitrobenzylidene) derivative **5b** showed lower activity compared with the parent thiourea **3b** (60%). It was observed that compound **4b** gave 30% protection and introduction of aryliden group at 5 position of thiazolidinone ring did not affect the activity (the protection percentage of compound **5b** was 30%).

Also, it was found that compounds **6b**, **6d**, **6e**, and **6g**, substituted with different iminothiazoline moities at the sixth position of coumarin showed varying anticonvulsant activity, however, compound 3-ethyl-4-(4-methoxyphenyl)-2-(2-oxo-2*H*-chromen-6-ylimino)thiazoline **6b** showed more potent activity comparing to the compound 3-ethyl-4-(4-bromophenyl)-2-(2-oxo-2*H*-chromen-6-ylimino)thiazoline **6g** (60% and 30%, respectively). Compound **6e** with *N*-phenyl substituent is slightly more active than compound **6d** with *N*-cyclohexyl group in the position 3 (30% and 20%, respectively).

Interestingly, it was found that the thiazoline-5-carboxylic acid ethyl esters **7b** and **7e** showed promising anticonvulsant activity (80% and 60%, respectively) especially compound **7b** that exhibited equipotent activity at a dose of 200 mg/kg to phenobarbital at a dose of 30 mg/kg (Table 12, Fig. 5) and its $LD_{50} = 1453.78$ mg/kg as calculated from (Table 13). Also it was observed that the compound 3,4-dimethyl-2-(2-oxo-2*H*-chro-

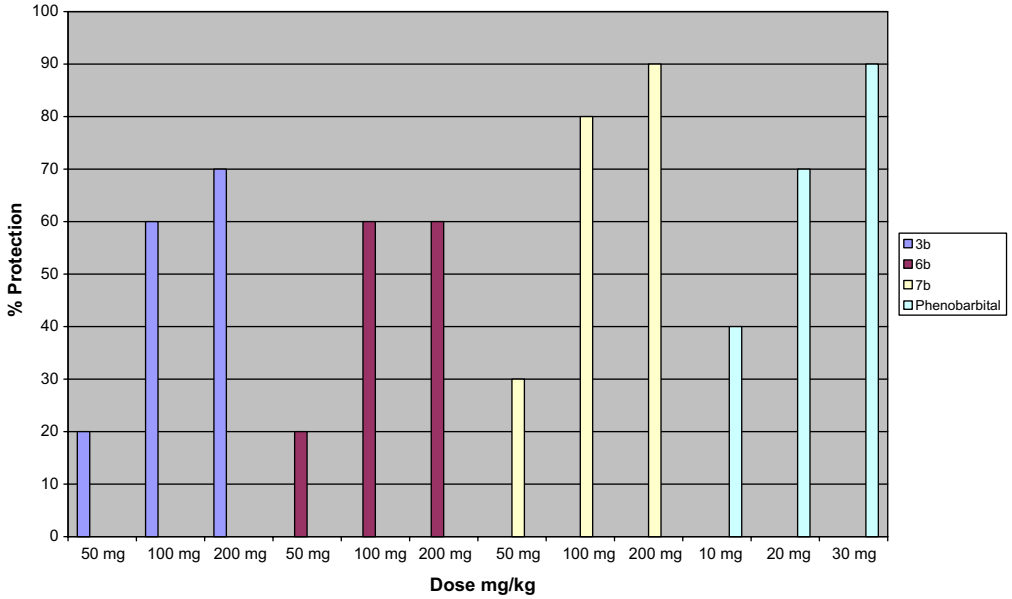


Figure 5. The bar diagram showing anticonvulsant activity (% protection) of compounds **3b**, **6b**, **7b** at doses 50, 100, 200 mg/kg and Phenobarbital at doses 10, 20, 30 mg/kg in PTZ test.

men-6-ylimino)thiazoline-5-carboxylic acid ethyl ester **7a** devoids anticonvulsant activity (0% protection) while compound 3-ethyl-4-methyl-2-(2-oxo-2*H*-chromen-6-ylimino)thiazoline-5-carboxylic acid ethyl ester **7b** gave the highest protection.

Also, compounds **8a** and **10a** that contain benzothiazole skeleton in their structure devoid any protection against PTZ while introducing thiazolidinone ring in **10a** showed anticonvulsant activity, for example, compound **11** 40% protection.

Although the convulsant mechanism of action of PTZ is poorly understood, it is reported that it is able to inhibit the chloride conductance by binding to sites of GABAA receptor complex.[37]

For the most significant compounds **3b**, **6b**, and **7b** it was thought to evaluate these compounds at three graded doses (50, 100, 200 mg/kg) and compared the results with that of standard drug phenobarbital at doses (10, 20, 30 mg/kg). Compound **7b** (PD_{50} = 95 mg/kg) at a dose of 200 mg/kg was found to be equipotent to phenobarbital (PD_{50} = 16 mg/kg) at a dose of 30 mg/kg (90% protection) (Table 12 and Fig. 5).

In the model of convulsion induced by strychnine (Table 14), all compounds being tested except compounds **3b**, **5b**, **6d**, **7e**, and **10a** significantly increased the average survival time in this model but less than that activity observed with phenytoin drug.

It is known that strychnine directly antagonize the inhibitory spinal reflexes of glycine.[37] All the compounds that increase the survival time therefore, might cause seizure suppression by acting on glycine inhibitory mechanisms.

3. Conclusion

Although the preliminary results revealed only marginal activity of the tested compounds, it is apparent from the data of PTZ test that:

- Coumarinylthiourea and coumarinylthiazoline with *N*-ethyl substituent showed more protection than the compounds having *N*-cyclohexyl or *N*-phenyl substituents.
- Cyclization of thiourea derivatives to thiazolidinone decreases the activity, while cyclization to thiazoline increases the activity.
- Substitution of the thiazoline derivatives at 4 and 5 positions with methyl and carboxylic acid ethyl ester, respectively, highly increases the activity.
- The tricyclic derivatives with free amino group at position 2 of thiazole ring devoid activity while introducing the thiazolidinone ring markedly increases the activity.
- Compound **7b** was found to be the most active compound, it devoid hypnotic and sedation and has LD_{50} equal to 1453.78 mg/kg which is higher than that of phenobarbital.

Preliminary results of strychnine seizure pattern test revealed that compounds **3e**, **3f**, **4c**, **5d**, **6b**, **6g**, **7b**, **8b**, and **11** were significantly different from respective strychnine treated group but not significantly different from respective phenytoin treated group.

Briefly, the coumarin iminothiazoline system serves as prototypic molecule for subsequent molecular modification in the search for novel anticonvulsants.

4. Experimental

4.1. Chemistry

Melting points °C were determined by open capillary tube method using Electrothermal 9100 melting point apparatus and were uncorrected. Elemental micro analyses were performed at the micro analytical center, Faculty of Science, Cairo University and National Research Center. The IR spectra were recorded as potassium bromide discs on Schimadzu-435 IR spectrophotometer and Bruker FT-IR spectrophotometer. The ^{1}H NMR spectra, in $^*CDCl_3$ or DMSO-d_6 as a solvent, were recorded on Varian Gemini 200 spectrophotometer at 200 MHz, Varian Mercury spectrophotometer at 300 MHz, and Jeol FX 90Q, 90 MHz. Chemical shifts are reported as δ (ppm) relative to tetramethylsilane (TMS) as internal standard. Mass spectra were performed on Fennigan MAT, SSQ 7000 mass spectrophotometer at 70 eV. The progress of the reaction was determined by thin layer chromatography, using Macherey–Nagel Alugram Sil G/UV_{254} silica gel plates with fluorescent indicator UV_{254} and chloroform-ethanol (9.5:0.5) as the eluting system and the spots were visualized using Vilber Lourmet ultraviolet lamp at λ = 254 nm.

4.1.1. General procedure for synthesis of *N*-substituted-3-(2-oxo-2*H*-chromen-6-yl)thioureas 3a–f. (Scheme 1, Tables 1, 2). A mixture of **2** (1.61 g, 10 mmol) and organic isothiocyanate (10 mmol) in absolute ethanol (25 mL) was heated under reflux for 8 h. The crude product was collected, washed with water, dried, and crystallized from suitable solvent.

4.1.2. General procedure for synthesis of 3-substituted-2-(2-oxo-2*H*-chromen-6-ylimino)thiazolidin-4-ones 4a–f. (Scheme 2, Tables 3, 4). A mixture of **3a–f** (10 mmol), monochloroacetic acid (0.95 g, 10 mmol), and anhydrous sodium acetate (2.0 g, 25 mmol) in glacial acetic acid (30 mL) was refluxed for 24–48 h. After cooling, the reaction mixture was poured onto ice-cold water. The precipitate was filtered, washed with water, dried, and crystallized from ethanol.

4.1.3. General procedure for synthesis of 3-substituted-5-(4-substituted benzylidene)-2-(2-oxo-2*H*-chromen-6-ylimino)thiazolidin-4-ones 5a–e (Scheme 2, Tables 5, 6). To a well stirred solution of **4a–c** (4 mmol) in acetic acid (35 mL) buffered with sodium acetate (1.0 g, 12 mmol), the appropriate aryl aldehyde (6 mmol) was added. The solution was refluxed for 48 h then

Table 1. Physical and analytical data of compounds **3a–f**

	Mol. formula	Mol. wt.	Yield %	MP (°C)	Microanalysis Calc. (Found)		
					C	H	N
a	$C_{11}H_{10}N_2O_2S$	234.28	84[c]	240–242	56.40 (55. 95)	4.30 (4.86)	11.96 (11.39)
b	$C_{12}H_{12}N_2O_2S$	248.31	77[a]	211–213	58. 05 (58.40)	4.87 (5.20)	11.28 (11.45)
c	$C_{13}H_{14}N_2O_2S$	262.33	80[a]	190–193	59.52 (60. 01)	5.38 (5.10)	10.68 (10.37)
d	$C_{13}H_{12}N_2O_2S$	260.32	64[a]	156–159	59.98 (60.00)	4.65 (4.70)	10.76 (10.77)
e	$C_{16}H_{18}N_2O_2S$	302.40	76[b]	233–234	63.55 (63. 56)	6.00 (6.57)	9.26 (9.21)
f	$C_{16}H_{11}ClN_2O_2S{\cdot}H_2O$	348.81	58[d]	241–245	55.10 (55.50)	3.76 (3.33)	8.03 (8.05)

Crystallization solvent: [a]Ethanol, [b]$CHCl_3$, [c]DMF/ethanol, [d]DMF/H_2O.

Table 2. IR and ^{1}H NMR spectral data of compounds **3a–f**

	IR (γ, cm^{-1})	^{1}H NMR (δ, ppm)
a■	3350, 3140(2NH), 3000(CHarom), 2950(CH_3), 1720(CO), 1620, 1570, 1520(NH, C=C), 1260(C=S)	2.94(s, 3H, C*H*$_3$), 6.50(d, 1H, C-3*H*), 7.38(d, 1H, C-7*H*), 7.57(d, 1H, C-8*H*), 7.74(s, 2H, C-5*H*, N*H*), 8.07(d, 1H, C-4*H*), 9.65(s, 1H, N*H*)
b	3334, 3139(2NH), 3070(CHarom), 2977(CH_3, CH_2), 1712(CO), 1620, 1545, 1520(NH, C=C), 1277(C=S)	1.14(t, 3H, C*H*$_3$), 3.42(q, 2H, C*H*$_2$CH$_3$), 6.42–8.10(m, 6H, aromatic*H*, N*H*), 9.5(s, 1H, N*H*, exchanged with D_2O)
c	3329, 3139(2NH), 2929, 2861(CH_3, CH_2), 1712 (CO), 1620, 1552, 1518(NH, C=C), 1285(C=S)	0.91(t, 3H, C*H*$_3$), 1.46–1.62(m, 2H, CH$_2$C*H*$_2$CH$_3$), 3.68(t, 2H, C*H*$_2$CH$_2$CH$_3$), 6.44–8.11(m, 6H, aromatic*H*, N*H*), 9.56(s, 1H, N*H* exchanged with D_2O)
d	3331, 3128(2NH), 2982(CH_2, CH), 1708(CO), 1620, 1544(NH, C=C), 1284(C=S)	4.12(d, 2H, C*H*$_2$CH=CH$_2$), 5.17(t, 2H, CH$_2$CH=C*H*$_2$), 5.78–5.89(m, 1H, CH$_2$C*H*=CH$_2$), 6.45–8.12(m, 6H, aromatic*H*,N*H*), 9.65(s, 1H, N*H* exchanged with D_2O)
e	3314, 3132(2NH), 3051(CHarom), 2972, 2928, 2851(CH_2, CH), 1713(CO), 1620, 1544, 1509(NH, C=C), 1278(C=S)	1.21–1.95(m, 10H, cyclohexyl*H* except C-1*H*), 4.09(m, 1H, cyclohexylC-1*H*), 6.47–8.09(m, 6H, aromatic*H*, N*H*), 9.43(s, 1H, N*H*)
f	3261(2NH), 3040(CHarom), 1707(CO), 1619, 1566, 1527(NH, C=C), 1283(C=S)	6.51–8.14(m, 9H, aromatic*H*), 10.01(s, 2H, 2N*H*, exchanged with D_2O)

■ MS: *m*/*z* (%) 234, M^+, (99.39).

Table 3. Physical and analytical data of compounds **4a–f**

	Mol. formula	Mol. wt.	Yield %	MP (°C)	Microanalysis Calc. (Found)		
					C	H	N
a	$C_{13}H_{10}N_2O_3S$	274.30	76	172–175	56.92 (57.22)	3.67 (3.91)	10.21 (10.48)
b	$C_{14}H_{12}N_2O_3S$	288.33	81	154–157	58.32 (58.30)	4.19 (4.00)	9.72 (9.47)
c	$C_{15}H_{14}N_2O_3S$	302.35	70	72–74	59.59 (59.94)	4.67 (5.00)	9.27 (8.85)
d	$C_{15}H_{12}N_2O_3S$	300.34	79	83–87	59.99 (60.00)	4.03 (4.10)	9.33 (9.33)
e	$C_{18}H_{18}N_2O_3S$	342.42	43	210–212	63.14 (63.15)	5.30 (5.34)	8.18 (7.93)
f	$C_{18}H_{11}ClN_2O_3S$	370.82	30	87–90	58.30 (58.69)	2.99 (3.09)	7.55 (7.46)

cooled to room temp. The separated solid was filtered, washed with water, dried and crystallized from ethanol.

4.1.4. General procedure for synthesis of 3-substituted-4-(4-substituted phenyl)-2-(2-oxo-2*H*-chromen-6-ylimino)thiazolines 6a–k (Scheme 3, Tables 7, 8). A mixture of **3a–f** (2 mmol), 4-substituted phenacyl bromide (2.5 mmol) in isopropanol (20 mL) was refluxed for 4 h, the solvent was filtered and the residue was triturated with 10% sodium acetate. The solution obtained was extracted several times with chloroform and the combined organic extracts were washed with water, dried, and evaporated. The solid formed was collected and crystallized from ethanol.

4.1.5. General procedure for synthesis of 3-substituted-4-methyl-2-(2-oxo-2*H*-chromen-6-ylimino)thiazoline-5-carboxylic acid ethyl esters 7a–e (Scheme 3, Tables 9, 10). The appropriate substituted thioureas **3a–e** (1 mmol) were dissolved in absolute ethanol (10 mL) and ethyl 2-chloroacetoacetate (0.16 g, 1 mmol) was added. The reaction mixture was refluxed for 24 h. The liquid was chilled and treated with 3 N ammonium hydroxide to pH 8 then diluted with water (30 mL). The precipitate formed was filtered, washed with water, dried, and crystallized from ethanol.

4.1.6. General procedure for synthesis of 2-substituted amino-7*H*-chromeno[6,5-*d*]thiazol-7-one 8a–c (Scheme 4). To a suspension of substituted thioureas **3a–c** (4 mmol)

Table 4. IR and ^{1}H NMR spectral data of compounds **4a–f**

	IR (γ, cm^{-1})	^{1}H NMR (δ, ppm)
a■	3053(CHarom.), 2991, 2934(CH_3, CH_2), 1731, 1706(2CO), 1630(C=N), 1562(C=C)	*3.32(s, 3H, CH_3), 3.86(s, 2H, COCH_2), 6.42–7.69(m, 5H, aromatic*H*)
b■■	3100(CHarom.), 2980, 2920(CH_3, CH_2), 1738, 1720(2CO), 1620(C=N), 1560(C=C)	1.23(t, 3H, CH_3), 3.8(q, 2H, CH_2CH$_3$), 4.07(s, 2H, COCH_2), 6.49–8.12(m, 5H, aromatic*H*)
c	3091(CHarom.), 2971, 2934, 2878(CH_3, CH_2), 1734(2CO), 1618(C=N), 1563(C=C)	0.83(t, 3H, CH_3), 0.99–1.07(m, 2H, CH$_2$CH_2CH$_3$), 3.72(t, 2H, CH_2CH$_2$CH$_3$), 4.07(s, 2H, COCH_2), 6.46–8.10(m, 5H, aromatic*H*)
d■■■	3070(CHarom.), 2990, 2930(CH_2, CH), 1735, 1710(2CO), 1640(C=N), 1560(C=C)	*3.87(s, 2H, COCH_2), 4.46(d, 2H, CH_2CH= CH$_2$), 5.28(t, 2H, CH$_2$CH=CH_2), 5.87–5.92(m, 1H, CH$_2$C*H*=CH$_2$), 6.42–7.69(m, 5H, aromatic*H*)
e	3059(CHarom.), 2928, 2853(CH_2), 1728 (2CO), 1628(C=N), 1563(C=C)	*1.16–2.34(m, 10H, cyclohexyl*H* except C-1*H*), 3.67(s, 2H, COCH_2), 4.29–4.45(m, 1H, cyclohexyl C-1*H*), 6.32–7.61(m, 5H, aromatic*H*)
f	3061(CHarom.), 2972, 2927(CH_2), 1728 (2CO), 1630 (C=N), 1565(C=C)	* 4.03(s, 2H, COCH_2), 6.41–7.75(m, 9H, aromatic*H*)

■ MS: *m/z* (%) 274, M$^+$, (62.37).
■■ MS: *m/z* (%) 288, M$^+$, (100).
■■■ MS: *m/z* (%) 300, M$^+$, (100).

Table 5. Physical and analytical data of compounds **5a–e**

	Mol. formula	Mol. wt.	Yield %	MP (°C)	Microanalysis Calc. (Found)		
					C	H	N
a	$C_{20}H_{13}N_3O_5S$	407.41	33	272–275	58.96 (59.09)	3.22 (3.21)	10.31 (10.21)
b	$C_{21}H_{15}N_3O_5S$	421.43	34	234–236	59.85 (59.81)	3.59 (3.21)	9.97 (9.95)
c	$C_{22}H_{17}N_3O_5S$	435.46	34	232–234	60.68 (60.84)	3.93 (4.08)	9.65 (9.68)
d	$C_{21}H_{15}ClN_2O_3S$	410.88	29	189–192	61.39 (61.45)	3.68 (3.50)	6.82 (6.78)
e	$C_{22}H_{17}ClN_2O_3S$	424.91	70	177–179	62.19 (62.18)	4.03 (4.15)	6.59 (6.91)

Table 6. IR and ^{1}H NMR spectral data of compounds **5a–e**

	IR (γ, cm^{-1})	^{1}H NMR (δ, ppm)
a	3073(CHarom), 2922, 2850(CH_3, CH), 1727, 1644(2CO), 1609(C=N), 1562(C=C), 1521, 1344(NO_2)	3.36(s, 3H, CH_3), 6.56–8.33(m, 10H, aromatic*H*, =C*H*)
b■	3063(CHarom.), 2927, 2852(CH_3, CH_2, CH), 1727, 1641 (2CO), 1610(C=N), 1562(C=C), 1520, 1340(NO_2)	1.3(t, 3H, CH_3), 3.49(q, 2H, CH_2CH$_3$), 6.49–8.22(m, 10H, aromatic*H*, =C*H*)
c	3064(CHarom.), 2933, 2873(CH_3, CH_2, CH), 1745, 1709 (2CO), 1646(C=N), 1609, 1561 (C=C), 1514, 1338(NO_2)	0.95(t, 3H, CH_3), 1.68–1.92(m, 2H, CH$_2$CH_2CH$_3$), 3.23 (t, 2H, CH_2CH$_2$CH$_3$), 6.55–8.19(m, 10H, aromatic *H*, =C*H*)
d	2950(CH_3, CH_2, CH), 1725, 1640(2CO), 1610(C=N), 1590, 1560(C=C).	*1.31(t, 3H, CH_3), 4.0(q, 2H, CH_2CH$_3$), 6.39–7.65(m, 10H, aromatic *H*, =C*H*)
e	3060(CHarom.), 2961, 2929, 2875, 2851(CH_3, CH_2, CH), 1729, 1637(2CO), 1607(C=N), 1562(C=C)	0.94(t, 3H, CH_3), 3.00–3.18(m, 2H, CH$_2$CH_2CH$_3$), 3.63 (t, 2H, CH_2CH$_2$CH$_3$), 6.59–7.76(m, 10H, aromatic*H*, =C*H*)

■ MS: *m/z* (%) 422, M$^+$+1, (13.64).

in chloroform (10 mL), bromine (4 mmol) in chloroform (5 mL) was added dropwise with stirring. The mixture was refluxed in a water bath for 30 min then solvent was evaporated. The product was treated with hydrochloric acid, filtered and the filtrate neutralized with ammonia. The precipitate obtained was filtered, dried and crystallized from ethanol.

4.1.6.1. 2-Methylamino-7*H*-chromeno[6,5-*d*]thiazol-7-one 8a. Yield 48%, mp 280–283 °C, IR: 3302 (NH), 3013 (CHarom.), 2938, 2893 (CH_3), 1685 (CO), 1615 (C=N), 1593, 1551 (NH, C=C) cm^{-1}. ^{1}H NMR: δ = 2.99 (d, 3H, CH_3), 6.54 (d, 1H, C-3*H*), 7.3 (d, 1H, C-7*H*), 7.64 (d, 1H, C-8*H*), 8.08 (d, 1H, C-4*H*), 8.15–8.16 (m, 1H, N*H* exchanged with D_2O). MS: *m/z* (%) 232, M$^+$, (33.61). Anal. Found: C 56.66, H 3.74, N 12.02. For $C_{11}H_8N_2O_2S$ (232.26): C 56.88, H 3.47, N 12.06.

4.1.6.2. 2-Ethylamino-7*H*-chromeno[6,5-*d*]thiazol-7-one 8b. Yield 54%, mp 219–222 °C, IR: 3292 (NH), 3022 (CHarom.), 2967, 2922, 2865 (CH_3, CH_2), 1713 (CO), 1617 (C=N), 1590, 1553 (NH, C=C) cm^{-1}. ^{1}H NMR: δ = 1.21 (t, 3H, CH_3), 3.41 (q, 2H, CH_2CH$_3$), 6.49 (d, 1H, C-3*H*), 7.25 (d, 1H, C-7*H*), 7.58 (d, 1H,

Table 7. Physical and analytical data of compounds **6a–k**

	Mol. formula	Mol. wt.	Yield %	MP (°C)	Microanalysis Calc. (Found)		
					C	H	N
a	$C_{20}H_{16}N_2O_3S$	364.43	66	117–120	65.92 (66.34)	4.43 (4.08)	7.69 (7.51)
b	$C_{21}H_{18}N_2O_3S$	378.45	98	149–152	66.65 (67.10)	4.79 (4.59)	7.40 (7.20)
c	$C_{22}H_{20}N_2O_3S$	392.48	66	117–120	67.33 (66.83)	5.14 (5.44)	7.14 (7.05)
d	$C_{25}H_{24}N_2O_3S$	432.54	75	229–231	69.42 (69.50)	5.59 (5.71)	6.48 (6.17)
e	$C_{25}H_{17}ClN_2O_3S$	460.94	25	120–122	65.14 (65.34)	3.72 (4.00)	6.08 (6.10)
f	$C_{19}H_{13}BrN_2O_2S$	413.30	70	218–223	55.22 (55.74)	3.17 (3.08)	6.78 (6.48)
g	$C_{20}H_{15}BrN_2O_2S$	427.32	88	201–205	56.22 (55.85)	3.54 (4.10)	6.56 (6.75)
h	$C_{21}H_{17}BrN_2O_2S$	441.35	68	172–176	57.15 (57.40)	3.88 (3.88)	6.35 (6.63)
i	$C_{21}H_{15}BrN_2O_2S$	439.33	98	196–199	57.41 (57.71)	3.44 (3.48)	6.38 (6.67)
j	$C_{24}H_{21}BrN_2O_2S$	481.41	70	200–201	59.88 (60.43)	4.40 (4.4 6)	5.82 (6.12)
k	$C_{24}H_{14}BrClN_2O_2S$	509.81	60	197–199	56.54 (56.80)	2.77 (3.00)	5.49 (5.45)

Table 8. IR and ^{1}H NMR spectral data of compounds **6a–k**

	IR (γ, cm^{-1})	^{1}H NMR (δ, ppm)
a	3066(CHarom.), 2949, 2925, 2829(CH_3, CH), 1711(CO), 1593(C=N), 1555(C=C)	3.30(s, 3H, NC*H*$_3$), 3.82(s, 3H, OC*H*$_3$), 6.16(s, 1H, thiazoline C-5*H*), 6.40–7.93 (m, 9H, aromatic*H*)
b	3104(CHarom.), 2966, 2926, 2833(CH_3, CH_2, CH), 1711(CO), 1595(C=N), 1553(C=C)	*1.20(t, 3H, C*H*$_3$), 3.82–3.88(m, 5H, C*H*$_2$CH$_3$, OC*H*$_3$), 5.71(s, 1H, thiazoline C-5*H*), 6.36–7.66(m, 9H, aromatic*H*)
c	3031(CHarom.), 2960, 2874, 2840(CH_3, CH_2, CH), 1708(CO), 1598(C=N), 1558 (C=C)	0.71(t, 3H, C*H*$_3$), 1.55–1.8(m, 2H, CH$_2$C*H*$_2$CH$_3$), 3.39(t, 2H, C*H*$_2$CH$_2$CH$_3$), 3.83(s, 3H, OC*H*$_3$), 6.12(s, 1H, thiazoline C-5*H*), 6.52–8.0(m, 9H, aromatic*H*).
d	3001(CHarom.), 2930, 2856(CH_3, CH_2, CH), 1724(CO), 1598(C=N), 1558(C=C)	*1.06–2.61(m, 10H, cyclohexyl*H* except C-1*H*), 3.81(s, 3H, OC*H*$_3$), 3.90–3.97(m, 1H, cyclohexyl C-1*H*), 5.59(s, 1H, thiazoline C-5*H*), 6.31–7. 62(m, 9H, aromatic*H*).
e	3066(CHarom.), 2920, 2846(CH_3, CH), 1727 (CO), 1601(C=N), 1559(C=C)	3.72(s, 3H, OC*H*$_3$), 6.47(s, 1H, thiazoline C-5*H*), 6.52–8.06(m, 13H, aromatic*H*).
f	2913(CH_3, CH), 1712(CO), 1587(C=N), 1558 (C=C)	3.7(s, 3H, C*H*$_3$), 6.25–8.07(m, 10H, aromatic*H*, thiazoline C-5*H*)
g■	3100(CHarom.), 1710(CO), 1610(C=N), 1590, 1560(C=C)	*1.22 (t, 3H, C*H*$_3$), 3.87(q, 2H, C*H*$_2$CH$_3$), 5.79(s, 1H, thiazoline C-5*H*), 6.39–7.7(m, 9H, aromatic*H*).
h	3027(CHarom.), 2962, 2933, 2873(CH), 1710(CO), 1608(C=N), 1586, 1558 (C=C)	0.72(t, 3H, C*H*$_3$), 1.42–1.78(m, 2H, CH$_2$C*H*$_2$CH$_3$), 3.74(t, 2H, C*H*$_2$CH$_2$CH$_3$), 6.24(s, IH, thiazoline C-5*H*), 6.42–8.08(m, 9H, aromatic*H*).
i	3030(CHarom.), 1710(CO), 1610(C=N), 1590(C=C)	4.42(d, 2H, C*H*$_2$CH=CH$_2$), 4.92(d, 1H, CH$_2$CH= C*H*$_a$H$_b$), 5.11(d, 1H, CH$_2$CH=CH$_a$*H*$_b$), 5.85–5.90(m, 1H, CH$_2$C*H*=CH$_2$), 6.33(s, 1H, thiazoline C-5*H*), 6.44–8.04(m, 9H, aromatic*H*).
j	3097(CHarom.), 2928, 2848(CH_2, CH), 1731 (CO), 1607(C=N), 1559 (C=C)	*1.04–2.62(m, 10H, cyclohexyl*H* except C-1*H*), 3.60–3.72(m, 1H, cyclohexyl C-1*H*), 5.61(s, 1H, thiazoline C-5*H*), 6.3–7.61(m, 9H, aromatic*H*).
k■■	3080(CHarom.), 2920(CH), 1725(CO), 1610 (C=N), 1580(C=C)	6.4–8.0(m, 14H, aromatic*H*, thiazoline C-5*H*).

■ MS: *m*/*z* (%) 426, M^+, (27.89) and 428, M^++2, (29.94).
■■ MS: *m*/*z* (%) 508, M^+, (18.34) and 510, M^++2, (24.23).

Table 9. Physical and analytical data of compounds **7a–e**

	Mol. formula	Mol. wt.	Yield %	MP (°C)	Microanalysis Calc. (Found)		
					C	H	N
a	$C_{17}H_{16}N_2O_4S$	344.39	69	150–152	59.29 (59.46)	4.68 (4.82)	8.13 (8.10)
b	$C_{18}H_{18}N_2O_4S$	358.42	93	146–150	60.32 (60.37)	5.06 (4.75)	7.82 (7.76)
c	$C_{19}H_{20}N_2O_4S$	372.45	88	96–100	61.27 (61.50)	5.41 (5.66)	7.52 (7.50)
d	$C_{19}H_{18}N_2O_4S$	370.43	89	121–122	61.61 (61.85)	4.90 (5.00)	7.56 (7.51)
e	$C_{22}H_{24}N_2O_4S$	412.51	31	168–170	64.06 (64.21)	5.86 (5.74)	6.79 (6.79)

Table 10. IR and ^{1}H NMR spectral data of compounds **7a–e**

	IR (γ, cm^{-1})	^{1}H NMR (δ, ppm)
a■	3065(CHarom.), 2981, 2921, 2851(CH_3, CH_2), 1735, 1697(2CO), 1617(C=N), 1562 (C=C)	*1.29(t, 3H, $COOCH_2CH_3$), 2.61(s, 3H, CH_3), 3.5(s, 3H, NCH_3), 4.23 (q, 2H, $COOCH_2CH_3$), 6.4–7.69(m, 5H, aromatic *H*)
b	2981, 2917, 2852(CH_3, CH_2), 1717, 1690 (2CO), 1590 (C=N), 1558(C=C)	*1.15–1.31(m, 6H, NCH_2CH_3, $COOCH_2CH_3$), 2.53(s, 3H, CH_3), 3.95(q, 2H, NCH_2CH_3), 4.13(q, 2H, $COOCH_2CH_3$), 6.29–7.61(m, 5H, aromatic *H*)
c	3046(CHarom.), 2965, 2933, 2875(CH_3, CH_2), 1718, 1692(2CO), 1600 (C=N), 1561(C=C)	1.12–1.28(m, 8H, $NCH_2CH_2CH_3$, $COOCH_2CH_3$), 2.61(s, 3H, CH_3), 3.67(q, 2H, $NCH_2CH_2CH_3$), 4.16(q, 2H, $COOCH_2CH_3$), 6.42–7.61(m, 5H, aromatic*H*)
d	3056(CHarom.), 2979, 2925, 2852(CH_3, CH_2, CH), 1715(2CO), 1599(C=N), 1560 (C=C)	1.21(t, 3H, $COOCH_2CH_3$), 2.75(s, 3H, CH_3), 4.17(q, 2H, $COOCH_2CH_3$), 4.7(d, 2H, $CH_2CH=CH_2$), 5.2(t, 2H, $CH_2CH=CH_2$), 5.8–6.2(m, 1H, $CH_2CH=CH_2$), 6.43–8.10(m, 5H, aromatic *H*)
e	3078(CHarom.), 2921, 2852(CH_3, CH_2, CH), 1721, 1698(2CO), 1592 (C=N), 1562 (C=C)	1.17–1.82(m, 13H, cyclohexyl *H* except C-1*H*, $COOCH_2CH_3$), 2.66 (s, 3H, CH_3), 4.11–4.22(m, 3H, $COOCH_2CH_3$, cyclohexyl C-1*H*), 6.5–8.1 (m, 5H, aromatic*H*)

■ MS: *m*/*z* (%) 344, M^+, (47.87).

C-8*H*), 8.02 (d, 1H, C-4*H*), 8.16 (t, 1H, N*H* exchanged with D_2O). Anal. Found: C 58.44, H 4.26, N 11.35. For $C_{12}H_{10}N_2O_2S$ (246.29): C 58.52, H 4.09, N 11.37.

4.1.6.3. 2-Propylamino-7*H*-chromeno[6,5-*d*]thiazol-7-one 8c. Yield 75%, mp 135–139 °C, IR: 3217 (NH), 3080 (CHarom.), 2961, 2908 (CH_3, CH_2), 1725 (CO), 1628 (C=N), 1556 (NH, C=C) cm^{-1}. ^{1}H NMR: δ = 0.95 (t, 3H, CH_3), 1.56–1.90 (m, 2H, $CH_2CH_2CH_3$), 3.62 (t, 2H, $CH_2CH_2CH_3$), 6.40–8.2 (m, 5H, aromatic*H*, N*H* exchanged with D_2O). Anal. Found: C 60.05, H 4.60, N 10.75. For $C_{13}H_{12}N_2O_2S$ (260.32): C 59.98, H 4.65, N 10.76.

4.1.7. General procedure for synthesis of 2-chloro-*N*-(7-oxo-7*H*-chromeno[6,5-*d*]thiazol-2-yl) acetamide/propionamide 10a, b. (Scheme 5). A mixture of **9** (0.33 g, 1.5 mmol), the corresponding chloroacylchloride (1.5 mmol), and potassium carbonate (0.41 g, 3 mmol) in dry benzene (20 mL) was refluxed for 24–36 h. The hot solution was filtered, the solvent was evaporated under reduced pressure and the residue was crystallized from the appropriate solvent.

4.1.7.1. 2-Chloro-*N*-(7-oxo-7*H*-chromeno[6,5-*d*]thiazol-2-yl)acetamide 10a. Yield 90%, crystallized from $CHCl_3$, mp 257–260 °C, IR: 3170 (NH), 3070 (CHarom.), 2988, 2939 (CH_2), 1735, 1659 (2CO), 1622 (C=N), 1578, 1542 (NH, C=C) cm^{-1}. ^{1}H NMR: δ = 4.50 (s, 2H, $COCH_2$), 6.48–8.19 (m, 4H, aromatic*H*), 9.61(s, 1H, N*H*). MS: *m*/*z* (%) 294, M^+, (34.35) and 296, M^++2, (15.83). Anal. Found: C 49.20, H 2.70, N 9.43. For $C_{12}H_7ClN_2O_3S$ (294.72): C 48.91, H 2.39, N 9.51.

4.1.7.2. 2-Chloro-*N*-(7-oxo-7*H*-chromeno[6,5-*d*]thiazol-2-yl)propionamide 10b. Yield 85 %, crystallized from ethanol, mp 237–239 °C, IR: 3191 (NH), 3055 (CHarom.), 2986, 2920, 2851 (CH_3, CH), 1729, 1697 (2CO), 1619 (C=N), 1579, 1542 (NH, C=C) cm^{-1}. ^{1}H NMR: δ = 1.69 (d, 3H, CH_3), 4.86 (q, 1H, C*H*), 6.59 (d, 1H, C-3*H*), 7.49 (d, 1H, C-7*H*), 7.98 (d, 1H, C-8*H*), 8.31 (d, 1H, C-4*H*), 12.94 (t, 1H, N*H* exchanged with D_2O). Anal. Found: C 50.88, H 3.54, N 8.95. For $C_{13}H_9ClN_2O_3S$ (308.75): C 50.57, H 2.94, N 9.07.

4.1.8. Synthesis of 2-(4-oxothiazolidin-2-ylideneamino)-7*H*-chromeno[6,5-*d*]thiazol-7-one 11. (Scheme 5). A mixture of **10a** (0.29 g, 1 mmol) and ammonium thiocyanate (0.15 g, 2 mmol) in absolute ethanol (50 mL) was refluxed for 10 h, filtered, washed with water, and crystallized from ethanol. Yield 93%, mp >300 °C, IR: 3105 (NH), 3049 (CHarom.), 2917, 2808 (CH_2), 1720 (2CO), 1618 (C=N), 1575 (C=C) cm^{-1}. ^{1}H NMR: δ = 4.06 (s, 2H, CH_2), 6.55 (d, 1H, C-3*H*), 7.42 (d, 1H, C-7*H*), 7.90 (d, 1H, C-8*H*), 8.12 (d, 1H, C-4*H*), 12.32 (t, 1H, N*H* exchanged with D_2O). Anal. Found: C 49.19, H 2.44, N 13.21. For $C_{13}H_7N_3O_3S_2$ (317.35): C 49.20, H 2.22, N 13.24.

4.2. Anticonvulsant Screening

Eighteen selected compounds from the target compounds **3–8**, **10**, and **11** were evaluated for anticonvulsant activity against PTZ and strychnine induced seizures in mice.

4.2.1. PTZ seizure pattern test. Male albino mice weighing 25–30 g were housed in groups of **10**. The animals were acclimated to their environment for at least 2 days before the experiments and were allowed free access to food and water before being tested. The tested compounds were suspended in water and 2% Tween 80 and administered to animals at a dose of 100 mg/kg ip. 1 h after the administration of the tested compound, mice were injected PTZ (112 mg/kg) ip. Animals devoid of generalized convulsions were considered to be protected and results were represented as percentage protection.[38] Standard drug used was phenobarbital sodium at the dose of 30 mg/kg (Table 11, Fig. 4).

Compounds, **3b**, **6b**, and **7b**, that gave the highest protection at a dose of 100 mg/kg were studied at different doses (50, 200 mg/kg) to calculate the PD_{50} which was determined by log linear regression analysis from the

Table 11. Anticonvulsant activity of the tested compounds using PTZ at a dose of 100 mg/kg and their log *P* values

Compound	Protection (%)	Log *P*
3b	60	2.12 ± 0.47
3e	30	4.01 ± 0.47
3f	20	3.33 ± 0.47
4b	30	2.20 ± 0.47
5b	30	4.57 ± 2.10
6b	60	4.99 ± 0.47
6d	20	6.19 ± 0.47
6e	30	7.03 ± 0.47
6g	30	5.94 ± 0.47
7a	0	2.20 ± 0.47
7b	80	2.54 ± 0.47
7e	60	3.75 ± 0.47
8a	0	2.52 ± 0.47
10a	0	2.46 ± 0.47
11	40	2.82 ± 0.47
Phenobarbital	90	1.80 ± 0.47

dose response curves to compare with PD_{50} of phenbarbital (10, 20, and 30 mg/kg) (Table 12, Fig. 5).

4.2.2. Determination of LD_{50}. Male albino mice weighing 25–30 g were divided into groups each of 8 animals. Preliminary experiments were done to determine the minimal dose that kills all animals (LD_{100}), and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses, each dose was injected in a group of eight animals, the number of dead animal in each group after 24 h was recorded and the LD_{50} was calculated according to *Spearman Karber method.*[39] (Table 13) as seen in the following formula

$M = X_k + 1/2\ d - dr/N$
M = Log LD_{50}
X_k = log dose causing 100% mortality.
d = logarithmic interval of doses.
r = Sum of the number of dead animals at each of the individual dose levels.
N = Number of animals at each of the dose level.

4.2.3. Strychnine seizure pattern test. Male albino mice, weighing 25–30 g were divided into control group and test groups ($n = 6$ per group). The control group received 2% Tween 80 in water at a dose of 10 mL/kg po while the test groups received the test compounds or phenytoin at the dose of (100 mg/kg po) 45 min before ip injection of strychnine (2 mg/kg). The average survival time (min) was recorded.[40,41]

Results of the experiments and observations are expressed as mean (M) ± standard deviation (SD) of survival time in min. The significance of differences between groups was determined using one-way analysis of variance (ANOVA) (Table 14).

4.3. Determination of partition coefficient (log *P*)

ChemDraw Ultra 7.0 software was used for calculation of log *P* of the tested compounds for antiepileptic screening (Table 11).

4.4. Determination of ionization constant (pK_a)

A spectrophotometric method was used for the determination of the pK_a of biologically tested compounds **3b**, **6b**, and**7b**. The spectra of the ionized and unionized forms were recorded in 0.1 M HCl and 0.1 M NaOH with DMF. Wavelengths were chosen where the difference in absorbance between the ionized and unionized forms was maximal ($\lambda = 336$, 341, 273, and 324 nm, respectively). The absorbance values at these wavelengths and at different pH (7.6, 7.4, 7, and 6.8) were measured in a phosphate buffer (Na_2HPO_4/KH_2PO_4, 0.15 M) with DMF.[42]

Table 13. Determination of the LD_{50} of compound **7b** in PTZ test

Dose (mg/kg)	Log dose	No. of dead animals
400	2.60	0
800	2.90	2
1600	3.20	3
3200	3.50	8

Table 14. Anticonvulsant activity of the tested compounds using strychnine at a dose of 100 mg/kg

Compound	Survival time (min) after strychnine administration (M ± SD)
Control	3 ± 1.10
Phenytoin	13.8 ± 4.50
3b	3.92 ± 1.20
3e	5.02 ± 1.15
3f	5.33 ± 0.516
4c	5.40 ± 0.548
5b	4.85 ± 1.19
5d	5.18 ± 2.21
6b	7.55 ± 1.14
6d	4.50 ± 0.495
6g	8.36 ± 2.44
7b	5.00 ± 0.935
7e	3.14 ± 1.09
8b	6.26 ± 1.23
10a	4.40 ± 0.946
11	5.44 ± 2.31

Table 12. % Protection and PD_{50} of compounds **3b**, **6b**, **7b** at dose 50, 100, and 200 mg and comparison with phenobarbital at dose 10, 20, and 30 mg in PTZ test

	50 mg	100 mg	200 mg	10 mg	20 mg	30 mg	PD_{50} (mg/kg)
3b	20	60	70	—	—	—	125
6b	20	60	60	—	—	—	138
7b	30	80	90	—	—	—	95
Phenobarbital	—	—	—	40	70	90	16

The pK_a values were calculated according to the following equation:
pK_a = pH + log (Ai−A/A−Au)
A = absorbance of the drug at a given pH
Ai = absorbance of the ionized drug
Au = absorbance of unionized drug

The mean value was then determined and standard deviations calculated for each compound. The spectra and absorbance values were recorded on a Kontron UVIKON 941 spectrophotometer.

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