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COUMARIN INCORPORATED TRIAZOLES: A NEW CLASS OF ANTICONVULSANTS

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Abstract: A series of coumarin incorporated 1,2,4- triazole compounds (1-14) were evaluated for their possible anticonvulsant and neurotoxic properties, log P values, pharmacophoric mapping and three dimensional structure analysis. Compound (6) with *para*-fluoro substitution showed significant anticonvulsant activity.

Keywords: coumarin, 1,2,4-triazoles, MES-test

Epilepsy is not a disease, but a syndrome of different cerebral disorders of central nervous system characterized by paroxysmal cerebral dysrhythmia, manifesting as brief episodes (seizures) of loss or disturbances of consciousness, with or without characteristic body movements called convulsions (1). Epilepsy is a common neurological condition, affecting 0.5–1% of the population worldwide. Every year approximately 250,000 new cases are added to this figure. Many patients have seizures that are resistant to available medical therapies. The convulsions of approximately 25% of epileptics are inadequately controlled by standard drug therapy (2). The number of drugs useful for the treatment of epilepsy is remarkably small. Fewer than twenty drugs are currently marketed in the United States and of these only five or six are widely used. Despite the optimal use of available antiepileptic drugs, many patients with epilepsy fail to experience seizure control and others experience the seizure control only at the expense of significant toxic effects (3). The search for new antiepileptic drugs with lower toxicities and fewer side effects continues to be an interesting area of investigation in medicinal chemistry.

In an attempt to develop anticonvulsant agents we have reported several five membered heterocyclic compounds which have showed significant anticonvulsant activities (4–6). The coumarins are a class of compounds presenting a range of biological applications and anticonvulsant activity (7, 8). Triazoles have also attracted much attention due to their significant anticonvulsant activity (9, 10). In the present study, we describe herein the anticonvulsant activity, log P, pharmacophoric mapping and three dimensional structure analysis of synthesized compounds 3-(4-{[(substituted phenyl) methylidene]amino}-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-2*H*-chromen-2-ones (**1-14**). These compounds have been obtained by incorporating triazoles with coumarin moiety, expected to have synergistic anticonvulsant effect.

EXPERIMENTAL

The pharmacological testing of all the compounds were performed according to the standard protocol given by epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by the Antiepileptic Drug Development (ADD) program (11). The phase I pharmacological screening comprised of MES, sc PTZ and neurotoxicity estimations. Compounds were administered intraperitoneally (*i.p.*) as a solution in polyethylene glycol (PEG). The anticonvulsant activity was assessed by two most adopted animal models i.e., MES – maximal electroshock seizure and chemo shock (sc PTZ) methods. All the synthesized compounds (**1-14**) were administered *i.p.* in mice using doses 30, 100 and 300 mg/kg and the observations were taken at two different intervals (0.5 h and 4.0 h).

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Neurological impairment was evaluated by rotorod method and data is presented in Table 1).

Maximal electroshock seizures test

Maximal electroshock seizures were elicited with a 60 cycle altering current of 50 mA intensity (5–7 times, necessary to elicit minimal electroshock seizures) delivered for 0.25 s *via* corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of the electrodes in order to prevent the death of the animal. Abolition of the hind limb tonic extensor component of the seizure is defined as protection, and results are expressed as number of animal protected/number of animals tested (12).

Subcutaneous pentylenetetrazole induced seizure test (sc PTZ)

The subcutaneous pentylenetetrazole test was performed according to the known protocol (13). This method utilizes pentylenetetrazole (75 mg/kg) that produces seizures in more than 95% of animals as a 0.5% solution subcutaneously in the posterior midline. The animal was observed for 30 min, failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection.

Neurotoxicity screen

The minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod of diameter 3.2 cm that rotates at 10 rpm. Trained animals were given *i.p.* injection of the test compounds (30, 100 and 300 mg/kg body mass). Neurological toxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the trials (14). A group of six mice each (statistically significant number) was used. The investigations were carried out on albino mice of either sex (25–30 g). The mice were kept under standard conditions at ambient temperature 25 ± 2°C and allowed free access to food and water except at the time they

Table 1. Anticonvulsant activity and minimal motor impairment of compounds **1**–**14**.

Compound	Intraperitoneal injection in mice[a]				Neurotoxicity screen	
	MES		scPTZ			
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
1	–	30	–	300	300	–
2	300	300	–	–	300	–
3	100	300	100	–	300	–
4	100	300	300	–	300	–
5	–	30	300	300	300	300
6	30	100	100	300	–	–
7	300	300	–	–	300	300
8	100	300	–	–	300	300
9	–	100	300	–	300	300
10	–	100	300	–	300	300
11	–	100	300	–	300	-
12	–	100	300	–	300	300
13	300	300	–	–	300	300
14	–	30	100	300	300	–
Phenytoin[b]	30	30	–	–	100	100
Ethosuximide[b]	–	–	300	–	–	–
Carbamazepine[b]	30	–	100	–	100	300
Valproic acid[b]	–	–	300	–	–	–

[a]Doses of 30, 100 and 300 mg/kg were administered, the figures in the table indicate the minimum dose whereby bioactivity was demonstrated in a half or more of the mice. The animals were examined 0.5 and 4.0 h after injections. The dash (–) indicates an absence of activity at maximum dose administered (300 mg kg). [b]Data from reference (6).

Table 2. Log P and CLOG P values of compounds **1**–**14**.

Compound	R	CLOG P (Log P)[a]
1	3-NO_2	4.49 (4.23)
2	$N(CH_3)_2$	4.75 (4.50)
3	4-OH	4.33 (4.15)
4	3-OH	4.19 (4.00)
5	2-NO_2	4.68 (4.45)
6	4-F	4.50 (4.70)
7	3,4-$(OCH_3)_2$	4.79 (4.50)
8	2-OH	4.69 (4.80)
9	4-OCH_3	4.54 (4.30)
10	2-Cl	4.70 (4.50)
11	3-Cl	5.27 (5.00)
12	4-Cl	5.37 (5.10)
13	H	5.33 (5.00)
14	4-NO_2	4.58 (4.70)

[a] Log P was determined by n-octanol : phosphate buffer method;
CLOG P was calculated using software ChemOffice 6.0.

were brought out of the cage. All the experimental protocols were carried out with the permission from Institutional Animal Ethics Committee (IAEC), form no. 304. Animals were obtained from Central Animal House Facility, Hamdard University, New Delhi-1100062. Registration number and date of registration of Animal House Facility: 173/CPC-SEA, 28, JAN-2000.

Log P determination

The partition coefficient between n-octanol and phosphate buffer was determined at room temperature (29°C) using a reported procedure (15). Ten mL of n-octanol and 10 mL of phosphate buffer (pH = 7.4) were taken in a glass stoppered graduated tube and 5 mg of accurately weighed drug was added. The mixture was then shaken with the help of mechanical shaker for 24 h at room temperature. The mixture was then transferred to a separating funnel and allowed to equilibrate for 6 h. The aqueous and octanol phase were separated and filtered through membrane filter and drug content in aqueous phase was analyzed by UV spectroscopy. The partition coefficient was calculated by equation; PC = (Ct – Ca)/Ca; where PC = partition coefficient, Ct = concentration of total drug, Ca = concentration of the drug in aqueous phase.

For the molecular mechanics calculations, the ACD/Chemsketch/3-D viewer Freeware version program was used for employing the Chemistry at Harvard Macromolecular Mechanics (CHARMM) force field. The three dimensional structural analysis of the synthesized compounds was performed using software Ortep3v2.

***In silico* studies (distance mapping)**

In conformational analysis of the clinically anticonvulsant drugs such as carbamazepine, phenytoin, lamotrigene, zonisamide, rufinamide, dezinamide, remacemide and diazepam, a molecular model was suggested on the basis of molecular dynamics distance estimations (16), according to which an electron donor (**D**) should be in a distance range of 3.2–5.1 Å to an aryl ring or any other hydrophobic unit (**R**) and of 3.9–5.5 Å to the hydrogen bonding domain (**HBD**). For the molecular mechanics calculations, the ACD/Chemsketch/3-D viewer 2.0 version program was used for employing the CHARMM force field (17).

Three-dimensional structure analysis

This was performed to define the role of three-dimensional structures of synthesized compounds for their anticonvulsant activity. Compound 3-(4-{[(4-fluoro phenyl)methylidene]amino}-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-2*H*-chromen-2-one (**6**), which represents the prototype structure of the synthesized compounds was chosen for study. It was performed using software Ortep3v2.

Syntheses of ethyl-2-oxo-2*H*-chromene-3-carboxylate (m. p. 120–122°C) and 2-oxo-2-*H*-chromene-3-carbohydrazide (m. p. 136–138°C) were carried out by standard procedures (7). The synthesis of 3-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2*H*-chromene-2-one (m. p. 200–202°C) was also carried out by reported method (18). Synthesis and analytical data of 3-(4-{[(substituted-phenyl)methylidene]amino}-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-2*H*-chromen-2-ones **(1-14)** has been published in our earlier publication (19).

RESULTS

In phase I of preliminary anticonvulsant screening, all the compounds showed some degree of protection in MES screen, which was the indicative of the good ability of these compounds to prevent seizure spread. Among these, compound **6** showed protection from seizures at the lowest dose (30 mg/kg) after 0.5 h. It also showed anticonvulsant activity at 4.0 h at higher dose of 100 mg/kg, which indicated the promising nature of the compound

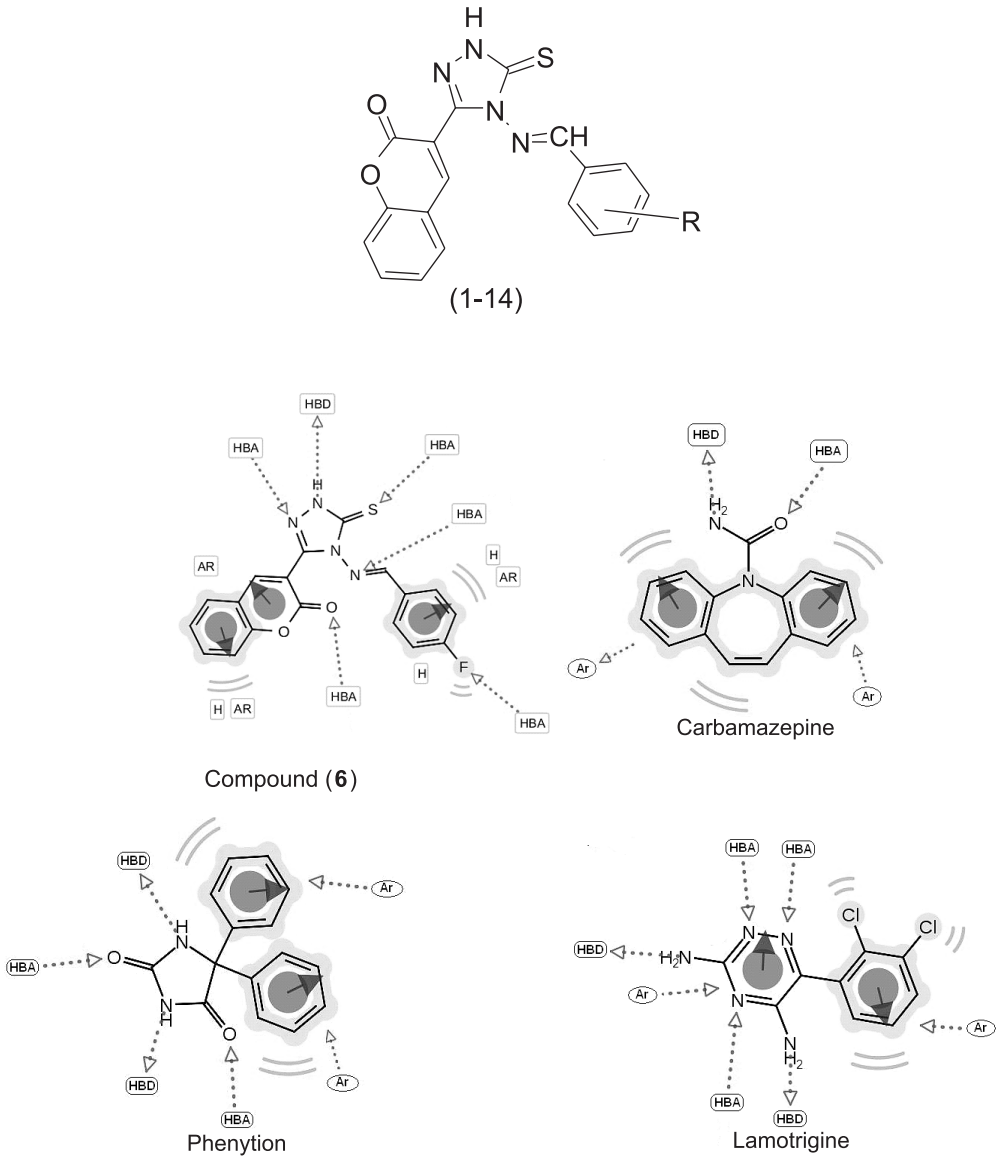

Figure 1. Anticonvulsant agents showing essential pharmacophoric elements present in their structure (HBA = hydrogen bond acceptor; HBD = hydrogen bond donor; Ar = lipophilic aryl ring)

Table 3. Distances range between the essential structure elements R, D and HBD (in Å).

Compound	R-HBD[a]	R-D[a]	D-HBD[a]
Carbamazepine	6.517	3.931	5.554
Phenytoin	3.042	3.868	2.497
Lamotrigine	5.807	3.301	4.598
Zonisamide	4.058	5.651	6.729
Rufinamide	2.407	7.474	5.209
Dezinamide	4.481	5.909	2.948
Remacemide	3.211	9.811	6.635
Diazepam	4.793	4.827	1.49
6	8.320	3.958	4.578

[a]Distance calculated for 3D optimized structures using ACD freeware 3D viewer 8.04 version.

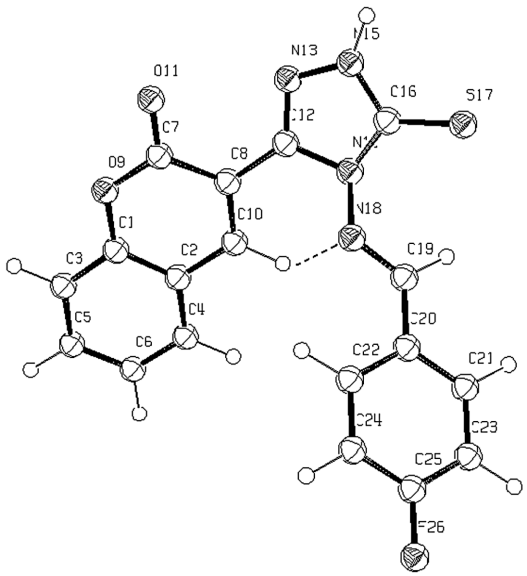

Figure 2. ORTEP diagram (50% probability) of 3-(4-{[(4-fluorophenyl)methylidene]amino}-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-2*H*-chromen-2-one (**6**) showing hydrogen bonding interaction (N…H) as dotted line

with quick onset and long duration of action. Compounds **1**, **5** and **14** also showed significant anticonvulsant action at low dose of 30 mg/kg but after 4.0 h. Compounds **3**, **4** and **8** showed anticonvulsant activity at a dose of 100 mg/kg at 0.5 h. Most of the compounds were active at a dose of 100 mg/kg after 4 h including compounds **9–12**.

In the sc PTZ screen, compounds that were found to be active included **3–6**, **9–12** and **14**. Among these compounds **3**, **6** and **14** were active at 100 mg/kg after 0.5 h. Compounds **6** and **14** were also found to be active after 4.0 h but at higher dose of 300 mg/kg. Compounds that were active at 300 mg/kg dose after 0.5 h were **4**, **5**, **9–12**. Compound **1** was also active at the same dose after 4.0 h.

In the neurotoxicity screening, compound **6** was devoid of minimal impairment at any dose. All other compounds showed some degree of neurotoxicity but were less toxic than the standard drug – phenytoin.

The dependence of biological activity in the set of congeneric agents has been shown in many types of drug action. In particular, the reports by Lien et al. indicated that the anticonvulsant activity of the different types of compounds were correlated with lipophilicity (20). In this study, we tried to correlate the anticonvulsant activity of the synthesized compounds with their log P value. The experimental log P values were determined using n-octanol-phosphate buffer method and the calculated log P values were obtained from the ChemOffice 6.0 software and results are shown in Table 2. All of the compounds have optimal log P values.

From the present study it was concluded that all the pharmacophoric elements were present in all investigated compounds (Fig. 1) that are essential for anticonvulsant activity (21). These are hydrophobic domain **R**, hydrogen bonding domain **HBD**, electron donor moiety **D** and distal hydrophobic domain **A.** All these elements are present in many antiepileptic drugs. The distal aryl ring increases the Van der Waal's bonding at the binding site and increases potency. To test this hypothesis the size of hydrophobic aryl ring has been varied.

Distance mapping involved the comparison of the structures for molecular modeling of well known compounds with anticonvulsant activity and the synthesized compounds to find out the structural elements essential for action. In case of the synthesized

compounds, the distances R-D, R-HBD and D-HBD were in conformity with that of active anticonvulsant drugs. The compounds selected for this comparison have at least one aryl hydrophobic domain (R), one electron donor (D) and a hydrogen bond acceptor/donor unit (HBD). Calculations on the basis of molecular mechanics, with the force field based on CHARMM parameterization were performed to obtain an overview on their minimum conformation for bioactivity. Analysis of the distance relationship showed that the synthesized compounds (**1-14**) fulfill the essential demands of the pharmacophore when compared with other known anticonvulsant drugs as shown in Table 3. The pharmacophore mapping of all these compounds was performed by using the software Ligandscout (3.0). It involves the identification of different pharmacophoric elements that are required for better pharmacological activity. These pharmacophoric elements include hydrogen bond donor (HBD), hydrogen bond acceptor (HBA) and hydrophobic aryl ring (Ar). Almost all the established antiepileptic drugs (AEDs) possess most of these elements that are responsible for their activity. The pharmacophore mapping led to the conclusion that all the synthesized compounds showed most of these essential pharmacophoric elements and significant pharmacological activities can be predicted from their structure.

The three-dimensional structural analysis was performed to delineate the role of three-dimensional structures of the synthesized compounds to their anticonvulsant activity. Compound 3-(4-{[(4-fluorophenyl)methylidene]amino}-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-2*H*-chromen-2-one (**6**), which represents the prototype structure of the synthesized compounds was chosen for this study. It was performed using the software Ortep3v2. The ORTEP diagram (50% probability) was drawn using this software and its characteristic features were studied. In the compound **6**, strong intramolecular hydrogen bonding occurred between the vinyl proton and the nitrogen atom as noted in Figure 2. It was observed between N^{18} and H^{10} where the distance between the donor and the acceptor atom (N…H) was found to be 2.138 Å.

CONCLUSION

A new series of 3-(4-{[(substituted phenyl) methylidene] amino}-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-2*H*-chromen-2-ones (**1**–**14**) have been studied. Compound **(6)** with *para*-fluoro substitution showed significant anticonvulsant activity as compared to standard antiepileptic drugs. It can serve as a lead for further research.

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REFERENCES

1. Wasteralin, C.: in Basic Neurochemistry: Molecular, Cellular and Medical Aspects, Siegel G.J., Agranoff B.W., Albers B.W. Eds., p. 797, Raven Press, New York 1989.
2. Mc Namara J.O.: in Drugs Effective in the Therapy of the Epilepsies, Hardman J.G., Limbrid L.E., Mollinoff P.B., Ruddon R.N. Eds., Goodman and Gilman's Pharmacological Basis of Therapeutics, 10th edn., p. 521, McGraw Hill, New York 2001.
3. Coatsworth J.J.: Studies on the clinical efficacy of marketed antiepileptic drugs, UNINDS Monograph No. 12, 73-15, HEW publication, U.S. Government Printing Office, Washington 1971.
4. Rana A., Siddiqui N., Khan S.A., Haque, S.E., Bhat M.A.: Eur. J. Med. Chem. 43, 1114 (2008).
5. Siddiqui N., Rana A., Khan S.A., Bhat M.A., Haque S.E.: Bioorg. Med. Chem. Lett. 17, 4178 (2007).
6. Siddiqui N., Pandeya S. N., Khan S.A., Stables J., Rana A., Alam M., Arshad M.F., Bhat M.A.: Bioorg. Med. Chem. Lett. 17, 255 (2007).
7. Bhat M.A., Siddiqui N., Khan S.A.: Acta Pol. Pharm. Drug Res. 65, 235 (2008).
8. Siddiqui N., Bhat M.A., Khan S.A., Ahsan W., Alam M.S.: J. Chinese Chem. Soc. 55, 1326 (2008).
9. Almsirad A., Tabatabai S.A., Faizi M.: Bioorg. Med. Chem. Lett. 14, 6057 (2004).
10. Shiradkar M.R., Ghodake M., Bothara K.G., Bhandari S.V., Nikalje A., Akula K.C. Desai N.C., Burange P.J.: Arkivoc 14, 58 (2007).
11. Anticonvulsant screening project, Antiepileptic Drug Development Program. National Institutes of Health, DHEW publ. (NIH), (US), pp. 78–1093 (1978).
12. Kupferberg H.J., Stables J.P.: in Challenge Epilepsy – New Antiepileptic Drugs. Stefen H.,

Kramer G., Mamoli B. Eds., p. 7, Blackwell Sciences, Berlin 1998.
13. Clark C.R., Wells M.J.M., Sansom, R.T., Norris G.N., Dockens R.C., Ravis W.R.: J. Med. Chem. 27, 779 (1984).
14. Yogeeswari P., Sriram D., Saraswat V., Ragavendran J. V., Kumar M.M., Murugesan S., Thirumurugan R., Stables J.P.: Eur. J. Pharm. Sci. 20, 341 (2003).
15. Farar V. A., Ciechanowicz-Rutkowska M., Grochowski J., Serda P., Pilati T., Filippini G., Hinko C.N. et al.: J. Med. Chem. 36, 3517 (1993).
16. Wong M.G., Defina J.A., Andrews P.R.: J. Med. Chem. 29, 562 (1986).
17. Brooks B.R., Bruccoleri R.E., Olafson B.D., States D.J., Swaminathan S., Karplus M.: J. Comput. Chem. 4, 187 (1983).
18. Cacic M., Trkovnik M., Cacic F., Has-Schon E.: Molecules 11, 134 (2006).
19. Bhat M. A., Siddiqui N., Khan S.A., Mohamed M.I.: Acta Pol. Pharm. Drug Res. 66, 625 (2009).
20. Lien E.J., Liao R.C.H., Shinouda H.G.: J. Pharm. Sci. 68, 463 (1979).
21. Pandeya S.N., Yogeeswari P., Stables J.P.: Eur. J. Med. Chem. 35, 879 (2000).

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