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Short Communication

Evaluation of Anti-Convulsant Activity of Novel Series of Benzopyran-2-One Derivatives by PTZ Induced Seizure Model in Mice

PM RONAD[1], VS MADDI[1], BC KOTI[2*], YV KURHE[2], AHMV SWAMY[2], AHMT SWAMY[2], MS JAJI[2]

[1]Department of Pharmaceutical Chemistry, K.L.E.S College of Pharmacy, Vidyanagar, Hubli, Karnataka, INDIA

[*2]Department of Pharmacology, K.L.E,S College of Pharmacy, Vidyanagar, Hubli, Karnataka, INDIA

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ABSTRACT

Newly synthesized benzopyran-2-one derivatives i.e., 7-(4-hydroxy-3-methoxy benzylideneamino)-4-methyl-2H-chromen-2-one (comp-I), 7-(furan-2-methylene amino)-4-methyl-2H-chromen-2-one (comp-II) and 7-(4-flurobenzyl ideneamino)-4-methyl-2H-chromen-2-one (comp-III) were assessed for their effects on the convulsant activity by pentylenetetrazole (PTZ) induced seizure model. The results clearly indicated a significant prolongation of onset of seizures and reduced the duration of seizures, where only comp-I was found to be effective in preventing the mortality rate in mice. The anti-convulsant activity was further supported by estimation of serum GABA levels in mice. Comp-I was found to be more potent than comp-II, where as comp-III was less effective as compared to comp- II.

INTRODUCTION

Epilepsy continues to be a neurological disorder awaiting safer drugs with improved anticonvulsant and anti-epileptogenic effectiveness as currently available drugs fail to provide adequate control of epileptic seizures in about one-third of patients and do not prevent progressive epileptogenic changes are not well understood. This fact has stimulated a considerable number of researches of new anti-epileptic drugs[1]. Coumarins owe their class name to "coumarou", the vernacular name of the tonka bean (Dipteryx odorata Willd., Fabaceae) from which coumarin itself was isolated in 1820. Coumarins are 2H-1-benzopyran-2-ones which may be considered, on first approximation, to be the lactones of the 2-hydroxy-2-cinnamic acids[2].

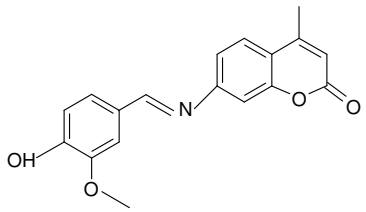
Review of literature reveals several pharmacological activities of coumarin derivatives including anti-cancer[3], anti-tubercular[4], anti-inflammatory[5], anti-mycobacterial[6], anti-fungal[7] and anti-coagulant[8] activities. Chemically synthesized derivatives of coumarins have been reported earlier for anti-anxiety[9], anti-convulsant[10], analgesic[11] and anti-inflammatory[12] activities.

Author for Correspondence:

Email: bc_koti@yahoo.com

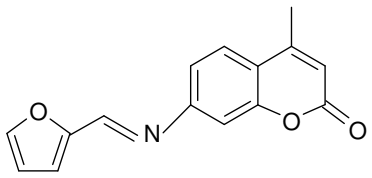
Following are the structures of benzopyran-2-one derivatives used in the present study.

Compound I



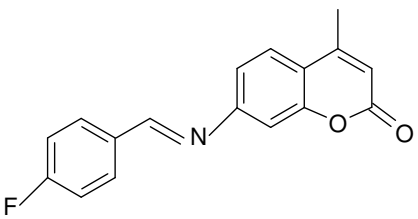
7-(4-hydroxy-3-methoxybenzylideneamino)-4-methyl-2H-chromen-2-one

Compound II



7-(furan-2-ylmethyleneamino)-4-methyl-2H-chromen-2-one

Compound III



7-(4-fluorobenzylideneamino)-4-methyl-2H-chromen-2-one

Hence, the present attempt deals with the investigation of the anti-convulsant effect of novel benzopyran-2-one derivatives such as 7-(4-hydroxy-3-methoxy benzylideneamino)-4-methyl-2H-chromen-2-one (comp-I), 7-(furan-2-methyleneamino)-4-methyl-2H-chromen-2-one (comp-II) and 7-(4-flurobenzylideneamino)-4-methyl-2H-chromen-2-one (comp-III) in mice.

All the benzopyran-2-one derivatives i.e., 7-(4-hydroxy-3-methoxy benzylideneamino)-4-methyl-2H-chromen-2-one (comp-I), 7-(Furan-2-methyleneamino)-4-methyl-2H-chromen-2-one (comp-II) and 7-(4-Flurobenzylideneamino)-4-methyl-2H-chromen-2-one (comp-III) were synthesized by earlier reported method[13].

Diazepam 1 mg/kg p.o. (Azardo Pharma, India), PTZ 80 mg/kg i.p. (Sigma Chemical Co.,USA) were used. A suspension of 5% sodium carboxyl methyl cellulose (CMC) at a dose of 5 ml/kg p.o. was used as control. All the three test compounds comp-I, comp-II, comp-III were administered as a suspension of 5% sodium carboxyl methyl cellulose (CMC).

Swiss albino mice of either sex (5-6-weeks-old) weighing 20-25g obtained from the animal house of K.L.E.'S College of Pharmacy, Hubli-31, Karnataka, India were used. The animals were housed under standard laboratory conditions (relative humidity 55-56 %, room temperature 23.0 ± 2.0 ^{0}C and 12 h light: dark cycle).The animals were fed with standard diet and water ad libitum. They were fasted overnight prior to experiment. All the animal experiments were approved from the animal ethical committee of K.L.E.'S College of Pharmacy, Hubli, Karnataka, India.

The toxicity test showed clinical signs and mortality of the animals at higher doses 2000 mg/kg and 300 mg/kg p.o. for all the test compounds. Further, next dose i.e., 200 mg/kg p.o. dose was found to be non-lethal and hence, 1/10th of this non-lethal dose i.e., 20 mg/kg p.o. dose was selected for the anti-convulsant activity for all three benzopyran-2-one derivatives (comp-I, comp-II and comp-III). Toxicity study was carried out as per the OECD/OCDE guide lines no. 423.

The mice (20-25 g) were divided into five groups of 6 animals each. Group-I served as control which received 5% sodium CMC (5 ml/kg p.o.) 30 min before administration of PTZ (80 mg/kg i.p.). Group-II received diazepam (1 mg/kg p.o.) dissolved in a suspension of 5% sodium CMC as a standard reference drug. Groups-III, IV and V received comp-I, comp-II and comp-III at a dose of 20 mg/kg p.o dissolved in suspension of 5% sodium CMC respectively 30 min before the administration of PTZ (80 mg/kg i.p.). Each animal was placed into an individual plastic cage for observation lasting 1 h. The time taken before the onset of clonic convulsions, the duration of clonic convulsions and percentage of mortality protection were recorded[14].

The GABA level in serum was assayed by paper chromatography method. The serum sample (0.1 ml) was added to 1.5 ml of absolute alcohol and centrifuged at 3000 g for 15 minutes. The upper layer was aspirated and 0.3 ml was put on Whatman's filter paper which was dipped in phenol solution for 24 hours and subsequently dried in air. Thereafter, ninhydrin salt solution was sprayed on chromatography paper and heated at 65^{o}C for 10 minutes. The spot, developed due to chromatographic mobility of GABA, was cut and put in 3 ml solution of absolute alcohol for elution.

The optical density was measured using a spectrophotometer at wavelength of 509 nm and compared with standard solution[15].

$$\frac{\text{O.D. of test}}{\text{O.D. of std}} \times 1000 = \text{pmol/ml}$$

Note: 1ug = 1000pg. Hence, to convert values into picogram (pg), values must be multiplied by 1000.
Concentration of Standard GABA solution: 0.1 ug/ml

The statistical analysis of result was carried out by using one-way ANOVA followed by Dunnet's multiple comparison tests using graph pad prism in stat 5 Demo and all the results obtained in the study were compared with the vehicle control group. P values ($p<0.05$) were considered statistically significant.

All the benzopyran-2-onederivatives i.e., comp-I, comp-II and comp-III prolonged the onset of seizures and reduced the duration of seizures after 60 min of treatment. But, only comp-I was found to be effective in protection of mortality in mice (Table 1).

Table 1: Effect of 7-Substituted Amino Coumarin Derivatives on Pentylenetetrazole (PTZ) - Induced Seizure in Mice

Sl.No.	Treatment	Dose (mg/kg p.o.)	Onset of Seizures(sec)	Duration of seizure(sec)	Percentage of mortality protection
1	Control (5% sodium CMC+ PTZ)	5 ml/kg + 80 mg/kg p.o.	13.33 ± 0.49	14.00 ± 0.36	0.00 ± 0.00
2	Compound-I	20	51.50 ± 1.11***	8.73 ± 0.54***	83.33 ± 16.6***
3	Compound-II	20	40.33 ± 0.71***	11.02 ± 0.37***	0.00 ± 0.00
4	Compound-III	20	26.0 ± 0.96***	11.85 ± 0.22***	0.00 ± 0.00
5	Diazepam	1	596.8 ± 1.37***	0.00 ± 0.00***	100.0 ± 0.00***
	p value		< 0.0001	< 0.0001	< 0.0001
	F value		66040	152.8	46.00

GABA levels were significantly increased in the treated group as compared to that of control. (Table 2). GABA levels in the treated groups were found to be less than that of the standard GABA solution (0.1ug/ml). GABA levels of group treated with comp-I was found to be higher than that of comp-II and comp-III.

Table 2: GABA Levels of All Three Benzopyran-2-One Derivatives Was Assayed and Compared With the Levels of Standard GABA

SL. No.	Treatment	Optical Density (OD) at 509 nm	GABA levels pmol /ml
1	Control	0.02	153.8
2	Compound-I	0.09	692.3
3	Compound-II	0.05	384.6
4	Compound-III	0.03	230.7
5	Diazepam	0.10	769.2
6	Std. GABA	0.13	1000

Anti-convulsant activity in mice was determined by PTZ induced seizure model. All the three test derivatives were found effective in altering the onset and duration of seizures. Whereas, only comp-I was effective in protecting mortality in mice. From the results of GABA assay it clearly indicates that the test compounds may have exerted the possible anti-convulsant effect by reducing T-type Ca^{2+} currents, increasing the GABA levels and enhancing the $GABA_A$ receptor mediated inhibitory neurotransmission[16], enhancing and facilitating GABA-mediated synaptic transmission and inhibition, an effect mediated either by a pre- or post- synaptic action. In presence of GABA, the $GABA_A$ receptor is opened, thus allowing an influx of Cl^- ions, which in turn increases membrane polarization [17].

In conclusion, benzopyran-2-one derivatives i.e., 7-(4-hydroxy-3-methoxy benzylideneamino)-4-methyl-2H-chromen-2-one (comp-I), 7-(furan-2-methyleneamino)-4-methyl-2H-chromen-2-one (comp-II) and 7-(4-flurobenzylideneamino)-4-methyl-2H-chromen-2-one (comp-III) possess anti-convulsant activity, where comp-I was found to be more potent than comp-II whereas, comp-III was less potent than comp-II.

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