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ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF SYNTHESIZED 3(SUBSTITUTED) CHROMEN-2-ONE

B. Lakshmi Narayanan*[1], L. A. Pradeep Rajkumar [1], A. Arulanandham [2], N. Satheesh Babu [3] T. Gayathri [4] and A. Raju [5]

Karpagam College of Pharmacy [1], Coimbatore- 641 032, Tamil Nadu, India
K.M. College of Pharmacy [2], Madurai- 625 107, Tamil Nadu, India
Lincoln University College [3], Kuala Lumpur- 47301, Malaysia
P.S.G. College of Pharmacy [4], Coimbatore- 641 004, Tamil Nadu, India
Shri Rawatpura College of Pharmacy [5], Datia, Madhya Pradesh- 475 661, India

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Correspondence to Author:

B. Lakshmi narayanan

M. Pharm, (Ph. D)., Asst. Professor, Dept. of Pharmaceutical Chemistry, Karpagam College of Pharmacy, S.F.No:762, Othakkalmandapam, Coimbatore- 41032, Tamil Nadu, India

ABSTRACT

This study was designed to investigate the *in vitro* anti-oxidant and *in vitro* anti-inflammatory potential of synthesized 3(Substituted) Chromen-2-one. Anti-oxidant activity has done by DPPH, NO and H_2O_2 free radical scavenging activity, ascorbic acid was used as a standard. Anti-inflammatory activity has done by membrane stabilization method; aceclofenac sodium was used as standard. The presence of OCH_3 group in aromatic ring system of the compound prompted us to evaluate its anti-oxidant and anti-inflammatory activity. Some of the synthesized compounds which contains OCH_3 group has been found to be having high anti-oxidant and anti-inflammatory activity by various *in vitro* models.

INTRODUCTION: Numerous compounds with biological activity have been investigated. However many of them are not suitable for therapeutic use due to their toxic, carcinogenic and mutagenic properties. Nowadays, it is possible to make modifications of active chemical structures, in order to synthesize compounds with improved therapeutic activity and reduced toxicity.

Coumarins have important effects in plant biochemistry and physiology, acting as anti-oxidants and enzyme inhibitors. The coumarins and its derivatives display a remarkable array of biochemical and pharmacological actions, some of which suggest that certain members of this group of compounds may significantly affect the function of various mammalian cellular systems. A lot of biological parameters should be evaluated to increase understanding of mechanisms by which these coumarins act. The coumarins are extremely variable in structure, due to the various types of substitutions in their basic structure, which can influence their biological activity. The coumarins and its derivatives have long been recognized to possess anti-inflammatory, analgesic, anti-fungal, anti-bacterial, anti-viral, anti-psychotic, anti-tuberculosis, anti-HIV, and anti-carcinogenic activities [1].

In recent years, a large number of new therapeutic agents have been produced by medicinal chemist, working generally as a part of interdisciplinary teams. The systemic research in pharmaceutical laboratory has led to the introduction of more and more synthetic drugs in the modern times. The present study was designed to investigate the *in vitro* anti-inflammatory and *in vitro* anti-oxidant potential of some synthesized coumarin derivatives of 3 (substituted) chromen-2-

one. Oxidative stress induced by reactive oxygen species including free radicals such as hydroxyl radicals, nitric oxide radicals, superoxide anions, are believed to be a primary pathogenesis of various degenerative disease [2, 3]. Anti-oxidants may offer major defence against radical mediated toxicity by protecting the damages caused by free radicals. Anti-oxidant based drugs for the prevention and treatment of complex diseases, like atherosclerosis, stroke, diabetes, and cancer, have appeared in last decade's [4]. These properties were attracted the researchers in a great deal of research interest in anti-oxidants.

There are evidences for the participation of reactive oxygen species in the etiology and pathophysiology of human disease, such as neurodegenerative disorders, inflammation, viral infections, autoimmune gastro-intestinal inflammation and gastric ulcer [5]. Drugs with multiple mechanism of protective action, including anti-oxidant activity, may be highly effective in minimizing tissue injury in human diseases. It has been demonstrated that many drugs and formulations possess potent anti-oxidant action.

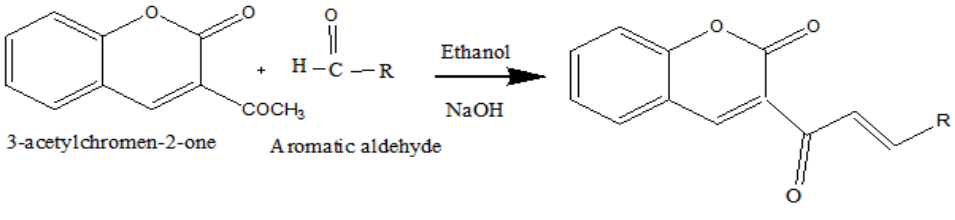
The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane [6]. Therefore as the membrane stabilizers, it interferes with the release and/or action of mediators like histamine, serotonin, prostaglandins, and leukotrienes etc [7].

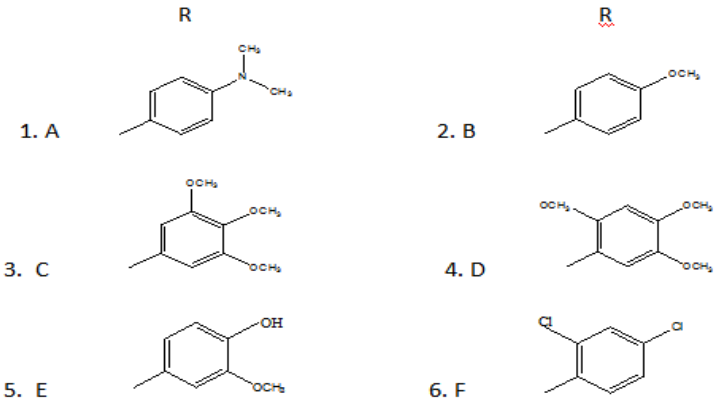
The present study was carried out to determine the anti-inflammatory effect of 3(Substituted) Chromen – 2-one by membrane stabilization method.

MATERIALS AND METHODS:

Chemicals and reagents: Coumarin, Ethanol, Sodium hydroxide, 1, 1 - diphenyl-2-picrylhydrazyl (DPPH), Hydrogen peroxide (H_2O_2), Sodium nitroprusside, Methanol, were purchased from Sigma Aldrich, India, Griess reagent were obtained from Himedia Laboratories Pvt. Ltd., India, Ascorbic acid, citric acid, sodium citrate, dextrose and sodium chloride were obtained from SD Fine Chemicals Ltd, India. Disodium hydrogen phosphate and potassium dihydrogen phosphate were obtained from Loba Chemie Pvt. Ltd, Mumbai, India. All the reagents used in the study were of analytical grade.

General procedure for the preparation of 3-(substituted) Chromen-2-one (a-f): Different synthetic compounds of 3-(substituted) chromen-2-one (a-f) were prepared by the method of Lakshmi Narayanan B *et al.,* [1]. A mixture of 3-acetylchromen-2-one (0.0304mol) and appropriate aldehyde (0.0304mol) was stirred in water (40 ml) and ethanol (25 ml) in presence of Sodium hydroxide (0.0302mol) for 5 hours. The reaction mixture was kept overnight in an ice bath. Then the above mixture was poured into a beaker containing water. The precipitated product was filtered and recrystallized from ethanol **(Scheme 1).**





SCHEME 1: SYNTHESIS OF SOME NEW 3 (SUBSTITUTED) CHROMEN-2-one (A-F)

All the synthesized compounds were characterized by physical, chemical and spectroscopic analysis including IR, NMR and Mass spectroscopy and it was reported [1]. All the synthesized compounds were used to determine anti-oxidant and anti-inflammatory activity.

NAMES OF THE SYNTHESIZED COMPOUNDS

S. No.	Compound code	Compound Name
1	A	3-(3(4-dimethylamino)Phenylacryloyl)-Chromen-2-one
2	B	3-(3(4-methoxy)Phenylacryloyl)-Chromen-2-one
3	C	3-(3(3, 4, 5-trimethoxy)Phenylacryloyl)-Chromen-2-one
4	D	3-(3(2, 4, 5-trimethoxy) Phenylacryloyl)-Chromen-2-one
5	E	3-(3(4-hydroxy-3-methoxy)Phenylacryloyl)-Chromen-2-one
6	F	3-(3(2, 4-dichloro)Phenylacryloyl)-Chromen-2-one

***In vitro* Anti-oxidant activity:**

1. **DPPH Free Radical Scavenging Activity:** The free radical scavenging activity of synthesized compounds were measured by decrease in absorbance of methanolic solution of DPPH.[8] 0.1ml of Synthesized compounds (100 µg/ml) and standard were added to 3 ml of a 0.004% Methanolic solution of DPPH. Absorbance of all the synthesized compounds and standard were determine after 30 minutes at 517 nm and the percentage inhibition of activity was calculated by using the formula $[(A_0 – A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, A_1 is the absorbance of the synthesized compounds / standard. Ascorbic acid was used as standard radical scavenger.

2. **Nitric oxide (NO) Radical Scavenging Activity:** Nitric oxide radical scavenging activity was measured by using Griess reagent.[9] Sodium nitroprusside (5mmol/L) in phosphate buffered saline pH 7.4, was mixed with different synthesized compounds (100 µg/ml) in methanol and incubated at 25^{o}C for 30 minutes. A control without the test compound, but with an equivalent amount of methanol, was taken. After 30 minutes, 1.5 ml of the incubated solution was taken and diluted with 1.5 ml of Griess reagent. Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1 naphthylethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured and calculated by using the above formula. Griess reagent consists of 1% sulphanilamide, 2% phosphoric acid and 0.1% N-1 naphthylethylene diamine dihydrochloride.

3. **Hydrogen peroxide (H_2O_2) radical scavenging activity:** A solution of hydrogen peroxide (40mmol/L) was prepared in phosphate buffer (pH 7.4). Different synthesized compounds (100µg/ml) and standard were added to the hydrogen peroxide solution (0.6ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide [7]. The percentage scavenging activity was measured and calculated by using the above formula.

***In vitro* Anti-inflammatory activity**

Preparation of reagents:

- **Alseviers solution:** 2 gm dextrose, 0.8 gm sodium citrate, 0.05 gm Citric acid and 0.42 gm sodium chloride were dissolved in distilled water. The final volume was made up to 100 ml with distilled water.
- Hypotonic Saline was prepared by dissolving 0.36 gm of sodium chloride in 100 ml of distilled water.
- Isotonic Saline was prepared by dissolving 0.85 gm of sodium chloride in 100 ml of distilled water.
- **Phosphate Buffer (pH 7.4, 0.15 M):** 2.38 gm disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8 gm of sodium chloride were dissolved in 100 ml of distilled water.
- **Erythrocyte suspension:** Whole blood was collected from rats under ether anaesthesia and heparin was added to prevent clotting. The blood was washed three times with 0.9% saline. 3 ml of Blood suspension and 3ml of Alseviers solution was mixed and centrifuge at 3000 rpm for 20 minutes. Packed cells were washed with isotonic saline. 10% V/V suspension of the packed cells was made with isotonic saline

Experimental Method: The synthesized compounds were used in the dose of 100µg/ml. Aceclofenac sodium (100µg/ml) was taken as a standard. The reaction mixtures (4.5ml) consists of 2ml of hypotonic saline (0.25% NaCl), 1ml of 0.15 M phosphate buffer (pH 7.4,0.15M), and 1ml of sample solution (100 µg/ml) in normal saline, 0.5 ml of 10% V/V erythrocyte suspension in normal saline were added. For test control, 1 ml of isotonic saline was used instead of sample solution while product control tests lacked red blood cells. All the mixtures were incubated at 56°C for 30 minutes. Then they cooled under running tap water and centrifuged at 3000 rpm for 20 minutes. The absorbances of the supernatants were read at 560 nm. Percentage membrane stabilizing activity was calculated as follows;

$$\text{Percentage stabilization} = 100 - \left\{ \frac{\text{OD of sample - OD of Product Control}}{\text{OD of test control}} \right\} X\ 100$$

Statistical analysis: Values were represented as mean ± SEM using one way analysis of variance (ANOVA) followed by student "t" test and p values <0.05 were considered as significant.

RESULTS: The results of anti-oxidant activity were given in **Table 1** and anti-inflammatory activity was given in **Table 2**.

DPPH Free Radical Scavenging Activity: DPPH is usually used as a reagent to evaluate free radical scavenging activity of anti-oxidant. It is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical with synthesized compound is determined by decrease in absorbance at 517 nm induced by anti-oxidant. Ascorbic acid is the reagent used as standard. The synthesized compounds are able to reduce the stable DPPH radical to the yellow colored di-phenyl picryl hydrazine. The scavenging effect of synthesised compounds with DPPH radical was found to be in the following order: compound C > D > B > E > F > A.

Nitric Oxide Radical Scavenging Activity: Nitric oxide is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. The scavenging effect of synthesized compound against nitric oxide was found to be compound C > E > D > B > F > A.

Hydrogen Peroxide Radical Scavenging Activity: Hydrogen peroxide free radicals are not very reactive, but sometimes it is toxic to cell because it may give rise to hydroxyl radical in the cells. Therefore removing of hydrogen peroxide free radicals is very important for anti-oxidant defence in cell or food system. The scavenging ability of synthesised compounds with hydrogen peroxide is shown in **fig. 1** and compared with standard ascorbic acid.

TABLE 1: *IN VITRO* ANTI-OXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS

Compounds	Conc.(µg/ml)	% inhibition		
		DPPH	NO	H_2O_2
A	100	61.71	58.12	60.83
B	100	72.51	69.38	71.53
C	100	85.12	88.73	84.31
D	100	78.31	69.72	65.72
E	100	83.33	87.08	81.23
F	100	63.28	62.84	58.93
Ascorbic acid	100	63.28	62.84	58.93

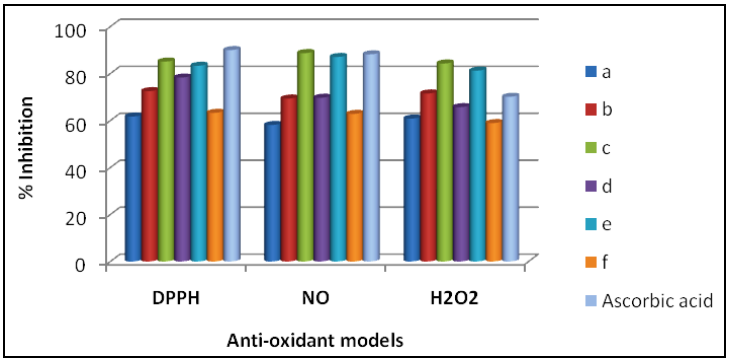


FIGURE 1: *IN VITRO* ANTI-OXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS

Anti-inflammatory Activity: From anti-inflammatory activity studies, it was found that few synthesized compounds showed highly significant activity and it was given in **Table 2**.

TABLE 2: *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF SYNTHESIZED COMPOUNDS

Treatment	Dose (µg/ml)	Membrane Stabilization (Mean ± SEM)
STD	100	80.46 ± 0.3714
A	100	13.32 ± 0.2988*
B	100	30.15 ± 0.2654**
C	100	78.45 ± 0.2412***
D	100	69.46 ± 0.2927***
E	100	61.98 ± 0.2249***
F	100	22.26 ± 0.3125*

P < 0.005 indicates the highly significant compared with standard; *** = Highly Significant; ** = significant * = Non Significant

DISCUSSION: Free radicals contribute more than one hundred disorders in humans. In this study that the compound "C" and the compound "D" has good anti-oxidant property due to the presence of methoxyl group in 3, 4, 5^{th} and 2, 4, 5^{th} position in benzene ring respectively. The compound "E" also has good free radical scavenging activity due the presence of phenolic hydroxyl group in benzene ring.

In case of anti-inflammatory activity, the compound "C", compound "D" and the compound "E" has highly significant activity due to presence of OCH_3 group at aromatic ring system, the compound "B" has significant activity and the compound "A" and compound "F" has non significant activity when compared to that of standard Aceclofenac sodium.

CONCLUSION: Anti-oxidant activities of various synthesized compounds were observed at the concentration of 100 µg/ml by DPPH, Nitric oxide and Hydrogen peroxide free radical scavenging models. The present study revealed that the presence of methoxyl group which was resembled to phenolic hydroxyl group in the compound "C" and the compound "D" showed good anti-oxidant activity. Also, the compound "E" contains phenolic OH group showed good anti-oxidant activity. It was already reported that phenolic compounds have high free radical scavenging property.

Anti-inflammatory activity of various synthesized compounds was studied at the concentration of 100 µg/ml by membrane stabilization method. From anti-inflammatory activity evaluations, it was found that the compounds "C, D, E" showed significant activity when compared to standard. Perhaps the presence of OCH_3 group at aromatic ring system and the presence of heterocyclic ring system may be the responsible for both anti-oxidant and anti-inflammatory activity.

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