

3-HYDROXY-2-(SUBSTITUTED PHENYL)-4H-CHROMEN-4-ONE DERIVATIVES- SYNTHESIS, SPECTRAL CHARACTERIZATION AND PHARMACOLOGICAL SCREENING

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ABSTRACT

A novel series of 3-hydroxy-2-(substituted phenyl)-4H-chromen-4-one derivatives (**IIa-d**) were synthesized by Algar-Flym-Oyamada reactions, o-hydroxy acetophenone and substituted aromatic aldehyde were reacted in ethanol followed by addition of sodium hydroxide (40%) which leads to the formation of substituted chalcone (**Ia-d**). synthesized compounds (**Ia-d**) were further reacted with ethanol, sodium hydroxide and hydrogen peroxide for 2-3 hours at room temperature which give the titled compound 3-hydroxy-2-(substituted phenyl)-4H-chromen-4-one (**IIa-d**). The structures of all these compounds have been established on the basis of analytical and spectral data. Synthesized compounds were characterized by their IR, ¹H-NMR, elemental and mass spectroscopic data. Synthesized compounds were then screened for anti-oxidant, anti-inflammatory and ulcerogenic activity. The anti-oxidant activity was performed by DPPH free radical scavenging method using ascorbic acid as standard and compound **IIa**, **IIc** and **II d** showed significant free radical scavenging activity. The anti-inflammatory activity was performed using carrageenan induced rat paw edema method and compound **IIa** and **II d** showed most significant anti-inflammatory activity. The acute ulcerogenic activity was performed on albino-wistar rats by ceoli et al method using aspirin as standard drug. Among the synthesized compounds only **II d** was found to have significant antioxidant and potential anti-inflammatory activity with no ulcerogenic effect. Among the synthesized compounds significant activity were shown by the compounds having substitution such as- 4-nitro, 3-nitro and 2, 6-dichloro respectively on the phenyl ring of the flavonol nucleus.

Keywords: Antioxidant, anti-inflammatory, flavonol, ulcerogenic.

INTRODUCTION

The flavonoids or bioflavonoids are a ubiquitous group of polyphenolic substances which are present in most plants. They also occur as glycosides. Chemically, flavonoids show a fifteen-carbon skeleton (C₆-C₃-C₆) which consists of two phenyl rings connected by a three carbon bridge. Flavonoids lead to potent antioxidant activity, the most important function of flavonoids to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals. Multiple combinations of hydroxyl groups, sugars, oxygen and methyl groups attached to these structures create the various classes of flavonoids, flavonols, flavonones, flavones, flavon-3-ols (catechins), anthocynins and isoflavones [1]. Flavonoids have been shown antitumor [2], antibacterial [3], antimicrobial [4], antimalarial [5] and antineoplastic [6] activities. Chalcones is an intermediate compound in the biosynthesis of flavonoids, which are substances widespread in plants and with an array of biological activities [7]. Chalcone also show antibacterial, antifungal, antitumor and anti-inflammatory properties [8].

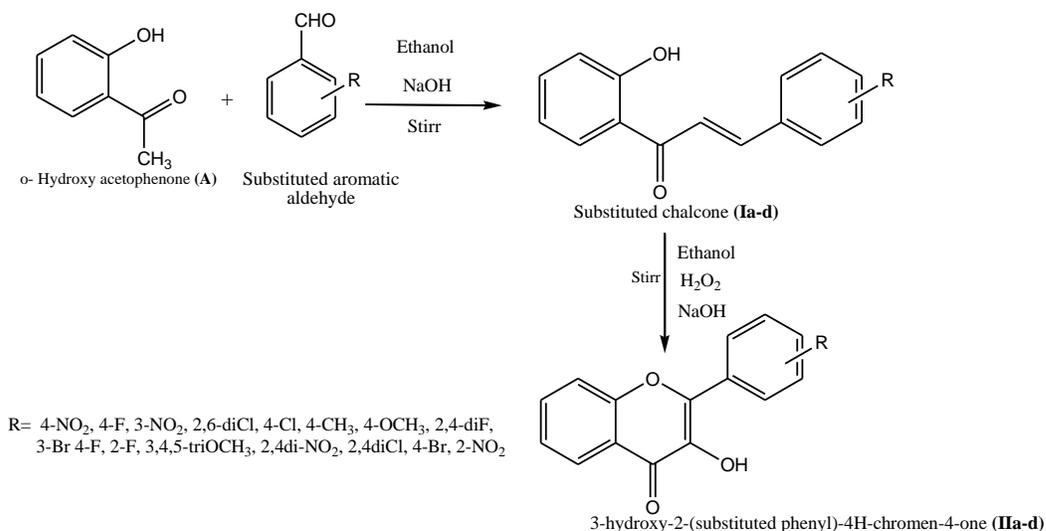
Oxidation is one of the most important processes, it produce free radicals. In turn, these radicals start chain reactions that damage cells and cause oxidative stress. This oxidative stress in cells results in the development of a wide range of diseases like alzheimer's disease [9-10], parkinson's disease [11], the pathologies caused by diabetes [12,13], rheumatoid arthritis [14] and neurodegeneration in motor neuron [15]. Antioxidants have been studied specifically as they terminate the chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols [16]. Living

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organisms themselves have a complex network of antioxidant metabolites and enzymes that work in synchronized manner to prevent oxidative damage to cellular components like DNA, proteins and lipids [17,18]. In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell. However, since reactive

tetramethylsilane (TMS) as the internal standard. Chemical shift values are expressed in ppm. Mass spectra were obtained using a Kratos-AEI MS-902S instrument. Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus (CDRI, Lucknow). The results of the elemental analysis (C, N, and S) were within $\pm 0.4\%$ of the calculated amounts.



Scheme 1

oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level [19].

MATERIAL AND METHODS

2.1.1 Chemistry

All the chemicals and reagents were obtained from Sigma (Germany) and CDH (India) and were recrystallized / redistilled as necessary. The melting points were determined by the open capillary tube method. The purity of the compounds was checked on thin layer chromatography (TLC) plates, which were precoated with silica gel G using solvent system "Ethyl acetate: n hexane" (3:7v/v). The spots were located under iodine vapors and ultraviolet (UV) light. Infrared (IR) spectra were recorded using KBr on Fourier transform infrared (FTIR) Shimadzu 8400S IR spectrophotometer (Japan). A JEOL AL300 FTNMR 300 MHz spectrometer was used to acquire High Resolution Nuclear Magnetic Resonance (1H NMR) spectra with Acetone as the solvent and

General procedures for the preparation of compounds

2.1.2 Synthesis of chalcone derivatives (I): General procedure

A mixture of o-hydroxy acetophenone (0.01mole) and substituted aromatic aldehyde (0.01mole) was stirred in ethanol (15ml) and then sodium hydroxide solution (40%) was added till highly turbid solution was obtained. Completion of mixture was monitored by TLC. The mixture was kept overnight at room temperature and it was poured on crush ice and acidified with dilute hydrochloric acid. The precipitate obtained was washed with ethanol and recrystallized with appropriate solvent.

2.1.2.1 Synthesis of 1-(2-hydroxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one (Ia):

It was obtained from stirring compound (A) and nitro benzaldehyde in ethanol and then sodium hydroxide solution was added. IR (KBr):- 3514.06 cm⁻¹ (OH str.), 1517.87 cm⁻¹(C=C str.), 1691.46 cm⁻¹

(>C=O str.), 1342.36 cm⁻¹(-O-N=O str.), 738.69 cm⁻¹ (Aromatic region monosubstituted). ¹HNMR (EtOD,

300 MHz, δ ppm): 6.92-8.0 (m, 8H, Ar-H), 7.81 (s, 1H, ethylene), 8.01 (s, 1H, ethylene), 5.2 (s, 1H, OH);

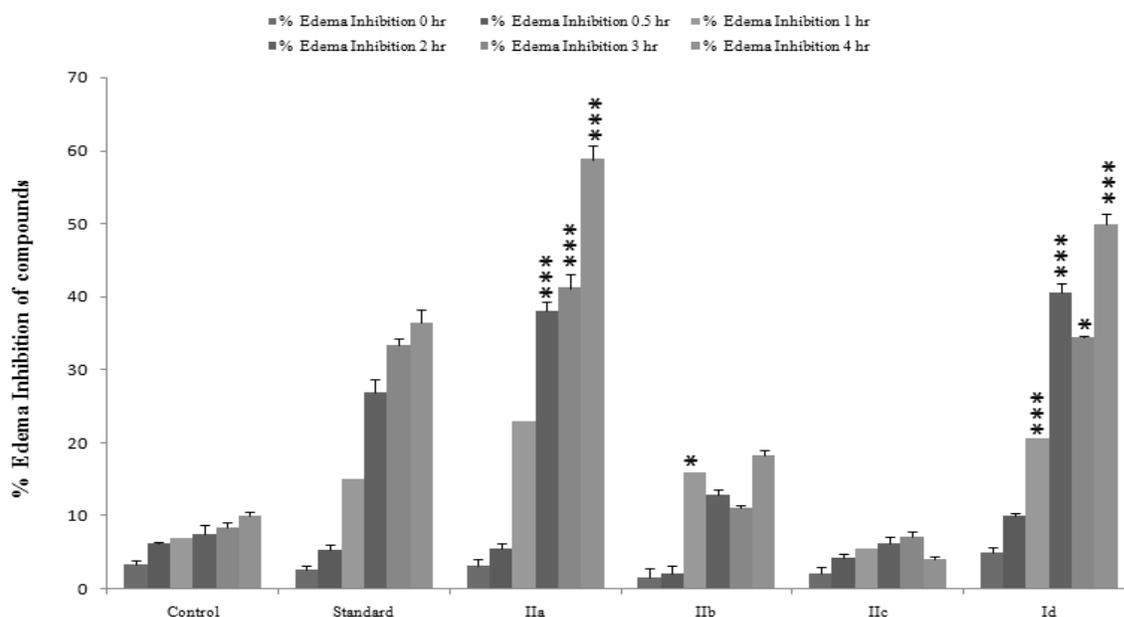


Figure 1: Graph showing percentage edema inhibition of synthesized compounds (IIa-d). (n=8), *p<0.05, **p<0.01, *p<0.001 when compared with standard group (using student t-test).**

300 MHz, δ ppm): 6.93-7.66 (m, 8H, Ar-H), 7.85 (s, 1H, ethylene), 8.04 (s, 1H, ethylene), 5.2 (s, 1H, OH); EIMS-m/z: 269.07 (M⁺).

2.1.2.2 Synthesis of 3-(4-fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (Ib).

It was obtained from stirring compound (A) and p-fluoro benzaldehyde in ethanol and then sodium hydroxide solution was added. IR(KBr):- 3585.42 cm⁻¹ (OH str.), 1512.09 cm⁻¹(C=C str.), 1693.38 cm⁻¹ (>C=O str.), 740.61 cm⁻¹ (Aromatic region monosubstituted); ¹HNMR (EtOD, 300 MHz, δ ppm): 6.92-7.64 (m, 8H, Ar-H), 7.56 (s, 1H, ethylene), 7.90 (s, 1H, ethylene), 5.2 (s, 1H, OH); EIMS-m/z: 242.07(M⁺).

2.1.2.3 Synthesis of 1-(2-hydroxyphenyl)-3-(3-nitrophenyl) prop-2-en-1-one (Ic):

It was obtained from stirring compound (A) and m-nitro benzaldehyde in ethanol and then sodium hydroxide solution was added. IR(KBr):- 3635.57 cm⁻¹ (OH str.), 1529.45 cm⁻¹(C=C str.), 1693.38 cm⁻¹ (>C=O str.), 1352.01 cm⁻¹(-O-N=O str.), 742.54 cm⁻¹ (Aromatic region monosubstituted); ¹HNMR (EtOD,

EIMS-m/z: 269.07(M⁺).

2.1.2.4 Synthesis of 3-(2, 6-dichlorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one (Id):

It was obtained from stirring compound (A) and 2, 6-dichloro benzaldehyde in ethanol and then sodium hydroxide solution was added. IR (KBr):- 3566.14 cm⁻¹ (OH str.), 1558.38 cm⁻¹(C=C str.), 1645.17 cm⁻¹ (>C=O str.), 775.30 cm⁻¹ (Aromatic region disubstituted). ¹HNMR (EtOD, 300 MHz, δ ppm): 6.92-7.64 (m, 7H, Ar-H), 7.38 (s, 1H, ethylene), 8.17 (s, 1H, ethylene), 5.2 (s, 1H, OH); EIMS-m/z: 294.01(M⁺).

2.1.3 Synthesis of 3-hydroxy-2-(substituted phenyl)-4H-chromen-4-one compounds (IIa-i): General procedure

Substituted compound (Ia-i) (0.01mole) was stirred continuously with ethyl alcohol (15ml), sodium hydroxide solution (5ml, 1.25N) and 5ml solution of hydrogen peroxide (30%) for 2-3 hours at room temperature. Completion of mixture was monitored by TLC. It was then diluted with ice cold water and acidified with dil. HCl. The precipitate

obtained was washed with ethanol and recrystallized with appropriate solvent.

2.1.3.1 Synthesis of 3-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one (IIa):

It was obtained from stirring of compound (Ia) with ethyl alcohol, sodium hydroxide solution and hydrogen peroxide. IR(KBr):- 3446.56 cm^{-1} (OH str.), 1515.94 cm^{-1} (C=C str.), 1699.17 cm^{-1} (>C=O str.), 1108.99 cm^{-1} (C-O-C str.), 1558.38 cm^{-1} (-O-N=O str.), 754.12 cm^{-1} (Aromatic region monosubstituted); ^1H NMR (EtOD, 300 MHz, δ ppm): 6.92-8.14 (m, 8H, Ar-H), 15.0 (s, 1H, OH); EIMS-m/z: 283.05(M⁺).

2.1.3.2 Synthesis of 2-(4-fluorophenyl)-3-hydroxy-4H-chromen-4-one (IIb):

It was obtained from stirring of compound (Ib) with ethyl alcohol, sodium hydroxide solution and hydrogen peroxide. IR(KBr):- 3523.70 cm^{-1} (OH str.),

2.1.3.4 Synthesis of 2-(2, 6-dichlorophenyl)-3-hydroxy-4H-chromen-4-one (IIc):

It was obtained from stirring of compound (Ic) with ethyl alcohol, sodium hydroxide solution and hydrogen peroxide. IR(KBr):- 3529.49 cm^{-1} (OH str.), 1560.30 cm^{-1} (C=C str.), 1735.81 cm^{-1} (>C=O str.), 1137.92 cm^{-1} (C-O-C str.), 779.19 cm^{-1} (Aromatic region disubstituted); ^1H NMR (EtOD, 300 MHz, δ ppm): 6.92-7.64 (m, 7H, Ar-H), 15.0 (s, 1H, OH); EIMS-m/z: 305.99(M⁺).

2.2 Pharmacological screening

2.2.1 Antioxidant Activity

Antioxidant activity of synthesized compounds was determined on the basis of free radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) [21]. The stock solutions of synthesized compounds were prepared (100 $\mu\text{g}/\text{ml}$)

Table 1: ANTIOXIDANT ACTIVITY OF SYNTHESIZED (IIa-d) COMPOUNDS

Compounds	% Scavenging of test compounds				
	10 $\mu\text{g}/\text{ml}$	20 $\mu\text{g}/\text{ml}$	30 $\mu\text{g}/\text{ml}$	40 $\mu\text{g}/\text{ml}$	50 $\mu\text{g}/\text{ml}$
Standard	70.75 \pm 1.32	73.00 \pm 2.32	80.50 \pm 3.21	88.00 \pm 1.12	90.50 \pm 0.77
IIa	73.25 \pm 2.13***	30.25 \pm 0.76	47.25 \pm 0.63	39.00 \pm 0.67	52.01 \pm 1.24**
IIb	12.35 \pm 0.34	42.34 \pm 0.84	42.60 \pm 0.76	54.00 \pm 0.71	36.83 \pm 0.56
IIc	53.50 \pm 1.22*	64.76 \pm 1.27**	66.70 \pm 1.24**	65.25 \pm 1.33**	68.50 \pm 1.44**
IIc	75.20 \pm 2.71***	79.75 \pm 1.89***	70.10 \pm 2.21	82.25 \pm 2.20***	83.50 \pm 2.28***

Values are expressed as Mean \pm S.E.M. n=4, *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001 when compared with standard group (using student t-test.)

1512.09 cm^{-1} (C=C str.), 1693.38 cm^{-1} (>C=O str.), 1147.57 cm^{-1} (C-O-C str.), 748.33 cm^{-1} (Aromatic region monosubstituted); ^1H NMR (EtOD, 300 MHz, δ ppm): 6.92-7.64 (m, 8H, Ar-H), 15.0 (s, 1H, OH); EIMS-m/z: 256.05(M⁺).

2.1.3.3 Synthesis of 3-hydroxy-2-(3-nitrophenyl)-4H-chromen-4-one (IIc):

It was obtained from stirring of compound (Ic) with ethyl alcohol, sodium hydroxide solution and hydrogen peroxide. IR(KBr):- 3564.21 cm^{-1} (OH str.), 1602.74 cm^{-1} (C=C str.), 1693.38 cm^{-1} (>C=O str.), 1116.71 cm^{-1} (C-O-C str.), 1529.45 cm^{-1} (-O-N=O str.), 742.54 cm^{-1} (Aromatic region monosubstituted); ^1H NMR (EtOD, 300 MHz, δ ppm): 6.92-8.23 (m, 8H, Ar-H), 15.0 (s, 1H, OH); EIMS-m/z: 283.05(M⁺).

and diluted to various concentrations of 10, 20, 30, 40 and 50 $\mu\text{g}/\text{ml}$ in methanol. Further, 1ml of DPPH solution was mixed with 1ml of different concentration of test and standard (ascorbic acid). This solution was kept for 30 min in dark and optical density was measured at 517 nm using methanol (1ml) with DPPH solution (0.002%, 1ml) as blank. The optical density was recorded and % inhibition was calculated by the following formulae:

$$\% \text{ Inhibition} = \frac{[(\text{Abs of control} - \text{Abs of test}) / \text{Abs. of blank}] \times 100}{100}$$

2.2.2 Animals

Albino-wistar rats weighing (150-200 gm) were used for studying in-vivo anti-inflammatory and ulcerogenic activity. Animals were maintained under standard laboratory conditions (24 \pm 2 $^{\circ}\text{C}$; relative

humidity 60-70%). Study protocol was approved by the institutional Animals Ethics Committee for the purpose of control and supervision on Experiments on Animals (IAEC, approval No. 711/02/a/CPCSEA) before experiment. Albino-wistar rats from laboratory Animals House section, Department of pharmaceutical Technology, Meerut Institute of Engineering & Technology, Meerut were used in the study. The Animals were kept in polypropylene cages and provides normal pellet diet (NPD) and water ad libitum.. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and in accordance with the Local Animals care and use committee.

2.2.3 Anti-inflammatory activity

All the synthesized compounds were screened for their pharmacological activities that included in-vivo anti-inflammatory activities. After the administration of carrageenan injection in rats the effect of standard and test compounds was

measured at the interval of 0, 0.5, 1, 2, 3 and 4 hr [21,22].

$$\% \text{Inhibition of edema} = \frac{\text{Mean edema of control group} - \text{Mean edema of treated group}}{\text{Mean edema of control group}} \times 100$$

2.2.4 Ulcerogenic Activity

All the synthesized compounds will be screened for their ulcerogenic activity. For screening the ulcerogenic activity all the rats were divided in six rats, housed in individual cages and fasted for 24 h before administration of the test drugs or aspirin. The animals were euthanited after 12 h of drug treatment. The stomach was opened along the greater curvature. The contents of the stomach was washed and cleaned with the saline. Then with the help of a 4x binocular magnifier gastric mucosa of the rats was examined and the number of ulcer spots will be examined [23].

2.2.5 Statistical analysis

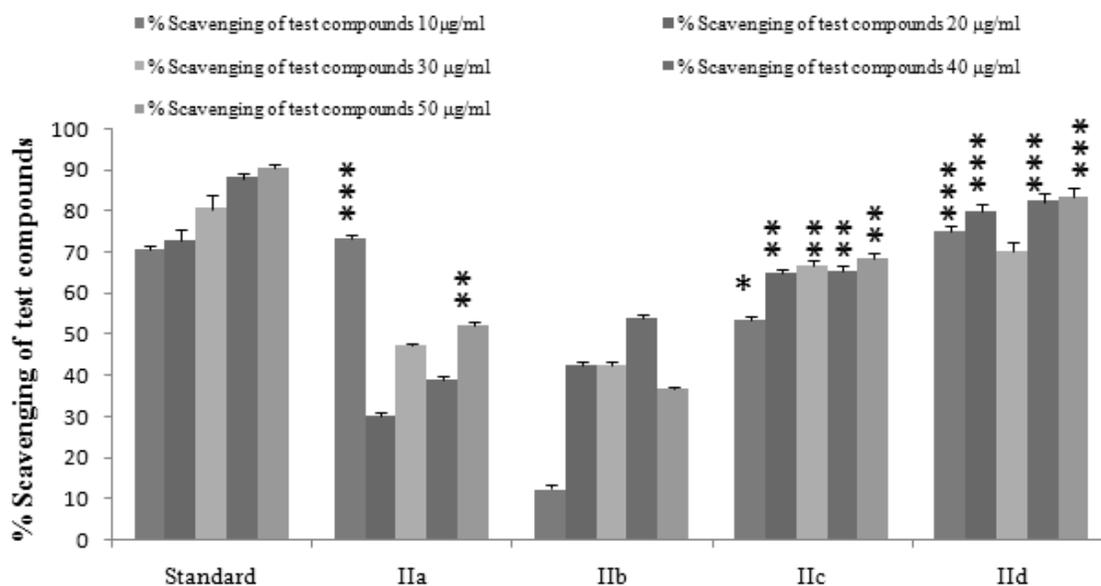


Figure 2: Graph showing percentage free radical scavenging activity of synthesized compounds (IIa-d). n=4, *p≤0.05, **p≤0.01, ***p≤0.001 when compared with standard group (using student t-test).

Results were shown as Mean±standard error of mean (S.E.M.) for each group. Statistical analysis was

solution and hydrogen peroxide for 2-3 hours at room temperature which gave 3-hydroxy-2-

Table 2: PHYSICAL DATA OF THE SUBSTITUTED CHALCONES (Ia-d)

Compound Code	R	Molecular Formula	Mol. Wt.	M.P. (°C)	Yield (%)	R _f
Ia	4-NO ₂	C ₁₅ H ₁₁ NO ₄	269.25	104-106	57.14	0.89
Ib	4-F	C ₁₅ H ₁₁ FO ₂	242.25	100-102	34.36	0.69
Ic	3-NO ₂	C ₁₅ H ₁₁ NO ₄	269.25	116-118	20.27	0.93
Id	2,6-di Cl	C ₁₅ H ₁₀ Cl ₂ O ₂	293.14	68-70	70.40	0.80

performed using Jandel Sigma Stat (Version 2.03) statistical software. Significance of difference between two groups was evaluated using Student's t-test. For multiple comparisons, One-way analysis of variance (ANOVA) was used. In case ANOVA showed

(substituted phenyl)-4H-chromen-4-one (**IIa-d**). The physical data of these synthesized compounds are summarized in table 1 and table 2.

3.2 Pharmacological screening

Table 3: PHYSICAL DATA OF THE 3-HYDROXY-2-(SUBSTITUTED PHENYL)-4H-CHROMEN-4-ONE (IIa-d)

Compound Code	R	Molecular Formula	Mol. Wt.	M.P. (°C)	Yield (%)	R _f
IIa	4-NO ₂	C ₁₅ H ₉ NO ₅	283.24	158-160	23.60	0.43
IIb	4-F	C ₁₅ H ₉ FO ₃	256.23	92-94	65.08	0.48
IIc	3-NO ₂	C ₁₅ H ₉ NO ₅	283.24	126-128	69.80	0.59
IId	2,6-di Cl	C ₁₅ H ₈ Cl ₂ O ₃	307.13	176-178	51.11	0.50

significant differences, post hoc analysis was performed with Tukey's test.

RESULT

3.1 Chemistry

For the synthesis of targeted compounds, the reaction sequence outline in scheme 1, were followed. A mixture of o-hydroxy acetophenone and substituted aromatic aldehyde was stirred in ethanol and then sodium hydroxide solution was added which gave a highly turbid solution. The mixture was kept overnight at room temperature and it was poured on crush ice and acidified with dilute hydrochloric acid yielded substituted chalcone (**Ia-d**). Then, this newly synthesized compounds was stirred continuously with ethyl alcohol, sodium hydroxide

3.2.1 Antioxidant Activity

The *in vitro* antioxidant activity was performed by using DPPH free radical scavenging method. Among the synthesized compounds, compound **IIa**, **IIc** and **IId** showed most significant free radical scavenging activity (Table 3 and figure 1).

3.2.2 Anti-inflammatory activity

The anti-inflammatory activity was performed by using carrageenan induced paw edema method (Table 4 and figure 2). Among the synthesized compounds, compound **IIa** and **IId** showed most significant anti-inflammatory activity.

3.2.3 Ulcerogenic Activity

The acute ulcerogenic activity was performed on albino-wistar rats using aspirin as standard drug and

activity, compound **Ila** and **Ild** showed significant activity as compared with the standard drug. Then,

Table 5: ANTI-INFLAMMATORY DATA OF SYNTHESIZED COMPOUNDS

Compound	% Edema Inhibition					
	0 hr	0.5 hr	1 hr	2 hr	3 hr	4 hr
Control	03.40±0.56	06.20±0.22	07.00±1.21	07.50±1.22	08.5±0.71	10.00±0.51
Standard	02.76±0.44	05.30±0.79	15.01±2.11	26.93±2.11	33.43±0.77	36.39±1.88
lia	03.30±0.89	05.60±0.65	22.98±1.31	38.00±1.31***	41.20±1.84***	58.80±1.93***
lib	01.60±1.21	02.20±0.98	16.02±0.98*	12.87±0.98	11.20±0.22	18.30±0.77
lic	02.10±0.89	04.30±0.54	05.5±0.49	06.20±0.49	07.20±0.76	04.20±0.33
ld	05.00±0.67	09.98±0.42	20.70±0.65***	40.60±0.65***	34.40±0.33*	49.90±1.43***

Values are expressed as Mean±S.E.M. (n=8), *p≤0.05, **p≤0.01, ***p≤0.001 when compared with standard group (using student t-test.)

only compound **Ild** is found to be ulcerogenic activity (Table 5).

Table 4: ULCEROGENIC RESPONSE OF THE VARIOUS GROUPS OF COMPOUNDS TESTED ON THE STOMACH OF RATS.

S.No.	Groups	No. of ulcer spots
1.	Control	3.00 ± 0.89
2.	lia	2.00 ± 0.67
3.	lid	11.10 ± 0.76**

Values are expressed as Mean±S.E.M. (n=8), *p≤0.05, **p≤0.01, ***p≤0.001 when compared with standard group (using student t-test.)

DISCUSSION

A novel series of 3-hydroxy-2-(substituted phenyl)-4H-chromen-4-one derivatives (**Ila-d**) were synthesized and characterized. The overall results indicated that the tested compounds showed promising antioxidant and anti-inflammatory activity. Among the synthesized compounds, compound **Ila**, **Ilc** and **Ild** showed significant antioxidant activity having substitution 4-nitro, 3-nitro and 2, 6-dichloro respectively on the phenyl ring of the flavonol nucleus. Further, all the test compounds evaluated for their anti-inflammatory

these compounds were screened for their ulcerogenic activity and the compound **Ild** was found to be ulcerogenic in nature. Compound **Ila** and **Ild** did not show any ulcer which shows that compound **Ila** showed significant anti-inflammatory activity with no duodenal ulcer. Thus, it may be possible that in the upcoming future this synthesized compound may become potent anti-inflammatory agent with lesser side-effects. So, it warrants further attention for complete elucidation of this compound for its pharmacological activity.

ACKNOWLEDGEMENT

I am extremely thankful to MIET, Meerut for providing all facilities and encouragement for successful completion of this work.

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