

Synthesis and Anti-inflammatory Activity of 7-Non-steroidal Anti-inflammatory Drugs Substituted Flavone

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ABSTRACT

Aim: Inflammation shows an important character in illnesses such for example cardiovascular diseases, asthma, malignant and diabetes. Food can guidance different phases of inflammation and can have an important influence on numerous inflammatory illnesses. Increasing scientific suggestion has displayed that polyphenolic compounds, such as flavonoids, which are originate in vegetables, fruits, cocoa or legumes, can have anti-inflammatory activity. Current researches prevent enzymes or transcription factors significant for regulatory intermediaries involved in inflammation. Flavonoids are also famous as effective antioxidants with the potential to weaken tissue injury or fibrosis. Subsequently, frequent studies *in vivo* and *in vitro* models shows that flavonoids have the prospective to prevent the beginning and development of inflammatory activity. **Materials and Methods:** In the present research work, a series of flavones merge with Non-steroidal Anti-Inflammatory Drugs were synthesized from resorcinol through Baker-Venkataraman Method, characterized and calculated there

in vitro anti-inflammatory activity by protein denaturation technique.

Results: The outcome is protein denaturation studies shown that the compound FS found to be most potent anti-inflammatory activity. The percentage inhibitions were 97.66%.

Key words: Albumin Denaturation, Baker Venkataraman transformation, Cyclooxygenase, Flavone, NSAIDS.

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INTRODUCTION

Inflammation is difficult in collective number of illnesses demanding the improvement of novel, effective and harmless managements of the patient. Non-steroidal anti-inflammatory medications need helpful in several instances; Non-steroidal anti-inflammatory drugs (NSAIDs) are the agents which cause their therapeutic effects by inhibiting prostaglandins synthesis. Prostaglandins are involved in the development of pain and inflammation as well as regulation of body temperature. However they only prevent cyclooxygenase, but not show any effect in cytokines. As a replacement for, several natural flavonoids have numerous anti-inflammatory properties, together with cyclooxygenase (COX) inhibition and a considerable harmless profile. Also much research increasing evidence shows that inflammation plays a life-threatening character in the pathogenesis of several illnesses that further more in volve mast cells. Therefore, the essential for novel, active and innocuous anti-inflammatory medications are more urgent.

Inflammation is well-thought-out as a principal physiologic protection mechanism that supports body to defend themselves against infection, toxic chemicals, burn, allergens or other harmful inducements.¹ Although, it is a defence mechanism, the difficult events and intermediaries involved the inflammatory response can maintain, induce or aggravate numerous illnesses.² Presently used anti-inflammatory medications are related through certain severe adverse effects. Consequently, the improvement of effective anti-inflammatory medications with less adverse effects is necessary. Flavonoids are a enormous type of naturally available polyphenolic compounds, extensively existing in fruits, vegetables and beverages derived from plants. These flavonoids are structurally separated

into subdivisions of flavones, flavones, dihydroflavonols, flavonols and chalcones.^{3,4} Various research recommended flavones might be beneficial for the prevention of a various type of ailments.

Based on this view a series of flavones merge with Non-steroidal Anti-Inflammatory Drugs. Flavones shows continuing attention for the reason of their wide-ranging pharmacological activities namely anti-inflammatory, anticancer, antifungal and antiviral, antioxidant, anti-osteoporotic effects cardiovascular, hepatoprotective, anti-allergic and antithrombotic effects.

With this evidence the aim of our research is too synthesized from resorcinol via Baker-Venkataraman transformation⁵ some newer flavones by merging with Non-Steroidal Anti-Inflammatory Drugs by esterification on 7th position of 7-Hydroxy flavones and also its *in-vitro* evaluation of Anti-inflammatory effect by Protein Denaturation Method.⁶ Flavones merged with NSAIDs may show more therapeutic activity by various mechanisms as compared with NSAIDs alone. So, newly synthesized compounds with effective Anti-inflammatory activity can be used for further studies. The synthesised Novel compounds were evaluate *in vitro* anti-inflammatory activity by protein denaturation method.

MATERIALS AND METHODS

All the chemicals and reagents, and solvents utilize for this effort were obtained from Merck Chemicals Pvt Limited Bengaluru, India.

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Melting points were found out by the electro thermal melting point apparatus with open capillary tube. The finishing point of the synthesis and pureness of the synthesized derivatives were observed by Thin Layer Chromatography. Infrared (IR) spectra were confirmation for the derivative on JASCO 4100 FT-IR using Potassium Bromide pellet disc technique. NMR spectra were confirmation on a Bruker Advance spectrometer.

Synthesis of 7- NSAID Substituted Flavones

Step I: Synthesis of Resacetophenone from Resorcinol

About 16.5 g of anhydrous zinc chloride was dissolved in 15.8 ml of glacial acetic acid with aid of heat (about 120°C), the hot mixture was placed on sand bath temperature not exceeding 159°C and about 11 g of resorcinol was added. The flame was disconnected then the hot solution was allowable cooled to room temperature by placing on a soil bath without additional heating for a period of about 20 min. The cooled mixture was diluted through 25 ml of concentrated hydrochloric acid then 25 ml of water. The red (dark) mixture was positioned in an ice then cooled at 5-10°C. The precipitate was collected in addition to wash with 100 ml diluted hydrochloric acid for free from zinc salts. The finally collect Orange yellow product was a resacetophenone.⁷ Percentage Yield: 65%w/w, MP: 145-150°C

Step II: Preparation of 2-acetyl 5-hydroxy phenyl benzoate from resacetophenone

Place 11.4g of resacetophenone in a conical flask then add mixture of benzoyl chloride and pyridine (4:5) ratio agitation to mix the content (use magnetic stirrer) which developed slightly heat after 20 min transfer the reaction solution 360 ml of 1M hydrochloric acid containing beaker through continuous stirring after that add 150 g of cursed ice. Filter the product in the help of suction pump and this resulting precipitated rinse through 15 ml of cold methyl alcohol and 15 ml of water. The percentage Yield: 53%, MP: 110-115°C.

Step III: Preparation of 1-(2, 4-dihydroxyphenyl)-3-phenylpropane 1,3dione from 2-acetyl 5-hydroxy phenyl benzoate

About 5.4g of 2-acetyl 5-hydroxy phenyl benzoate dissolved in 18 ml of pyridine in flask then boil reaction mixture to 50°C. Add with mechanical stirring 1.7g of crushed potassium hydroxide continue to stir for 15 min than maintain the reaction mixture at room temperature and acidify it by adding 25 ml of 10% acetic acid slowly drop by drop with vigorous stirring resulting pale yellow precipitate obtained and filtered by suction pump. The percentage Yield: 25%w/w, MP: 140-145°C.

Step IV: preparation of 7-hydroxy flavones from 1-(2, 4-dihydroxy phenyl)-3-phenylpropane-1, 3-dione

Dissolve 2.7 g of 1-(2, 4-dihydroxyphenyl)-3-phenylpropane-1, 3-dione in 15 ml of glacial acetic acid in flask then add 0.6 ml of con. Sulphuric

acid with vigorous shaking than attached with reflux condenser with intermittent shaking for one hour. Transfer the hot solution 150 g cursed ice with stirring on about and allow melt the ice the rustling creamy light brown 7-hydroxy flavones filter off and separated, wash it with water.⁸ The percentage Yield: 75% w/w; MP: 153-158°C.

Step V: Esterification of 7-hydroxy flavones with Acid derivatives

Place 8.00 g of various marketed available NSAIDS (Mefenamic Acid, Salicylic Acid, Diclofenac Sodium, Aspirin.) and 25 ml (0.617mol) of 7-hydroxy flavones in 100 ml round bottom flask, then carefully transferred 3 ml of concentrated sulphuric acid, The Resulting Solution refluxed in water bath for 30 min after that Cooled the reaction mixture under running water until precipitate occurs and filtered the crude product and dried.⁹

In vitro Anti-inflammatory Activity by Protein Denaturation Method

The anti-inflammatory activity of synthesized compounds activities are was carried out by inhibition of protein (albumin) denaturation method which was carried out base on standard Procedure. The egg white was separated from the whole egg. The solvent control, reference, standard and test (each 1ml) were added to egg albumin (1ml, 1mM) in separate sample test tubes. Denaturation of protein was induced by keeping the reaction mixture at 70°C in water bath for 10 min. After cooling the supernatant, the turbidity was measured at 660nm. (UV Visible Spectrophotometer Shimadzu) The experiment was performed in triplicate. The percentage inhibition of protein (albumin) Denaturation were calculated by using the formula.¹⁰

The Percentage inhibition of protein (albumin) denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance Control} - \text{Absorbance Sample})}{\text{Absorbance control}} \times 100$$

Statistical Analysis

The outcomes are expressed in mean \pm standard error of the mean. ($n=6$) Statistical analysis was completed by one technique ANOVA, monitored by control. *P* vs. Dennett multiple comparisons test.

RESULTS

Various Ester derivatives of 7-Hydroxy flavones were prepared from various substituted Acid derivatives by esterification method. All the compounds obtained were if good yield ranging from 65-72%; the homogeneity of the synthesized compounds was observed by performing TLC by which R_f values were calculated. The solvent system used for all the compounds was Benzene: Pyridine: Ammonia (8:2:1). Compounds were found to be more lipophilic indicated by their log P values. Physicochemical data is given in Table 1 and spectral data are given in Table 2.

Table 1: Physicochemical Data of the Newly Synthesized Compounds.

S.No	Code	Structure (Ar)	Molecular formula	Molecular weight	Melting Point(°C)	Percentage Yield (%)	R_f value	Log P value
1	FA	Aspirin	$C_{24}H_{16}O_6$	400	412-415	68	0.75	4.14
2	FD	Diclofenac	$C_{20}H_{19}Cl_2NO_4$	516	589-593	65	0.78	7.08
3	FM	Mefenamic acid	$C_{30}H_{23}NO_4$	461	540-544	72	0.83	7.00
4	FS	Salicylic acid	$C_{11}H_{10}O_5$	358	445-449	70	0.89	4.17

*Solvent system; Ammonia: Pyridine: Benzene (1:2:8 Ratio)

Solubility: Benzene: TLC Spot Identification Method: UV Chamber

Table 2: Spectroscopic Data of the Newly Synthesized Compounds.

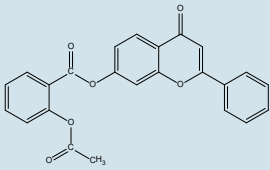
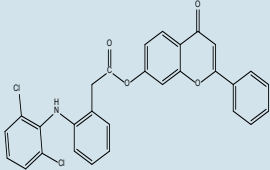
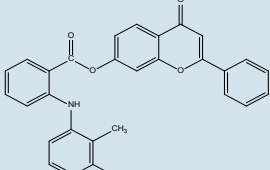
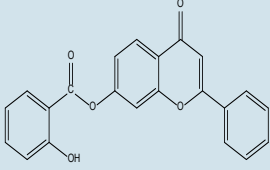
Comp. code	Molecular Structure Of The Synthesized Compounds	(IR) _{max} (KBr/ cm ⁻¹)	NMR (δppm)	MASS m/z
FA	 7-acetyl salicylate flavone	1499(C=Caryl)1737(C=O) 1055(C-O-C)1240(COO) 876(Di substituted aryl) 1021(-CH ₃)	7.63(CH,Ar),3.52(CH Ar) 2.08(CH ₃),8.13(C=O), 6.71(ethylene, 4.2(ArC-NH),6.85(C=O).	m/z: 401
FD	 7-(1-(2,6-dichloro anilino) phenyl) acetyl flavone	1505(C=C aryl)1739(C=O) 1132(C-O-C)1241(COO) 744(C-Cl)3336(N-H) 826(Tri substituted aryl)	7.61(CH,Ar),3.49(CHAR), 8.11(C=O)6.73(ethylene), 4.0(ArC-NH),6.93(C-Cl),6.34(C-N),6.82(C=O).	m/z: 515
FM	 7-N-(2,3,4,5-tetramethyl)anthranilate flavone	1499(C=Caryl),1737(C=O) 1079(C-O-C),1250(COO) 3344(N-H),1436(C-H) 841(Trisubstituted aryl)	7.26(CH,Ar),3.81(CHAR)2.11(CH ₃) ,8.17(C=O)6.78(ethylene),6.41(C-N),6.78(C=O),4.3(ArC-NH).	m/z: 461
FS	 7-(2-hydroxy benzoate) flavone	1499(C=C aryl)1737(C=O) 1065(C-O-C)3647(-OH) 1246(COO)876(Di substituted aryl)	7.66(CH,Ar),3.49(CHAR)2.35(CH ₃),8.12(C=O), 6.71(ethylene),6.82(C=O),5.0(ArC-OH)	m/z: 359

Table 3: In vitro anti-inflammatory activities of the synthesised ester derivatives of 7-hydroxy flavones by Protein Denaturation method.

Samples	Dose(µg/ml)	Absorbance at 660 nm	Percentage Inhibition
Solvent control	-	2.181±0.02	Nil
Diclofenac(Reference)	500	0.151±0.01**	93
FA	500	0.140±0.02**	93.58
FD	500	0.061±0.02**	97.20
FM	500	0.132±0.01**	93.94
FS	500	0.051±0.03**	97.66

Each value denotes the mean ± SD. n=3, Investigational collection were matched with control

**p<0.01, well-thought-out extremely significant.

DISCUSSION

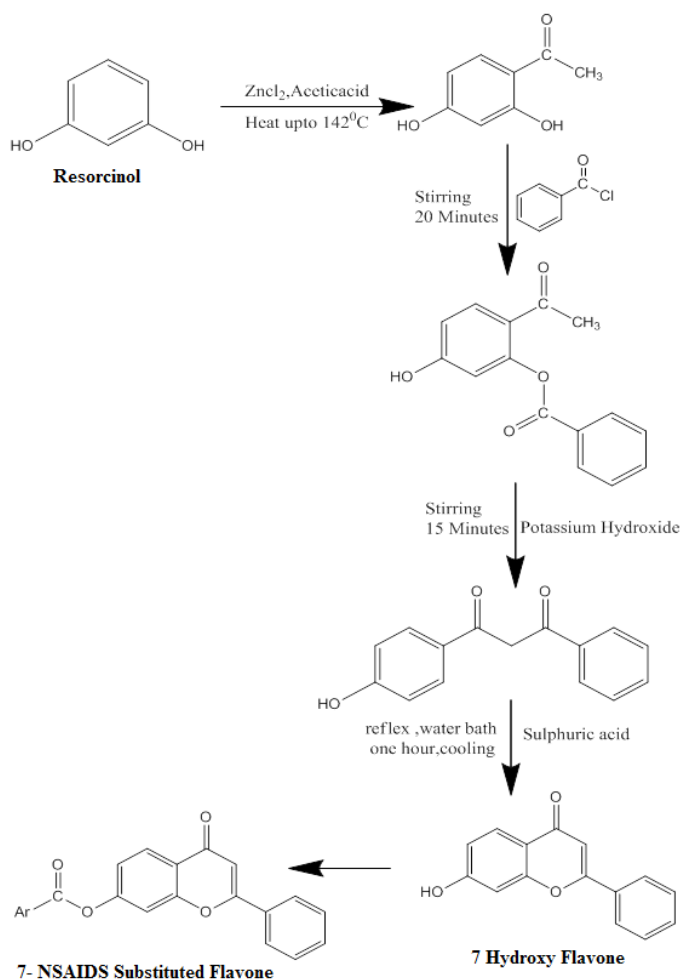
Chemistry

Various 7- NSAIDS Substituted Flavone was design and synthesized through the scheme 1. The structural arrangements of compounds were confirmed through by Infra-Red Spectroscopy, Nuclear Magnetic Resonance and Mass spectral data's. Resacetophenone was synthesized

in the help of resorcinol was added to the heating mixture of zinc chloride and glacial acetic acid and kept at 120°C, then temperature was raised 142°C for 20 min. The flavones were synthesized through resacetophenone by the one of the famous method of known as the Baker-Venkataraman transformation. 7 NSAIDS merged flavones were prepared from corresponding NSAIDS through shown in Scheme.

The resorcinol dissolved in the mixture of benzoyl chloride in pyridine afforded the 2-acetyl-5-hydroxy phenyl benzoate than was heated to 50°C with pyridine, to which added pulverized potassium hydroxide than stirred in the help of mechanical stirrer for 20 min at room temperature than resulting alkali solution was neutralized in the help of acetic acid to yield 1-(2, 4 dihydroxy phenyl)-3-phenyl propane-1, 3-dione was refluxed after addition of Concentrated Sulphuric acid and acetic acid for 90 min with infrequent stirring obtained 7- hydroxyl Flavones. Finally 7- hydroxy flavones was reacted with various NSAIDS in the medium of pyridine so that 7 hydroxy group was esterified (stirred at 0-5°C for 1-2hr) to corresponding NSAIDS Merged in the seventh position of flavone as a final compound.

Physical and spectral characterization data of 7-NSAIDS Substituted Flavone derivatives are shown in Table 1 and 2. Newly synthesized structures were assigned on the base of their spectroscopic analysis like IR, Proton NMR and Mass spectroscopy. The lead compound flavone presented the absorption group at 3126 cm⁻¹ owing to the being there of -OH at 7 location of flavonoid nucleus any where as in newly



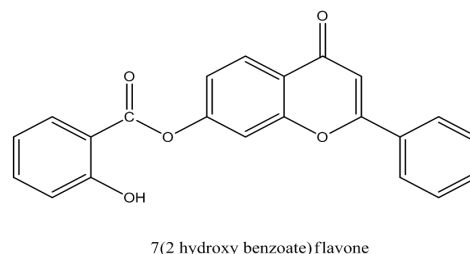
Scheme 1: Synthesis of 7 NSAIDS Substituted Flavones.

synthesized ester compounds that hydroxyl group absorption groups remained lacking in this area in its place the spectral bands were looked at 1749 cm^{-1} at $\text{C}=\text{O}$, this somewhat greater than common ester $\text{C}=\text{O}$ stretch 1740 cm^{-1} for the reason that of the phenyl group conjugation through alcoholic oxygen. The Spectral Peak at $1240\text{--}1250\text{ cm}^{-1}$ correspondingly specified the being there of $\text{C}-\text{O}$ stretch of $(\text{C}=\text{O})-\text{O}$. The Proton NMR spectrum, compounds FS displayed the wide-ranging singlet indications at 5.0 ppm , which be present Hydroxyl protons of phenolic group. The acquired molecular ion m/z principles of the concluding flavonoid derivatives were reliable through the molecular weight of the newly synthesized compounds and base peak owing to cleavage of $[\text{C}_8\text{H}_7]^+$ at $m/z=103$ and additional intense peaks at m/z 146 due to breakup after base peak by comparative intensity of 58%.

The synthesized NSAIDS merged flavone derivatives, were evaluated for anti-inflammatory activities by protein denaturation method. The out comes be situated in Table 3. All the compounds showed good anti-inflammatory activities compared with standard. SAR (Structure-Activity Relationship) investigation showed that synthesized derivatives behavior phenolic hydroxyl group at seventh position of flavone nucleus shows an significant character for the biological character of the compounds FS the percentage of inhibition 97.66 %.

CONCLUSION

The above research is a one of the strong evidence from the outcomes that anti-inflammatory activity of newly synthesized compounds accompanying by way of substituent by the seventh position of flavones ring. In upcoming there is wide ranging opportunity of alterations are promising to enhance the activity for flavone, structural modification and Structure-Activity Relationship research can lead to new effective and extremely active anti-inflammatory compounds.



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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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None

ABBREVIATIONS

TLC: Thin layer chromatography; **FT-IR:** Fourier transform infrared; **NMR:** Nuclear magnetic resonance; **TMS:** Tetramethylsilane; **UV-Vis:** Ultraviolet-Visible, **NSAID:** Non-steroidal Anti-Inflammatory Drugs.

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