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 as 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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DOI: 10.5530/jyp.2020.12.81

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 themself against infection, toxic chemicals, burn, allergens or other harmful inducements. Although, it is a defence mechanism, the difficult events and intermediaries involved the inflammatory response can maintain, induce or aggravate numerous illnesses. 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, flavonol, dihydroflavonol, flavonol and chalcones. 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 transformation 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. 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.
Melting points were found out by the electro thermal melting point apparatus with open capillary tube. The finishing point of the synthesis and purity 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 Resorcinal**

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 100°C and about 11 g of resorcinol was added. The flame was disconnected then the hot solution was allowed 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. 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-1,3dione**

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 solution150 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. The percentage Yield: 75% w/w: MP: 153-158°C

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

Place 8.00 gof 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.

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. 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 ± 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 homogeneousness of the synthesized compounds was observed by performing TLC by which Rf 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 logP 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.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Code</th>
<th>Structure (Ar)</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Melting Point(°C)</th>
<th>Percentage Yield (%)</th>
<th>Rf value</th>
<th>Log P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FA</td>
<td>Aspirin</td>
<td>C9H6O4</td>
<td>400</td>
<td>412-415</td>
<td>68</td>
<td>0.75</td>
<td>4.14</td>
</tr>
<tr>
<td>2</td>
<td>FD</td>
<td>Diclofenac</td>
<td>C14H8Cl2NO4</td>
<td>516</td>
<td>589-593</td>
<td>65</td>
<td>0.78</td>
<td>7.08</td>
</tr>
<tr>
<td>3</td>
<td>FM</td>
<td>Mefnamic acid</td>
<td>C18H13NO4</td>
<td>461</td>
<td>540-544</td>
<td>72</td>
<td>0.83</td>
<td>7.00</td>
</tr>
<tr>
<td>4</td>
<td>FS</td>
<td>Salicylic acid</td>
<td>C9H6O3</td>
<td>358</td>
<td>445-449</td>
<td>70</td>
<td>0.89</td>
<td>4.17</td>
</tr>
</tbody>
</table>

*Solvent system; Ammonia: Pyridine: Benzene (1:2:8 Ratio)  
Solubility: Benzene: TLC Spot Identification Method: UV Chamber
**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 reflexed 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 flavones a final compound.

Table 2: Spectroscopic Data of the Newly Synthesized Compounds.

<table>
<thead>
<tr>
<th>Comp. code</th>
<th>Molecular Structure Of The Synthesized Compounds</th>
<th>(IR)max(KBr/cm−1)</th>
<th>NMR (δppm)</th>
<th>MASS m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td><img src="image" alt="Molecular Structure of FA" /></td>
<td>1499(C=Caryl)1737(C=O) 1055(C-O-C)1240(COO) 876(Di substituted aryl) 1021(-CH3)</td>
<td>7.63(CH,Ar),3.52(CH Ar) 2.08(CH3),8.13(C=O),6.71(ethylene, 4.2(ArC-NH),6.85(C=O).</td>
<td>m/z: 401</td>
</tr>
<tr>
<td>FD</td>
<td><img src="image" alt="Molecular Structure of FD" /></td>
<td>1505(C=C aryl)1739(C=O) 1132(C-O-C)1241(COO) 744(C-Cl)3336(N-H) 826(Tri substituted aryl)</td>
<td>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).</td>
<td>m/z: 515</td>
</tr>
<tr>
<td>FM</td>
<td><img src="image" alt="Molecular Structure of FM" /></td>
<td>1499(C=Caryl),1737(C=O) 1079(C-O-C),1250(COO) 3344(N-H),1436(C-H) 841(Trisubstituted aryl)</td>
<td>7.26(CH,Ar),3.81(CHAr),2.11(CH3 ),8.17(C=O)6.78(ethylene),6.41(C-N),6.78(C=O),4.3(ArC-NH).</td>
<td>m/z: 461</td>
</tr>
<tr>
<td>FS</td>
<td><img src="image" alt="Molecular Structure of FS" /></td>
<td>1499(C=C aryl)1737(C=O) 1065(C-O-C)3647(-OH) 1246(COO)876(Di substituted aryl)</td>
<td>7.66(CH,Ar),3.49(CHAr)2.35(CH3 ),8.12(C=O),6.71(ethylene),6.82(C=O),5.0(Ar C-OH)</td>
<td>m/z: 359</td>
</tr>
</tbody>
</table>

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

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

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

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

ACKNOWLEDGEMENT

The authors are thankful to Management and Principal of Karpagam College of Pharmacy, Coimbatore 641032, India, for providing facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Funding Support

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.

REFERENCES


Article History: Submission Date : 08-10-2020; Revised Date : 02-11-2020; Acceptance Date : 21-11-2020