Research Article

Synthesis and human RBC membrane stabilization activity of substituted 3-benzoyl flavone

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Abstract

Objective: Flavonoids are type of polyphenolics biosynthesized by plants, several times proved to have anti-inflammatory activity. Several attempts have been made to synthesize flavonoids with desired substitution at desired place in order to get therapeutically better entity with reduced side effects. In present research attempt, substituted 3-benzoyl flavone **3** was synthesized via modified Baker-Venkatraman reaction. **Material and methods:** the chemical structure of the newly synthesized compound was confirmed by ^{1}H NMR, MS, IR spectral data and evaluated for its anti-inflammatory activity following Human RBC membrane stabilization model. **Results and conclusion:** On screening, it was found that, compound **3** has significant and dose-dependent HRBC membrane stabilization activity as compared to standard, diclofenac. Topological polar surface area (TPSA) of compound **3** indicated that it exerts the membrane stabilization potential after being entered into it.

Keywords: Substituted 3-benzoyl flavone, Baker-Venkatraman reaction; HRBC membrane stabilization activity, Three-dimensional study

Introduction

Flavonoids are polyphenols type of plant secondary metabolites with wide variety of pharmacological activities exhibited through their ability to influence several cellular pathways. So far, more than 5000 flavonoids have been identified several appear in fruits and vegetables. They contain elementary nucleus–flavan, with 15 carbon atoms arranged in a C_6–C_3–C_6 configuration exhibiting two aromatic rings (A and B) linked by a three carbon unit which may or may not form a third ring (C) (Markham, 1982). Flavonoids other than chalcones form γ-pyrone moiety in the form of C-ring. Rings (A and C) are chromane ring bearing a second aromatic ring B in position 2, 3 or 4 (phenylchromone). Based on different functional groups and their relative positions on C_6–C_3–C_6 carbon skeleton, flavonoids are classified into several subgroups such as flavone, flavonol, flavanone, isoflavonoid, chalcones and anthocyanidin. Flavonoids have repeatedly proven pharmacological activities like antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic and vasodilator effects. Considering these wide range of pharmacological potentials, several attempts were made to synthesize the flavonoid derivatives substituted with different functional groups at desired positions and subsequently evaluated for their respective biological activity. Approaches employed in synthesis of flavonoids, so far, include various name reactions like Robinson synthesis, von Kostanecki synthesis, Baker-Venkatraman reaction, Wheeler method, Heck reaction, Vilsmeier-Haack reaction, Spath reaction, Baker-Ollis synthesis, Algar-Flynn-Olyamada synthesis (Agrawal, 2007) where the starting compounds already have A and B ring of flavonoid derivative to be synthesize whereas reaction remains limited to rearrangement to form C ring (Zheng et al., 2007; Mediavilla et al., 2007).

Any harmful stimuli can be replied in biologically complicated way to remove it including swelling, redness, rise in temperature and pain, collectively termed as inflammation (Ferrero-Miliani et al., 2007). As inflammation onsets, the cells undergo activation and release inflammatory mediators like histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), prostaglandins

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and some lysosomal enzyme systems such as the complement system, the clotting system, the fibrinolytic system and the kinin system (Perianayagam et al., 2006) this can also be considered as initiate the healing process.

Inflammation is treated with non-steroidal anti-inflammatory drugs which either inhibit lysosomal enzymes or stabilize lysosomal membrane. Due to similarity of HRBC (human red blood cell) membrane with lysosomal membrane, HRBC membrane could be used to determine membrane stabilizing aspect of anti-inflammatory agents under test as a function of anti-inflammatory activity *in vitro* (Varadarasu et al., 2007). As several researchers reported membrane stabilizing activity of extracts rich in flavonoids (Chopade et al., 2012; Bag et al., 2013), the present research work was aimed towards synthesis of electron-donating group substituted 3-benzoyl flavone by modified Baker-Venkatraman reaction and screening for its *in-vitro* anti-inflammatory activity evaluated by human RBC membrane stabilization model.

Materials and Methods

Chemicals

2'-Hydroxy-5'-methyl acetohenone was purchased from Sigma-Aldrich, USA while *p-N, N*-dimethylamino benzoyl chloride was purchased from Alfa Aesar, UK. Melting points (uncorrected) were determined on a Stuart scientific melting point apparatus in open capillary tubes.^{1}H NMR spectrum was recorded on a 400 MHz spectrometer (BRUKER AVANCE II-400 NMR SPCTROMETER) using DMSO as solvent with TMS as the internal standard. Chemical shifts are expressed in δ units; Coupling constants (*J*) are given in Hertz (Hz). Mass spectrum (MS) was acquired by electron-spray ionization (ESI) technique with the aid of Liquid-Chromatography-Mass Spectrometer (WATERS ZQ-4000).

Synthesis of 3[4'-Dimethylamino] benzoyl-6-methyl-2[4'-dimethylamino] phenyl-2, 3-dihydro-4*H*-chromen-4-one, 3

About 0.03M solution of solution 2'-Hydroxy-5'-methyl acetophenone **1** was stirred in dry acetone, to which then anhydrous potassium carbonate (K_2CO_3) was added. After 10 min stirring, *p-N, N*-Dimethylamino benzoyl chloride **2** (0.09 M) was added to mixture and stirred for additional half an hour. Then, after refluxing for 24 h, dry acetone was evaporated under reduced pressure; residue was cooled to room temperature and slightly acidified with dilute hydrochloric acid. Precipitate was separated by filtration and obtained as yellow solid (**3**, yield 64%) : mp 96 – 100 °C; ^{1}H NMR (DMSO,400 MHz) δ ppm 2.163 (s, 3H), 3.172 (bs, 12H), 6.670 (d, J =12.0 Hz, 2H), 6.734 (d, J = 10.0 Hz, 2H), 7.096 (s, 2H), 7.389 (d, J = 4.44 Hz, 2H), 7.658 (d, J = 8.51 Hz, 1H), 7.873 (d, J = 4.23, 1H), 8.017 (d, J = 8.69 Hz, 1H); IR: 37390, 2810, 1760, 1602, 1482, 1366, 1182, 1062, 824, 764, 731, 693, 666 cm^{-1}; MS : m/z calcd for $C_{27}H_{26}N_2O_3$ ([M+H]+) 426.506; found 425.73 (15 %).

Screening of *In-vitro* anti-inflammatory activity

In-vitro anti-inflammatory activity of compound 3 was screened by human red blood cell (HRBC) membrane stabilization method as described by Gadamsetty et al. (2013). To ensure the activity of compound 3, blood was collected from healthy volunteers who have not administered any non-steroidal ant-inflammatory drug within last 15 days. The blood so collected was then mixed with equal volume of Alsever solution (which comprises of dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100mL) and centrifuged with isosaline solution. To 1mL of this HRBC

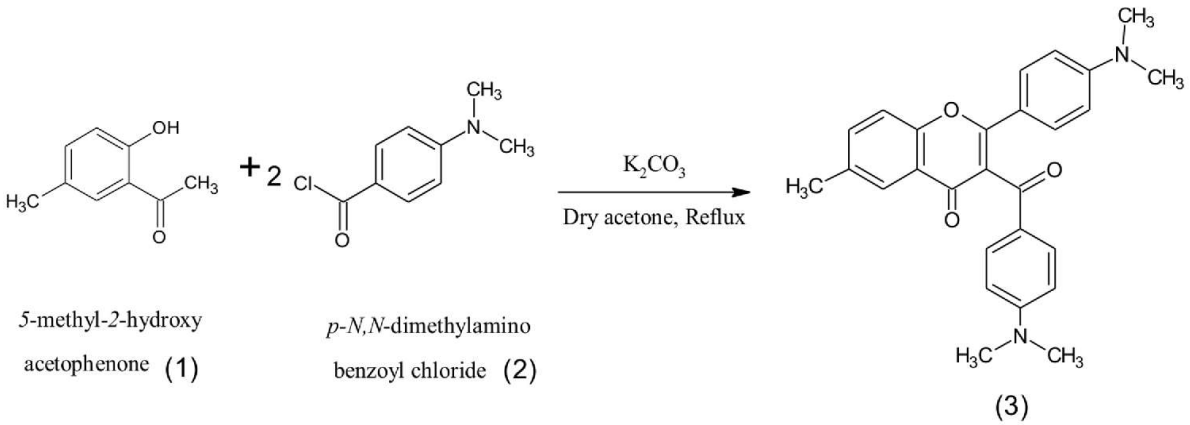

Figure 1. Synthesis of 3[4'-Dimethylamino] benzoyl-6-methyl-2[4'-dimethylamino] phenyl-2, 3-dihydro-4*H*-chromen-4-one

suspension, equal volume of test drug in three different concentrations, 100, 200, and 400µg/mL, was added; and these mixtures were incubated at 37°C for 30 minutes and centrifuged. Then, haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560nm against blank. Diclofenaac was used as standard in concentrations similar to that of test compound. The percentage of protection from haemolysis was calculated by the formula as given below:

% Protection from haemolysis = 100- ODt/ODc × 100

where, ODt and ODc were optical densities or absorbances of test and control solutions at 560 nm respectively.

Theoretical evaluation of Pharmacokinetic properties

According to 'Lipinski's Rule of Five' cell membrane permeability (by passive diffusion) is dependent upon molecular weight, log P, no. of hydrogen bond acceptors and no. of hydrogen bond donors. These parameters were determined using online Molinspiration property calculation programme.

Results and Discussion

Chemistry

In the present attempt, *p-N, N*-Dimethylamino substituted 3-benzoyl flavone **3** was synthesized by the modified Baker-Venkatraman reaction including condensation of 2'-Hydroxy-5'-methyl-acetophenone **1** with 2 molecules of *p-N, N*-Dimethylamino benzoyl chloride **3**. In alkaline medium of K_2CO_3, when 5-Methyl-2-hydroxyacetophenone **1** was treated with *p-N, N*-Dimethylamino benzoyl chloride **2**, addition of its first equivalent produces *O*-benzoyloxyacetophenone moiety which in the presence of a base, the enolate of the acetyl group was formed and attacks the carbonyl of the ester to give the hemiacetal moiety; which further undergo ring-opening to give the -diketone moiety, the Baker–Venkataraman rearrangement product. Its phenolic group went esterification with another equivalent of *p-N, N*-Dimethylamino benzoyl chloride **2**

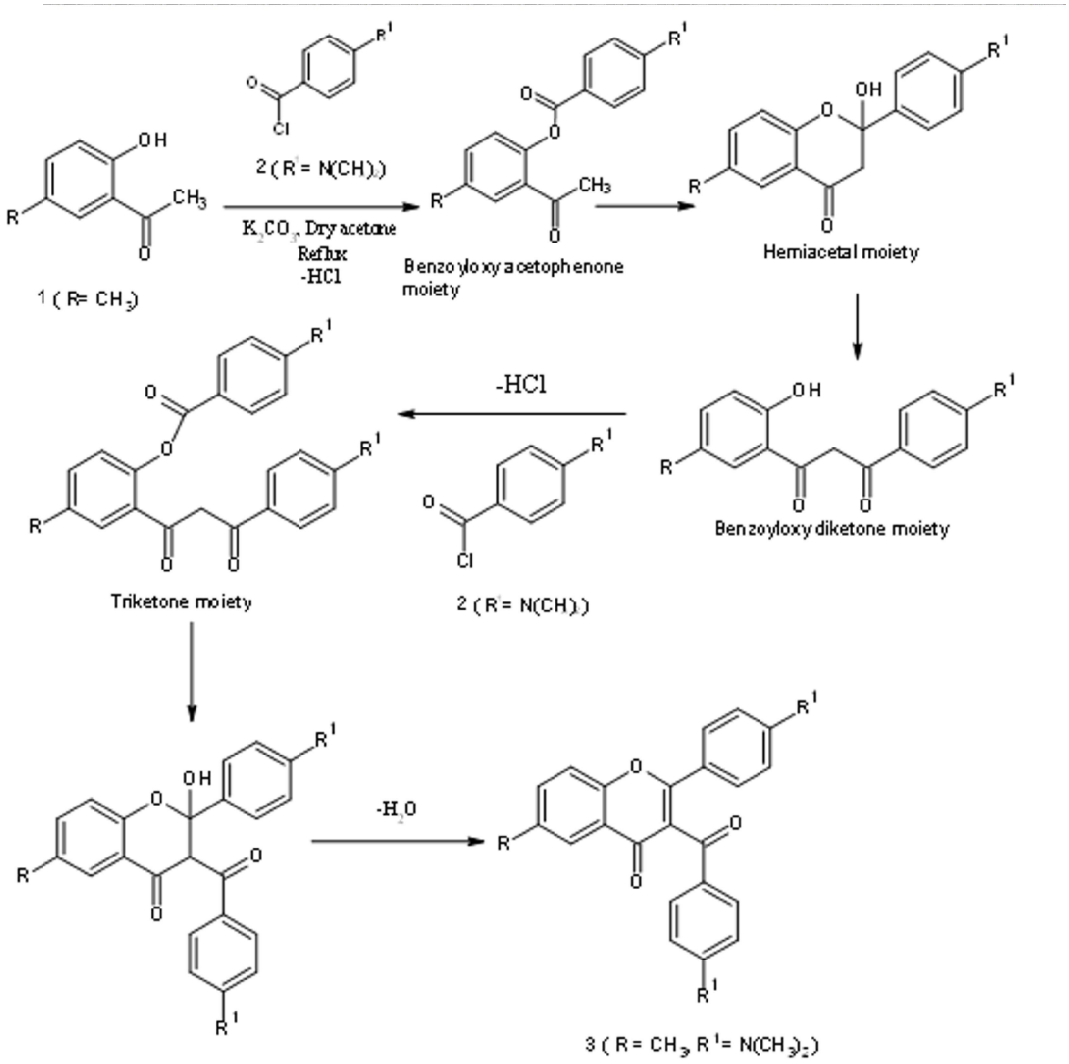

Figure 2. Synthesis of substituted 3- Benzoyl flavone

Table 1. HRBC membrane stabilization activity of compound 3

Sample	Concentration (µg/ml)	Absorbance (560nm)	Protection (%)
Control	-	0.115 ± 0.001	--
Diclofenac	100	0.098 ± 0.001*	16.96
	200	0.084 ± 0.001*	26.96
	400	0.057 ± 0.001*	50.43
Compound 3	100	0.101 ± 0.001*	12.17
	200	0.089 ± 0.001*	22.60
	400	0.068 ± 0.001*	40.86

Each value represents mean ± SEM; *: $P<0.001$ compared to control

forming benzoyloxydiketone intermediate, which further rearranged to get triketone intermediate which finally formed substituted 3-benzoyl flavone **3** by *in situ* cyclodehydration of hemiacetals. The proposed mechanism for synthesis of substituted 3-benzoylflavone is shown in Scheme 1.

***In-vitro* anti-inflammatory activity**

Compound 3 was found to have dose dependent *In-vitro* anti-inflammatory activity screened out using hypotonic solution induced lysis in human erythrocyte membrane (Table 1). At concentrations of 100, 200 and 400 µg/ml, compound 3 showed 12.17%, 22.60% and 40.86% protection against lysis of HRBC, respectively; which was again similar to standard diclofenac which had exhibited somewhat higher, 16.96%, 26.96% and 50.43% protection against HRBC lysis, tested in three concentrations similar to that of test compound 3. This in vitro method of evaluation of anti-inflammatory activity was found to be easy, flexible and time saving.

Theoretical evaluation of Pharmacokinetic properties

Molinspiration property calculation programme determined pharmacokinetic parameters to be considered in applying the Lipinski's Rule of Five to check cell membrane permeability (by passive diffusion) (Table 2).

Considering Lipinski's rule of five, it can be noted that rule is violated by only 1 parameter (Lipinski, 2000).In addition, Topological polar surface area (TPSA), described to be a predictive indicator of membrane penetration is also found to be positive for this compound (Ertl et al., 2000).

Table 2. Pharmacokinetic parameters

Parameters	Value
logP	5.74
Molecular Weight	426.50
nON (H-bond acceptors)	5
nOHON (H-bond donors)	0

Conclusion

Synthesized compound **3** was screened for *in-vitro* anti-inflammatory activity. Results obtained from screening were satisfactory as compared with corresponding standard Diclofenac used. Online determined Topological polar surface area (TPSA) was also found to be positive for compound **3**, indicating the penetration of compound across the membrane and exhibit membrane stabilization.

Collectively, these results show that flavonoid with electron donating substitution like Dimethylamino [-N $(CH_3)_2$] group has significant antioxidant activity. The strong antioxidant potential could allow this flavone derivative to be administered for prevention of numerous free radical based diseases.

In conclusion, this compound might be utilized for the development of novel anti-inflammatory leads which can be useful for the management of inflammatory diseases.

Conflict of Interest

We, authors of this research article declare no conflict of interest

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NMR spectral analysis of compound **3**, respectively.

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Graphical Abstract