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# DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NEW SUBSTITUTED 3-BENZOYL FLAVONE

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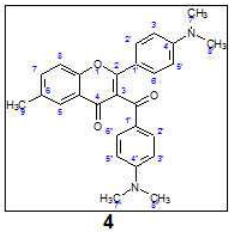
## ABSTRACT

Substituted 3- benzoyl flavone 4 was synthesized via modified Baker-Venkatraman reaction. The chemical structure of the newly synthesized compound was confirmed by $^1$H NMR, MS, IR spectral data. $LD_{50}$valueof synthesized compound was determined on Acute Oral toxicity studies carried out as per OECD guidelines-423 and were found to be 200 mg/ kg body weight. The compound was screened for Anti-inflammatory (Carrageenen-induced rat paw edema model) and Anti-oxidant activity (DPPH-radical scavenging model) and Brine Shrimp cyto-toxicity assay. On screening, it was found that 3-benzoyl flavone with electron donating substitutions (4) has significant Anti-oxidant potential ($IC_{50}$ 19.75µg/ml) and Anti-inflammatory activity (% reduction in edema 83.75± 5.30 %). Compound 4 also has highest cyto-toxicity on Brine Shrimp Nauplii ($IC_{50}$ 0.45 mg/ml). Also, screening of anti-cancer activity on 60 cell lines of 9 different types of cancers by NCI – NIH showed that compound 4 is active against renal cancer (cell line UO-31).

### *GRAPHICAL ABSTRACT*



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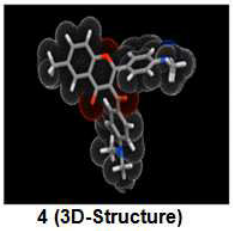

4 (3D-Structure)

**KEYWORDS: 3-benzoyl flavone, Baker-Venkatraman reaction, Brine-shrimp cyto-toxicity, anti-cancer activity**

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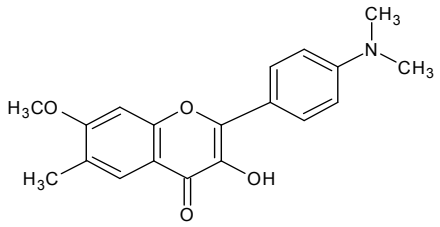
# INTRODUCTION

Flavones are the yellowish polyphenolic compounds belonging to class Flavonoids of plant secondary metabolites having 2-phenyl benzopyran functionality. These secondary metabolites are derived from amino acid Phenylalanine and exist as flavonoid glycosides in plants, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, red wine and fruit drinks). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, antitumor and antioxidant activities.[1]Of the many actions of flavonoids, antioxidant and antiproliferative effects stand out. This nutritious phenomenon has often been referred to as the 'French paradox'.[2]However, all these pharmacological properties of flavonoids depend on their antioxidant activity.[3-8] The role of free radicals is implied and established in various diseases such as aging, cancer, inflammation, rheumatoid arthritis and atherosclerosis. Antioxidants are gaining an importance as a panacea for these diseases. Flavonoids are the members of phenolic antioxidants. They are good free radical scavengers because they are highly reactive as hydrogen or electron donors.[9]In nature flavononids are present but in lesser quantity, thereby their processing cost increases. Hence, it is rational to synthesize flavonoids or their derivatives and thereby study their Quantitative Structure Activity Relationship (QSAR).Amino flavones are highly active molecules, wherein positions 5 and 7 are the most important and the most beneficial.[10] Flavonoids, bearing amino groups on the benzo- or pyranone ring have been reported to be potential antineoplastic agents [11-14]. It is now well established that such potency is mainly due to the ability of these aminoflavones to be competitive inhibitors of certain protein tyrosine kinases with respect to ATP.[15,16] *N,N*-dimethyl amino substituted flavonol**1** was also synthesized and on anti-microbial screening it was found that *N,N*-dimethyl amino substituted flavonol is a potent anti-microbial agent against Gram negative bacteria like *Shigella* spp. and *E.coli*and some Gram positive bacteria like *B.subtilis*and *S.aureus.*[17]

***Structure of 4'-N,N-Dimethylamino-3-hydroxy-6-methyl-7-methoxyflavonol (1)***




4'-*N,N*- Dimethylamino-3-hydroxy-6
-methyl-7-methoxy flavonol (**1**)

Flavonoids can be synthesized by any one of the methods - 1) Robinson synthesis,[18] 2) von Kostanecki synthesis,[18] 3) Baker-Venkatraman reaction,[18] 4) Wheeler method,[18]5) Heck reaction,[19] 6) Vilsmeier-Haack Reaction.[20] Traditionally, drug discovery processes were based on potency, regardless of the pharmacokinetic properties. As a result, the development of non-drug-like molecules was costly. In addition, these molecule would have high risk and low success rate. Recent attention has focused on early detection of ADME i.e. pharmacokinetic properties allowing medicinal chemists to consider these properties at the same time as they are optimizing potency.[21-23] The present research work was a successful attempt to synthesize 3-benzoyl flavone substituted with electron-donating substitutions, by following modified Baker-Venkatraman reaction. This

study also explored the feasibility and effectiveness of starting materials in synthesizing flavonoids. Synthesized compound was characterized by instrumental methods of analysis like IR spectroscopy, $^1$H NMR spectroscopy, Mass spectrometry. Finally, synthesized compound was screened for their pharmacological activities like Anti-oxidant and Anti-inflammatory activities. Cytotoxicity was determined by Brine Shrimp Cyto-toxicity Assay.

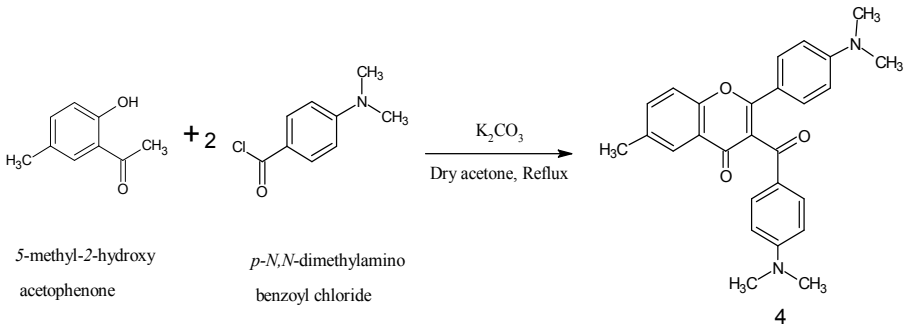
## MATERIALS AND METHODS

2'-Hydroxy-5'-methyl acetophenone was purchased from Sigma-Aldrich, USA while *p-N, N*-dimethylamino benzoyl chloride was purchased from Alfa Aesar**,** UK. Melting points (uncorrected) was determined on 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).

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

Anhydrous potassium carbonate ($K_2CO_3$) was added to a stirred solution 2'-Hydroxy-5'-methylacetophenone2 (0.03M) in dry acetone. The mixture was stirred independently at room temperature for 10 min and then *p-N,N*-Dimethylaminobenzoyl chloride**3** (0.09 M) was added drop wise and the mixture was stirred at room temperature for an additional half an hour. Reaction mixture was refluxed for 24 h. After refluxing for 24 h, the solvent was evaporated under reduced pressure. The residue was cooled to room temperature and acidified in a beaker with dilute hydrochloric acid to weak acidity. The precipitate formed was filtered off, dried affording 3[4'-Dimethylamino] benzoyl-6-methyl-2[4'-dimethylamino] phenyl-2,3-dihydro-4*H*-chromen-4-one, 4**,** as yellow solid (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$^{-1}$; LCMS : m/z calcd for $C_{27}H_{26}N_2O_3$([M+H]+) 426.506; found 425.73 (15 %).

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




*5*-methyl-*2*-hydroxy acetophenone

*p-N,N*-dimethylamino benzoyl chloride

4

### *2. Screening of Anti-oxidant activity*

The Anti-oxidant activity of compounds is screened by following DPPH-radical scavenging activity model. One and a half milliliter of 100 µM DPPH ethanol solution was mixed with 0.25 ml of the investigated compounds. After 10 min. of mixing, the absorbance at 517 nm against a blank sample (without Tested compound) was measured spectrophotometrically (Shimadzu UV-1800

UV-Vis spectrophotometer).[28] DPPH radical scavenging activity of the compound 4 was calculated according to the formula:[28]

$$\text{DPPH radical scavenging rate (\%)} = \frac{(ABS - ATS) \times 100}{ABS}$$

Where ABS = the absorbance of blank sample (t = 0min)
ATS = the absorbance of tested sample (t =10 min).

$IC_{50}$ was calculated from calibration graph extrapolation.

***3. Screening of Anti-inflammatory activity***

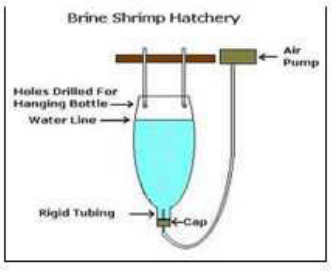
For evaluation of anti-inflammatory activity, it is necessary to determine its toxic level ($LD_{50}$ ).$LD_{50}$was calculated as per the OECD guidelines for Acute Oral Toxicity (OECD guidelines-423).[22] Then, anti-inflammatory activity was evaluated after the approval by Institutional Animal Ethics Committee (Registration No. 1153/PO/ac/08/CPCSEA).The animals are starved overnight. To insure uniform hydration, the rats receive 5 ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.1 ml of 1% solution of carrageenan into the plantar side of the left hind paw. Mice would be divided into 6 different groups. The paw is marked with ink at the level of the lateral malleolus and immersed in water up this mark. The paw volume is measured plethysmometrically immediately after injection, 1, 2, and 3 hrs after challenge.[29] Then, Percentage Reduction in Edema was determined using formula.[30]

$$\text{Percentage Reduction in Edema} = \frac{\text{Mean edema (Control)} - \text{Mean edema (Test)}}{\text{Mean edema (Control)}} \times 100$$

***4. Screening of Cyto-toxicity***

Compound 4was screened for Cyto-toxicity by Brine–Shrimp Cyto-toxicity Assay. Samples of three different concentrations 10, 100, 1000 µg/ml were prepared. Brine shrimp (*Artemia salina* Leach) nauplii were hatched in a specific tank as shown in Fig.1

**Figure 1**
***Brine Shrimp Hatchery***




Ten shrimps were transferred to each sample vial and then sea water was added to make the volume 5 ml. The vials were kept for 24 hours, thereafter the active nauplii were counted with the aid of a 3 x magnifying glass and the $IC_{50}$ values were determined graph extrapolation.[31]

### *5. Screening of Anti-cancer activity on human cell lines*

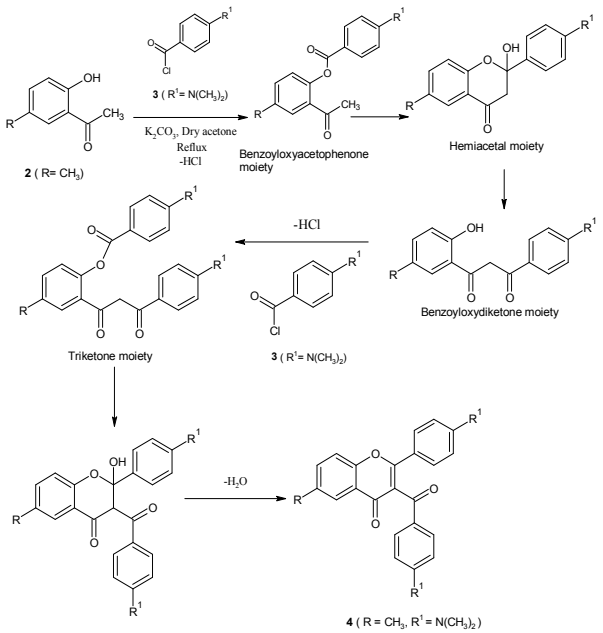
Structure of compound 4 was sent to National Cancer Institute (NCI) -National Institute of Health (NIH) online, under DTP anti-cancer drug discovery programme. National Cancer Institute (NCI) - National Institute of Health (NIH) selected it for screening of Anti-cancer activity on cell lines. Compound 4was then sent to Chemotherapeutic Agents Repository, Fisher Bio Services, 1592 Rockville Pike, Suite E, Rockville, MD 20852, USA, an institute affiliated to National Cancer Institute (NCI) - National Institute of Health (NIH) , USA.

## RESULTS AND DISCUSSION

### *1. Chemistry*

Substituted 3-benzoyl flavone 4was synthesized by condensation of 2'-Hydroxy-5'-methyl-acetophenone2 with 2 molecules of *p-N,N*-Dimethylaminobenzoyl chloride**3**, by the modified Baker-Venkatraman reaction. When 5-Methyl-2-hydroxyacetophenone2 was treated with *p-N,N*-Dimethylaminobenzoyl chloride3and $K_2CO_3$, addition of the first equivalent of *p-N,N*-Dimethylaminobenzoyl chloride3produces*O*-benzoyloxyacetophenone moiety, which in the presence of a base, the enolate of the acetyl group is formed and attacks the carbonyl of the ester to give the hemiacetal moiety, which undergo ring-opening to give the β-diketone moiety, the Baker–Venkataraman rearrangement product. In an excess of benzoyl chloride, the phenolic group of β-diketone moiety undergoes esterification with another equivalent of *p-N,N*-Dimethylaminobenzoyl chloride 3 to form benzoyloxydiketone moiety. Rearrangement of the benzoyloxydiketone moiety gives triketone intermediate which finally cyclodehydrates substituted 3-benzoyl flavone via *in situ* cyclodehydration of hemiacetals. The proposed mechanism for the formation of substituted 3-benzoylflavone is shown in Scheme 1.

***Scheme 1: Synthesis of substituted 3- benzoyl flavone***




### *2. Anti-oxidant Activity*

Table 1 illustrates a significant decrease of DPPH radical due to the scavenging ability of tested compound 4 which correlates with a dose dependence. Higher the concentrations of flavonoid, the

higher the percentage of DPPH radical scavenging rate. Compound 4 was found to have highest DPPH radical scavenging activity (87.79±0.490).

**Table 1**
***DPPH radical scavenging rate (%)of compound 9 and control at various concentration.***

| CONCENTRATION (µg/sample) | COMPOUND 4 | STANDARD |
|---|---|---|
| 200 | 87.79 ±0.490 | 93.00 ±0.176 |
| 100 | 86.06 ±0.385 | 93.05 ± 0.140 |
| 50 | 79.55 ±0.570 | 92.97 ± 0.055 |
| 25 | 71.25 ±0.051 | 92.25 ± 0.186 |

***Values are average of three separate experiments of three samples (mean ± SD)***

$IC_{50}$ values for synthesize compound 4 was determined and compared with standard i.e. Ascorbic acid (Table 2). While comparing with standard, it was observed that, 4 is most effective anti-oxidant, only19.75 µgof compound 4 is needed to scavenge 50% of ethanolic DPPH solution.

**Table 2**
***$IC_{50}$ values for anti-oxidant activity***

| COMPOUND | IC $_{50}$ (µg/ml) |
|---|---|
| Compound **4** | 19.75 |
| Ascorbic acid | 13.75 |

### *3. Anti-inflammatory Activity*

To determine in-vivo Anti-inflammatory potential of compound 4, it's toxic level ($LD_{50}$) was determined as per OECD guidelines-423.[24] $LD_{50}$ of tested compound 4 was found to be 200 mg/kg body weight. Then, role of test compound 4 on carrageenan induced acute inflammation model was evaluated at concentration 20 mg/kg body weight (10 % of $LD_{50}$ ).In carrageenan administered animals the severe swelling was reached at 1 hr. Compound 4 significantly reduced the inflammation after the third hour. Results (Table 3) showed that compound 4 has significant Percentage reduction (83.75 % ± 5.30)in Carrageenan-induced rat paw edema when compared with standard i.e. Diclofenac (86.81% ± 4.13).

**Table 3**
***% Reduction in edema for different compounds***

| GROUP CATEGORY (20 mg/kg) | AVERAGE RISE IN VOLUME AFTER- | | | % REDUCTION IN EDEMA |
|---|---|---|---|---|
| | 1Hr | 2 Hrs | 3 Hrs | |
| Compound **4** | 1.130 ± 0.31 | 0.408± 0.35 | 0.105 ± 0.03 | 83.75± 5.30 |
| Standard (Diclofenac) | 0.983± 0.04 | 0.553 ± 0.09 | 0.078± 0.03 | 86.81 ± 4.13 |
| Control (DMSO) | 1.298± 0.09 | 0.948 ± 0.08 | 0.645 ± 0.22 | - |

***Average rise in volume and % Reduction in edema values are expressed in Mean ± SD,***
**** (P<0.01) as compared with standard (Dunnett test).***

### *4. Brine Shrimp Cyto-toxicity Assay*

Results of Brine Shrimp cyto-toxicity of compound 4 are shown in Table 4. Number of live and dead nauplii was counted after 24 hrs of incubation of nauplii with different concentrations (0.01mg/ml, 0.1mg/ml, 1.0mg/ml) of synthesized compounds dissolved in DMSO. Results showed that compound 9has highest cyto-toxicity on Brine Shrimp Nauplii ($IC_{50}$ 0.45 mg/ml).

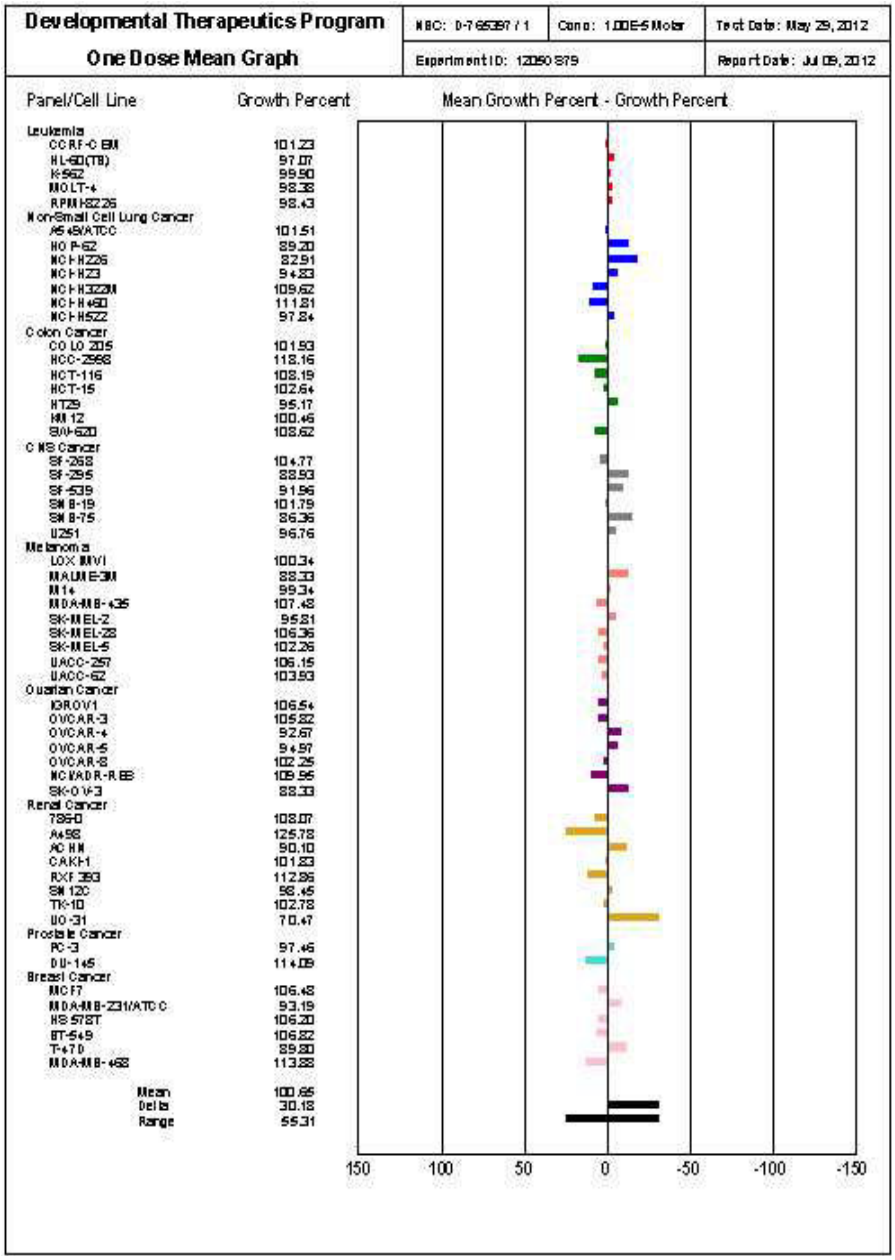
**Table 4**
***No. of dead and alive nauplii on treatment with compounds***

| COMPOUNDS | 0.01 mg | | 0.1 mg | | 1.0 mg | | $IC_{50}$ (mg/ml) |
|---|---|---|---|---|---|---|---|
| | ALIVE | DEAD | ALIVE | DEAD | ALIVE | DEAD | |
| DMSO | 10 | 0 | 10 | 0 | 10 | 0 | - |
| Compound **4** | 9 | 1 | 8 | 2 | 1 | 9 | 0.45 |

### *5. Anti-cancer Activity on Cell lines*

After structure based selection of compound 4 by National Cancer Institute (NCI) – National Institute Health (NIH), USA, on screening of Anti-cancer activity on cell-lines, it was found that compound 4 is active against renal cancer cell line (UO-31) with % growth inhibition of 29.47%. (One Dose Mean Graph of compound 4).

***One Dose Mean Graph of compound 4***



### *6. 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 Molinspiration property calculation programme (Table 5).[25]

**Table 5**
***Pharmacokinetic parameters***

| PARAMETER | VALUE |
|---|---|
| logP | 5.74 |
| Molecular Weight | 426.50 |
| nON (H-bond acceptors) | 5 |
| nOHON (H-bond donors) | 0 |

Lipinski deduced that only compounds with a molecular weight lower than 500, a logP less than 5, and less than 5 hydrogen bond donors and 10 hydrogen bond acceptors are likely to permeate efficiently across the cell membrane by passive diffusion. Also, highly lipophilic drugs may become sequestered in the cell, with little improvement in permeability - across the membrane.[26]Hence, from the data obtained, it is observed that, there is negligible violation of 'Lipinski's Rule of Five' by 1. 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.[27]

## CONCLUSION

Synthesized compound 4 was screened for pharmacological activities like Anti-oxidant activity, Anti-inflammatory activity and Cyto-toxicity on behalf of Anticancer activity. Results obtained from pharmacological screening are satisfactory as compared with corresponding standards used. Compound 4 has higher anti-oxidant potential when compared to Ascorbic acid. Compound 4has also shown highest anti-inflammatory activity. Coincidentally, 4 has significant Cyto-toxicity. 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 anticancer drug leads. Thus, the mechanism of selective cytotoxicity is needed to be discovered for further studies. Finally, it can be suggested that the future studies on flavonoids and their derivatives can be useful for the management of carcinogenic diseases.

## ACKNOWLEDGEMENTS

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