Heterocyclic Letters
Vol. 12| No.4|797-804|Aug-Oct|2022
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHESIS, MOLECULAR DOCKING, MOLECULAR PROPERTIES ESTIMATIONS AND ANTIINFLAMATORY ACTIVITY OF 5, 7-DIHYDROXY-3' PRENYL FLAVANONE

Badikela Rama Krishna[a], Rathnakar Ch[a], Durgaprasad Kemisetti[b], Vijayaraj Surendran[c] and Parameshwar Ravula[c,*]

[a]Department of Pharmaceutical Analysis & Quality Assurance, Guru Nanak Institutions Technical Campus-School of Pharmacy, Hyderabad, Telangana, India
[b]Faculty of Pharmaceutical Science, Assam Down Town University, Guwahati, Assam, India
[c]Department of Pharmaceutical Chemistry, Amity Institute of Pharmacy, Amity University, Gwalior, Madhya Pradesh, India
**Corresponding author Email: parmipharma@gmail.com*

ABSTRACT:
A novel compound 5, 7-dihydroxy-3'- prenyl flavanone was synthesized by introducing prenyl moiety at 3rd position of flavanone nucleus. The structures of synthesized compounds were confirmed by ^{1}H NMR and mass spectral data. The anti-inflammatory screening of the synthesized compound was performed by *in vivo* using carragenan induced paw oedema method. *In silico* prediction of molecular and drug-likeness properties of target compound was found to be promising. The results are obtained from anti-inflammatory activity and molecular docking suggested that test compound could be considered as drug candidate for anti-inflammatory activity.

KEY WORDS: Flavanone, Anti-inflammatory activity, Molecular docking, Synthesis.

INTRODUCTION:

Inflammation is the immune response of the body to irradiation, infection or foreign substances. It is defense mechanism of body in order to eliminate necrosed cells or limit the spread of injurious agent[i]. Non-steroidal anti-inflammatory drugs (NSAIDs) were identified as an essential class of biological agents that relieve pain and reduce inflammation[ii]. Many side effects such as gastrointestinal mucosal damage, bleeding, intolerance, renal toxicity and hepatotoxicity have been accompanied by long term use of NSAIDs[iii]. Thus, design and development of more selective novel anti-inflammatory compounds with reduced side effects is a major challenge to research groups.

Prenylated flavanone derivatives have attracted the attention of numerous researchers over many years due to their wide range of biological activities[iv]. An array of biological activities such antidiabetic[v], antibacterial[vi], antifungal[vii], antitumor[viii], anti-inflammatory agents[ix], and

antioxidant[x] has been reported to be shown by various flavones. It has been proved that these flavones compounds are effective as inhibitors of inflammatory mediators in intact cells, M. tuberculosis and human enterovirus[xi]. Besides, flavones derivatives also exhibited inhibitory activity towards tubulin polymerization, cyclin-dependent kinase and enzymatic assays on Src and Abl tyrosine kinases[xii]. Prompted by these claims and in continuing our research work on bioactive flavones, in the present study, a significant effort has been made to synthesize novel 5, 7-dihydroxy-3'- prenyl flavanone as per Scheme **1** and characterized by ^{1}H NMR and mass spectral data. The synthesized compound was tested for their *in vivo* anti-inflammatory activity by using carragenan induced paw oedema method[xiii].

Molecular docking studies play an essential role in the drug discovery by placing a ligand into the active site of the macromolecule[xiv]. In the present study, the synthesized compound was docked with the active site of the COX-2 enzyme (PDB ID: 3Q7D) with the help of Autodock software and subsequently to rationalize the achieved anti-inflammatory data. Furthermore, the target compound was subjected to Molinspiration Property and Molsoft tool kits[xv] to predictively calculate their molecular properties. Moreover, metric analysis was directed to predictably enumerate the lipophilicity (clogP), the water solubility (clogS), topological polar surface area (TPSA), number of rotatable bonds (NROTB), number of hydrogen bonds acceptors (NHBA) and number of hydrogen bond donators (NHBD) which are important for verification of drug-likeness of the novel compound.

EXPERIMENTAL SECTION:

All reagents were purchased from commercial sources. Melting points (m.p.) were uncorrected and determined in one end open capillary tubes using Analab melting point apparatus. The IR spectra were recorded on Shimadzu FTIR 8400 S spectrophotometer and expressed in wave numbers (cm^{-1}), using 1% potassium bromide disc. ^{1}H NMR spectra were recorded on Varian 400 MHz spectrometer and mass spectra were recorded on Agilent 6430 triple quadruple LC-MS system. TLC was performed using E.Merck 0.25 mm silica gel plates and visualization of spots was accomplished with UV light.

GENERAL PROCEDURE

SYNTHESIS OF 1-(2, 4, 6-TRIMETHOXYPHENYL) ETHANONE (2)

A solution of 1, 3, 5-trimethoxybenzene **1** (0.5 mol), acetic anhydride (5 ml) and borontrifluride diethyl etherate (3 ml) and dichloro methane as solvent (10 ml) taken in a round bottom flak and stirred for about 6 hr and then refluxed for 50 min at 70^{o}C. Further, the reaction mixture was cooled to room temperature and poured into ice cold water. The obtained compound was extracted with ethyl acetate, the combined organic solvent was washed with 10% sodium bicarbonate and dried over magnesium sulphate. The product was concentrated under reduced pressure and the reaction mixture was purified by flash column chromatography ($CHCl_3$–MeOH, 90:10) to afford 1-(2, 4, 6-trimethoxyphenyl) ethanone.

Yield 65 %, m.p 225°C. IR (cm^{-1}): 1696 (C=O). ^{1}HNMR (DMSO-d6, δ): 2.52 (s, 2H, -CH_3), 3.74-3.99 (m, 9H, 3-OCH3), 6.85-7.25 (m, 2H, Ar-H). MS (ESI): m/z 211 (M+1).

SYNTHESIS OF 2-(2-CHLOROPHENYL)-5,7-DIMETHOXYCHROMAN-4-ONE (3)

A solution of 1-(2,4,6-trimethoxyphenyl)ethanone **2** (0.01 mol) in dimethyl formamide (20 ml) was added 1-chloro-2-(hydroperoxymethyl)benzene (3 gm), Sodium carbonate (0.5 gm) and Sodium hydride (0.5 gm) in a three necked round bottom flask. Further, dry HCl gas was passed through it continuously for a period of 3 hr at 0 ^{o}C then the reaction mixture is cooled in a refrigerator for two days and then extracted with ether. The obtained product was concentrated under reduced pressure to afford crude compound.

Yield 75 %, m.p 185°C. IR (cm^{-1}): 1668 (C=O), 1616, 1458 (C=C aromatic) and 1154 (C–O).^{1}HNMR (DMSO-d6, δ): 2.74 (1H, dd, *J*=15.7 Hz and 2.5 Hz), 3.26 (1H, dd, *J*=15.2 Hz and 3.1 Hz), 3.84 (s, 6H, 2-OCH3), 5.42 (1H, dd, *J*=12.3 Hz and 3.2 Hz), 5.92-7.22 (m, 6H, Ar-H). MS (ESI): m/z 319 (M+1).

SYNTHESIS OF 2-(2-CHLOROPHENYL)-5,7-DIHYDROXYCHROMAN-4-ONE (4)

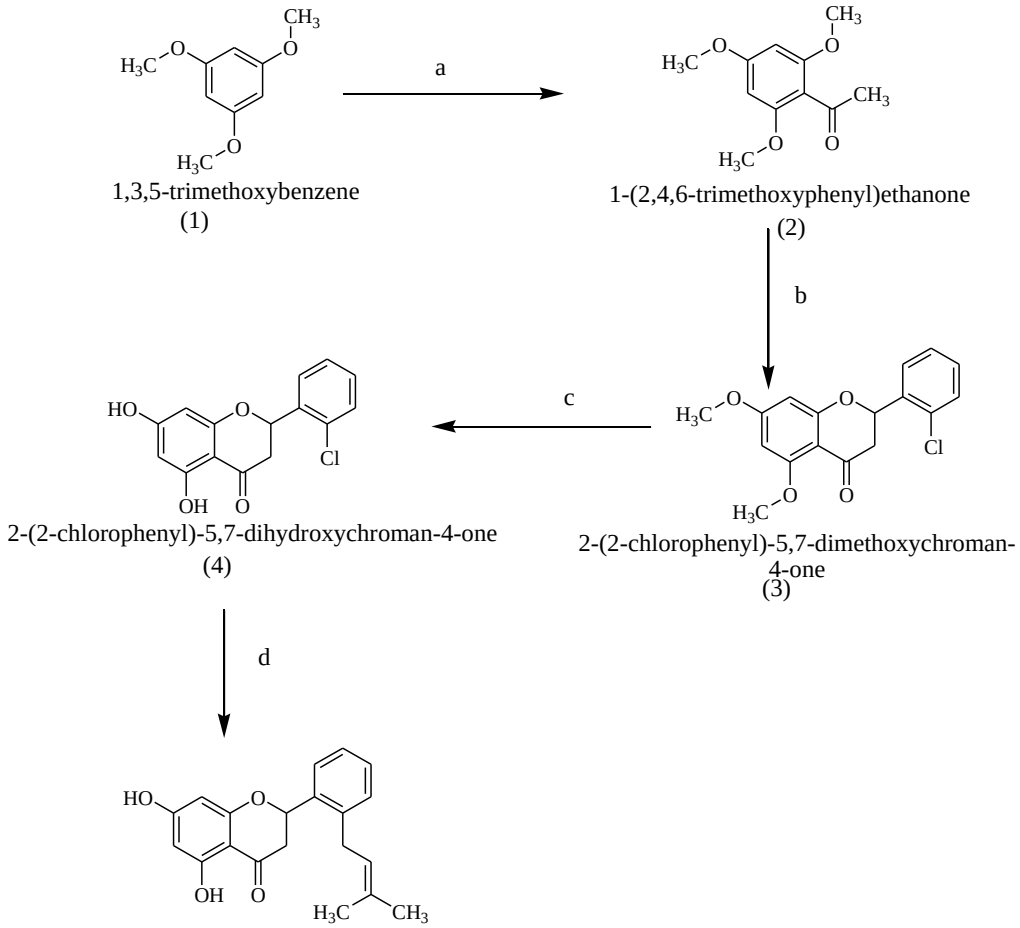
To a solution of 2-(2-chlorophenyl)-5, 7-dimethoxychroman-4-one (0.01 mol) **3** in pyridine (30 ml) was added HCl (0.5 ml) drop by drop as catalyst and then refluxed for 2 hr. The reaction mixture was quenched with ice and then obtained crude compound was filtered, washed with petroleum ether and dried under vacuum to afford 2-(2-chlorophenyl)-5,7-dihydroxychroman-4-one.

Yield 86 %, m.p 176°C. IR (cm^{-1}): 3452 (OH), 1672 (C=O), 1618, 1465 (C=C aromatic) and 1156 (C–O).^{1}HNMR (DMSO-d6, δ): 2.76 (1H, dd, *J*=15.8 Hz and 2.6 Hz), 3.28 (1H, dd, *J*=15.4 Hz and 3.2 Hz), 5.43 (1H, dd, *J*=12.4 Hz and 3.3 Hz), 5.90-7.21 (m, 6H, Ar-H), 8.45 (1H, bs, OH), 9.15 (1H, bs, OH). MS (ESI): m/z 291 (M+1).

SYNTHESIS OF 5,7-DIHYDROXY-2-(2-(3-METHYLBUT-2-ENYL)PHENYL)CHROMAN-4-ONE (5)

To a solution of 2-(2-chlorophenyl)-5,7-dihydroxychroman-4-one (0.005 mol) **5** in ethanol (10 ml) was added zinc isoprene and catalytic amount of nickel and stirred at room temperature for 8 hr. The obtained mixture was transferred in to round bottomed flask and boiled for 3 hr and cooled to room temperature. The excess of ethanol was distilled under reduced pressure to afford 5,7-dihydroxy-2-(2-(3-methylbut-2-enyl)phenyl)chroman-4-one.

Yield 56 %, m.p 146°C. IR (cm^{-1}): 3459 (OH), 1679 (C=O), 1609, 1458 (C=C aromatic) and 1161 (C–O).^{1}HNMR (DMSO-d6, δ): 1.55 (6H, s, $2CH_3$), 2.79 (1H, dd, *J*=15.1 Hz and 2.9 Hz), 3.12 (2H, d, J=6.3 Hz, $-CH_2$), 3.27 (1H, dd, *J*=15.2 Hz and 3.0 Hz), 5.29 (1H, m, H-allylic), 5.46 (1H, dd, *J*=12.6 Hz and 3.6 Hz), 5.82-7.02 (m, 6H, Ar-H), 8.43 (1H, bs, OH), 9.46 (1H, bs, OH). MS (ESI): m/z 325 (M+1).



Scheme 1: Synthesis of 5, 7-dihydroxy-3'-prenyl flavanone: Reagents and conditions (a) Ac_2O, BF_3OEt_2, DCM, 70°C, 7 hr (b) 1-chloro-2-(hydroperoxymethyl)benzene, Na_2CO_3, NaH, DMF, HCl, 0°C, 3 hr (c) Pyridine**,** reflux, 2 hr (d) Zinc isoprene, Ni, EtOH, reflux, 3 hr.

IN VIVO ANTIINFLAMMATORY ACTIVITY

The antiinflammatory activity of the synthesized compounds was assessed by *in vivo* using the carrageenan induced paw edema method. The experimental protocol for the pharmacological screening was done in accordance with the guidelines prescribed by an Institutional Animal Ethics Committee (Reg no: 1374/PO/RE/S/10/CPCSEA). The rats were divided into 3 groups of six animals each. The control group received 1 % aq.CMC. Ibuprofen sodium (100 mg/kg, standard) and test compound (**5**, 100 mg/kg) in 1% carboxy methyl cellulose (CMC) were administered orally 1 h before induction of inflammation. Inflammation was induced in to the sub-plantar region of the right hind paw by subcutaneous injection of 0.1 mL of freshly prepared 1% carrageenan in saline solution. The right paw thickness was measured using the plethysmometer at 0.5, 1, 2, 3 hrs after the carrageenan injection. The average value of edema was calculated by taking the average of six animals at different hours. Percentage inhibition of inflammation was calculated for each group with respect to the control group.

Inflammation was expressed as the change in paw volume

Edema = $T_t - T_0$

Where T_0 = Volume at '0' hrs

T_t = Volume at't' hrs

Percentage of edema inhibition = $(V_0 - V_t)/V_0 \times 100$

Where, V_0 = Volume of the paw of control at time't'
V_t = Volume of the paw of drug treated at time't'

RESULTS AND DISCUSSIONS:

CHEMISTY:

In the present study, a synthetic approach has been outlined for the preparation of 5, 7-dihydroxy-3'-prenyl flavanone in scheme-**1**. According to scheme-**1**, 1, 3, 5-trimethoxybenzene **1** was treated with borontrifluride diethyl etherate in acetic anhydride to give 1-(2,4,6-trimethoxyphenyl) ethanone **2** which was reacted with 1-chloro-2-(hydroperoxymethyl)benzene in DMF in the presence of HCl at 0°C to produce 2-(2-chlorophenyl)-5,7-dimethoxychroman-4-one **3**. Further, the compound **3** was reacted with pyridine to yield 2-(2-chlorophenyl)-5,7-dihydroxychroman-4-one **4**. The compound **4** was reacted with zinc isoprene in the presence of ethanol under reflux for 3 hrs to afford 5, 7-dihydroxy-2-(2-(3-methylbut-2-enyl) phenyl) chroman-4-one **5**. The structures of the all the synthesized compounds were confirmed based on their IR, ^{1}H NMR, and mass spectral data.

ANTIINFLAMMATORY ACTIVITY:

The synthesized compound was tested for their antiinflammatory activity by carageenan induced paw edema method. The results are summarized in Table 1. It was observed that 5, 7-dihydroxy-2-(2-(3-methylbut-2-enyl) phenyl) chroman-4-one **(5)** elicited potent activity (79.08% inhibition) after 3 hrs as results are comparable to the standard drug, ibuprofen (87.79 % inhibition of edema).

Table-1: Antiinflammatory effect of compounds on carageenan induced paw edema in rats

Compound	**0.5 hr**	**1 hr**	**2 hrs**	**3 hrs**	**% inhibition after 3 hrs**
Control	2.59 ± 0.01	3.79 ± 0.02	4.21 ± 0.04	4.59 ± 0.02	--
Ibuprofen	1.65 ± 0.02*	1.13 ± 0.04*	0.97 ± 0.03*	0.56 ± 0.05*	87.79
5	1.79 ± 0.03*	1.69 ± 0.02*	1.21 ± 0.04*	0.96 ± 0.03*	79.08

Each value represents mean ± SE of six animals; $^*P < 0.05$ as compared to control; using one way ANOVA was done by Dunnett's t-test. Ibuprofen and test compound were taken at a dose of 100 mg/kg body weight.

MOLECULAR DOCKING

Molecular docking was executed for further exploration of the mechanism of action of the target compound with COX-2 enzyme and to elucidate the obtained anti-inflammatory data. Docking of compound (**5)** exhibited four conventional hydrogen bond interactions such as ASN A: 39, GLN A: 42, GLU A: 465 and LYS A: 468 and another important building residues that formed pi-alkyl at CYS A: 36, CYS A: 47 and PRO A: 153 which are very similar to that of the interactions showed by the known COX-2 inhibitors[xvi]. The two dimensional and three-dimensional representations of target compound **5** were given under Figure-1.

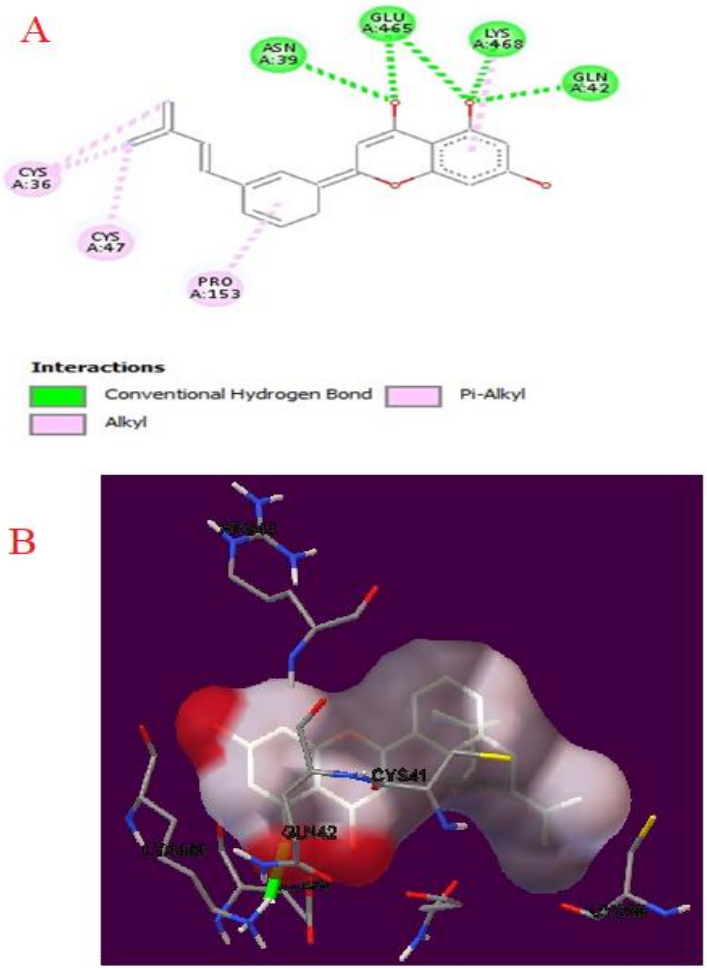


Figure-1: (A) Two-dimensional representation of the interacting mode of compound **5** with COX-2

(B) Three-dimensional representation of compound **5** into COX-2

ASSESSMENT OF MOLECULAR PROPERTIES AND LIPINSKI PARAMETERS

A computational study for prediction of ADME properties of the molecules was performed by determination of lipophilicity, topological polar surface area and lipinski in formulating "rule of five". The Lipinski rule states that molecule could be considered as a drug candidate should have logP <= 5, molecular weight <= 500, number of hydrogen bond acceptors <= 10, and number of hydrogen bond donors <= 5. Compounds violating any one of these rules are expected to have bioavailability problems[xvii-xix]. Molecular properties and Lipinski parameters of target compound presented in Table-2. Interestingly, the synthesized compound could be considered drug-like candidates for novel anti-inflammatory agents, as they obeyed the rule of five without violating any one of these rules.

Table-2: Molecular properties and lipinski parameters of synthesized compound.

Compound	**MW[a]**	**TPSA[b]**	**NROTB[c]**	**NHBA[d]**	**NHBD[e]**	**cLogS[f]**	**cLogP[g]**
5	324	66.7	3	4	2	-4.7	4.4

[a]Molecular weight.
[b]Topological polar surface area.
[c]Number of rotatable bonds.
[d]Number of hydrogen bonds acceptors.

[e]Number of hydrogen bond donators.
[f]Water solubility (cLogS).
[g]Lipophilicity (cLogP).

CONCLUSION:

A novel compound 5, 7-dihydroxy-3'- prenyl flavanone was synthesized by facile synthetic method and characterized by physical and spectral data. The anti-inflammatory screening of the synthesized compound was performed by *in vivo* using carragenan induced paw oedema method. *In silico* prediction of molecular and drug-likeness properties of synthesized compound was promising. Moreover, the synthesized compound showed significant docking interactions with COX-2 active site. Molecular docking results along with the biological data suggested that the tested compounds have the potential as valuable lead for antiinflammatory activity.

ACKNOWLEDGEMENT:

The authors would like to thank the management of Guru Nanak Institutions, Hyderabad for giving an opportunity to carry out this research.

REFERENCES:

i. Ravula P.; Babu H.; Manichandrika P.; Ramanachary N.; Narendra Sharath Chandra JN.; Microwave assisted synthesis, biological evaluation and docking studies of novel pyrazoline derivatives as potent antiinflammatory and antibacterial agents; Int. J. Chem. Sci.; 2016, **14**, 769.
ii. Swetha KS.; Parameshwar R.; Reddy BM.; Synthesis of novel pyrazolyltetrazoles as selective COX-2 inhibitors; Med. Chem. Res.; 2013, **22**, 4886.
iii. Silverman SL.; Schepman P.; Rice JB.; Beck CG.; Johnson M.; White A.; Robinson RL.; Emir B.; Drug utilization, clinical and economic outcomes of patients with osteoarthritis of the hip and/or knee treated with long-term use of traditional NSAIDs, topical NSAIDs, and COX-2 inhibitors. Curr. Med. Res. Opin .; 2022, **2**, 1.
iv. Mohamed Adib A.; Ahmad F.; Idris MS.; Synthesis and antimicrobial activity of 4′, 5, 7-trihydroxy-3′-prenylflavanone; J. chem. sci.; 2008, **120**, 469.
v. Shahinozzaman M.; Taira N.; Ishii T.; Halim MA.; Hossain MA.; Tawata S.; Anti-inflammatory, anti-diabetic, and anti-Alzheimer's effects of prenylated flavonoids from Okinawa propolis: an investigation by experimental and computational studies; Molecules.; 2018, **23**, 2479.
vi. Osorio M.; Carvajal M.; Vergara A.; Butassi E.; Zacchino S.; Mascayano C.; Montoya M.; Mejías S.; Martín MC.; Vásquez-Martínez Y.; Prenylated flavonoids with potential antimicrobial activity: synthesis, biological activity, and *in silico* study; Int. J. Mol. Sci.; 2021, **22**, 5472.
vii. Jin YS.; Recent advances in natural antifungal flavonoids and their derivatives; Bioorg. Med. Chem. Lett.; 2019, **29**, 126589.
viii. Kumar S.; Pathania AS.; Nalli YK.; Malik FA.; Vishwakarma RA.; Ali A.; Synthesis of new O-alkyl and alkyne–azidecycloaddition derivatives of 4′-methoxy licoflavanone: a distinct prenylated flavonoids depicting potent cytotoxic activity; Med. Chem. Res.; 2015, **24**, 669.
ix. Liu YP.; Yu XM.; Zhang W.; Wang T.; Jiang B.; Tang HX.; Su QT.; Fu YH.; Prenylatedchromones and flavonoids from Artocarpusheterophyllus with their potential antiproliferative and anti-inflammatory activities; Bioorg. Chem.; 2020, **101**, 104030.

x. Dufall KG.; Ngadjui BT.; Simeon KF.; Abegaz BM.; Croft KD.; Antioxidant activity of prenylated flavonoids from the West African medicinal plant Dorsteniamannii; J. Ethnopharmacol.; 2003, **87**, 67.
xi. Ahmad A.; Kaleem M.; Ahmed Z.; Shafiq H.; Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections—A review; Int. Food Res. J.; 2015, **77**, 221.
xii. Agullo G.; Gamet-Payrastre L.; Manenti S.; Viala C.; Rémésy C.; Chap H.; Payrastre B.; Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition; Biochem. Pharmacol.; 1997, **53**, 1649.
xiii. Achutha L.; Parameshwar R.; Reddy BM.; Babu VH.; Microwave-assisted synthesis of some quinoxaline-incorporated Schiff bases and their biological evaluation; J. Chem.; 2013, DOI:10.1155/2013/578438
xiv. Ravula P.; Vamaraju HB.; Paturi M.; Sharath Chandra JN.; Design, synthesis, *in silico* and antiproliferative evaluation of novel pyrazole derivatives as VEGFR-2 inhibitors; Arch. Pharm.; 2018, **351**, 1700234. DOI:10.1002/ardp.201700234.
xv. Schübler M.; Sadek B.; Kottke T.; Weizel L.; Stark H.; Synthesis, molecular properties estimations, and dual dopamine D2 and D3 receptor activities of benzothiazole-based ligands; Front. Chem.; 2017, **5**, DOI:10.3389/fchem.2017.00064.
xvi. Giles D.; Roopa K.; Sheeba FR.; Gurubasavarajaswamy PM.; Divakar G.; Vidhya T.; Synthesis pharmacological evaluation and docking studies of pyrimidine derivatives; Eur. J. Med. Chem.; 2012, **58**, 478.
xvii. Hadda TB.; Rastija V.; AlMalki F.; Titi A.; Touzani R.; Mabkhot YN.; Khalid S.; Zarrouk A.; Siddiqui BS.; Petra/Osiris/Molinspiration and Molecular Docking Analyses of 3-Hydroxy-Indolin-2-one Derivatives as Potential Antiviral Agents; Curr. Comput.-Aided Drug Des.; 2021, **17**, 123.
xviii. Kemisetti DP.; Manda SA.; Aukunuru JI.; Chinnala KM.; Rapaka NK.; Synthesis of prodrugs of mefenamic acid and their in vivo evaluation.; Int. J. Pharm. Pharm. Sci.; 2014, **6**, 437.
xix. Kemisetti D.; Manda S.; Rapaka NK.; Jithan A.; Synthesis of nimesulide conjugates, in vitro and in vivo evaluation.; Der. Pharma Chem.; 2014, **6**, 317.

Received on 13 August 2022.