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Synthesis and molecular docking studies of new chromane (2-(4-hydroxybenzyl) 3,5,7-trihydroxychroma-4-one) and its O-substituted analogues

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***Abstract*---**A series of 3-(O-R) substituted compounds (SI-SX) of 2-(4-hydroxybenzyl) 3,5,7-trihydroxychroma-4-one were synthesized from easily accessible starting materials such as, p-hydroxybenzaldehyde and ethyl bromopyruvate. All the ten derivatives (SI-SX) were synthesized in appropriate yields, and they were characterized by IR, ^{1}H NMR and C NMR. Molecular docking of all the derivatives were performed using Molegro virtual docker tool 6.0.2 (MVD) tool. CYCLOOXYGENASE-2 (COX2) or PROSTAGLANDIN SYNTHASE-2 was taken as the target protein and was downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (https://www.rcsb.org/structure/1CX2) with PDBID: ICX2, 10.2210/pdb1CX2/pdb). The 3D images of ligand protein interactions were extracted using the software, MVD 6.0.2 visualizer interface. Among all the derivatives, SII, SVI has shown good moldock score values -77.59 and -75.75 with high number of interactions (09) towards the target protein, indicating its ability to act as an anti-inflammatory and analgesic activity.

***Keywords*---**chroman-4-one, anti-inflammatory, analgesic, molecular docking, COX 2.

Introduction

Among the widespread heterocyclic molecules, the oxygen heterobicycles like chroman-4-one/Chromanone occupy a privileged place because of their extensive natural abundance as flavonols, flavone, flavanone, isoflavone and homisoflavonones with broad spectrum biological as well as pharmaceutical significance [1–3]. In the precise classes of chromane, 'chroman-4-one' scaffold represent a privileged nucleus well distributed in natural products with a broad spectrum of potent biological activities like anticancer, tumour necrosis factor-α (TNF-α) inhibitors, antidiabetic, antioxidant, antivascular, antifungal, antimicrobial, antiviral, insecticidal, antileishmanial, spasmolytic, analgesic, anti-inflammatory, estrogenic inhibitor, anti-acetylcholinesterase and anticoagulant activity [4]. Instead of these actions, several analogues of chromane are also available in market like taxifolin (antidiabetic), tocopherols (vitamin E), troglitazone (antidiabetic), tetrazole (antidiabetic), ormeloxifene (anticancer) and nebivolol (beta-blocker) with potent activity [5]. Generally, the presence of chromane ring-based structure in a molecule is often associated with its potency to prevent diseases. Moreover numerous synthetic derivatives of naturally occurring chromane such as hesperitin[6], Violacin A[7], dihydromyricetin[8], stilbin[9], silybin[10], 4'-O-Dimethylophiopogonanone E[11], Novel (E)-5,7-dimethoxy-3-(4'-hydroxybenzylidene) chroman-4-one [7], 3-arylidene-7-methoxychroman-4-one, Dihydroflavonols [3,5,7-Trihydroxy-2-(4′-fuorophenyl) chroman-4-one], [3,5,7-Trihydroxy-2-(pyridin-3-yl) chroman-4-one], [3,5,6,7-Tetrahydroxy-2-(3′,4′- dihydroxyphenyl) chroman-4-one][12], Homoisoflavones [(R)-3-(3,4-Dihydroxybenzyl)-7-hydroxy-5-methoxychroman-4-one], [1,3,6-Trihydroxy-2-methoxy-8-methylxanthen-9-one], 7-Dimethylamino-4-chromanones, Ovatifolionone acetate [13]and Pruinosanone A[14], containing chromanone nucleus were evaluated as analgesic and anti-inflammatory agents with good inhibitions whereas chroman-4-one containing stilbin and silybin are used clinically proved anti-inflammatory drugs[10]. Moreover, these isolated compounds further pave the way for synthesis of new anti-inflammatory drugs via inhibition of cyclooxygenase 2 (COX-2).

New Chromane(3,5,7-trihydroxy-2-(4-hydroxybenzyl)-chroman-4-one) was firstly isolated from dried Leaves of *Dillenia indica* (*D. indica*) Linn, Family Dilleniaceae [15] is structurally related to α-Tocopherol, Troglitazone, thiazolidinediones, quercetin, homisoflavonones having *chromane moiety*. A key feature is that the lipophilic nature of chromane/benzopyran derivatives helps to cross the cell membrane easily and O-H group substitution reported for analgesic and anti-inflammatory potential via COX 2.[16] Based on the above statement, the present study is based on the synthesis of novel new chromane and its O-substituted compounds which were evaluated for COX2 protein binding compared against diclofenac and quercetin as reference molecules.

Generally, SAR studies revealed that C-2 substitution of chromane nucleus with hydroxybenzylidine, arylidene, hydroxyphenyl, pyridine-3-yl and fluorophenyl

displayed the best anti-inflammatory activity with significant inhibition.[4] In this context, we have tried to identify new chemical fragments to develop more druggable COX 2 inhibitors. Based on the literature survey, our group designed O-alkylated or aryl alkylated and O-heteroaromatic derivatives of novel chroman-4-one. Table 1 illustrates the newly designed SI to SX compounds with their IUPAC which will further check for binding study with COX 2 protein because cyclooxygenases (COX) or prostaglandin endoperoxide synthases are the key enzymes in the synthesis of prostaglandins, the main mediators of inflammation, pain, and increased body temperature (hyperpyrexia).[17]

Material and Method

The melting point of the synthesised compounds were measured by capillary method using Melting point apparatus and expressed in °C. All reagents used in synthesis were of Laboratory grade (LR), synthetic grade (SR) and solvents were of Analytical grade (AR). The reactions were monitored by preparative TLC's, Kiese gel, 0.25 mm, 60 F254, Merck, Germany and spots were visualized by ultraviolet (UV) light absorption using a developing solvent system of ethyl acetate/hexane. IR spectra were measured in a KBr matrix using a Perkin Elmer FT-IR spectrometer. 1 H NMR spectra were recorded using an Advance Bruker NMR spectrometer at 400 MHz, whereas 13C NMR spectra were recorded at 100 MHz using Dimethylsulphoxide (DMSO) as the internal standard. The chemical shifts are expressed in δ ppm and splitting patterns are nominated as s: singlet; d: doublet; q: quartet; m: multiplet. Mass spectra (MS) was recorded using a Time-of-Flight Mass spectrometry (TOF-MS).

Molecular docking

Molecular docking was conducted by Molegro Virtual Docker 6.0.2 (MVD) software on new chromane and all designed derivatives of COX2 protein to determine the binding affinity of ligands with different kinds of amino acids. The 2D structures of the new chromane and relevant derivatives (S1 to SX) were prepared using ACD chem sketch tool.

Protein preparation

The 3-dimensional (3D) crystal structure of COX2 (CYCLOOXYGENASE-2 or (PROSTAGLANDIN SYNTHASE-2 (PDB ID: ICX2, 10.2210/pdb1CX2/pdb) was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (https://www.rcsb.org/structure/1CX2) **.**The crystal structure has a resolution of 3.00 °A. It also has a structural weight of 276.18 k Da and contains a unique chain (Chain A) out of four chains A, B, C and D. The enzyme was prepared by removing cofactors, water molecules and other attached ligands and imported in MVD workspace with labels. [18]

Ligand Preparation

The 2D structures of ligands were prepared using ACD chem sketch program. After preparation, the 2D structures were converted to 3D format and then these were energetically minimized by using a 3D optimization tool implemented in the

same and saved as MDL mole file (*.mol) which will later imported in active MVD workspace (1CX2) for docking purposes.

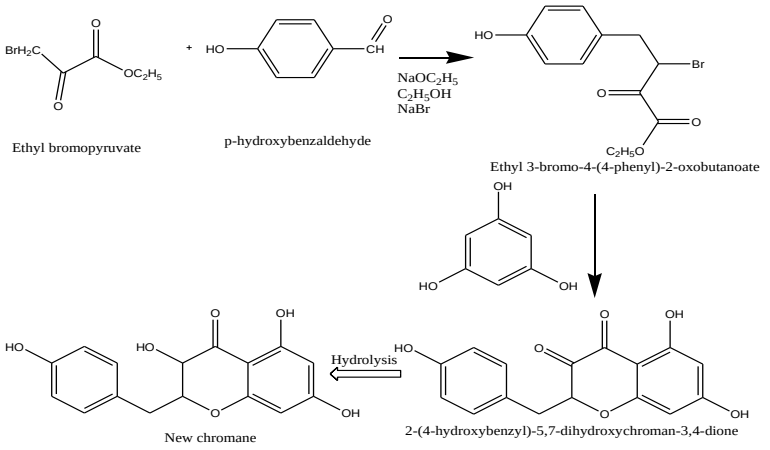
Cavity prediction

The cavity or the potential ligand binding site of PDB ID: 1CX2 was predicted in active MVD workspace. The cavity or potential ligand binding site of chain A (PDB ID: 1CX2) was predicted with defined volume 159.016 and surface area 472.071 in MVD docking wizard. The Mol Dock grid score was set to a grid resolution of 0.30 ^{0}A 1,500 maximum iteration and a minimum of 10 runs were accomplished for each compound having threshold energy of 100. The binding site was set inside a restriction sphere of 15 radius with the centre X: 42.37, Y: 33.47, Z: 34.98. The best pose of each compound was selected for evaluation of subsequent ligand-protein interaction energy.

Experimental work

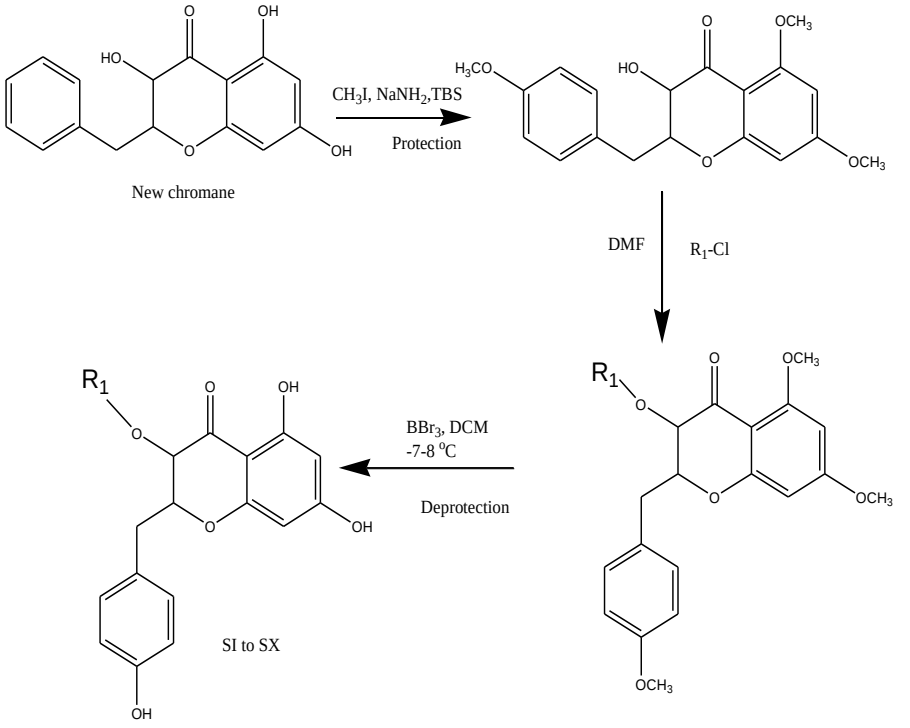
General procedure for the synthesis of new chromane:

- Charge mixture of 1.0 mole eq. of p-hydroxybenzaldehyde and 1.1 mole eq. ethyl bromopyruvate with 10 V ethanol and 1.0 mole eq. NaBr. Cool the reaction mass at 0–5-degree C. Add 1.2 mole eq $NaOC_2H_5$ slowly. Stirrer the mixture for 30 minutes and characterized the product using Thin Layer Chromatography (TLC).
- Add 1,3,5-trihydroxybenzene (1.0 mole eq.) with methanol (10V) and conc. sulphuric acid (0.5 cm3) in above reaction product results 2-(4-hydroxybenzyl)-5,7-dihydroxychroman-3,4-dione. Upon hydrolysis (sodium borohydride, $NaBH_4$) a pale-yellow colored product obtained. The final product (new chromane) was dried through vacuum filtration and characterized by TLC using ethyl acetate: hexane (4:6) as mobile phase.

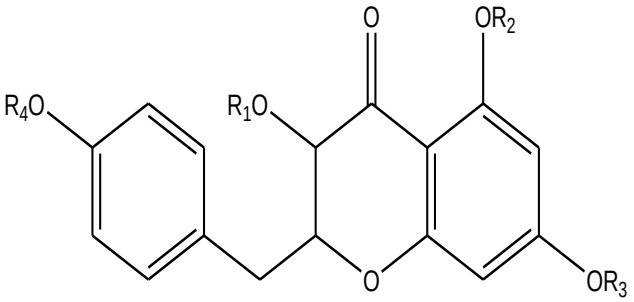


Synthesis of O- substituted compounds

All these compounds were synthesized using available reactants especially chloro-derivatives. Firstly, the protection of phenolic groups has been introduced with the help of tributyl silyl (TBS), methyl iodide and sodium azide. After that hydroxyl group (C-3) is able to attack by chloroderivatives and O-alkylation can be taken place using DMF solvent on stirring only. In the end, deprotection help to synthesize final product. [19-23]

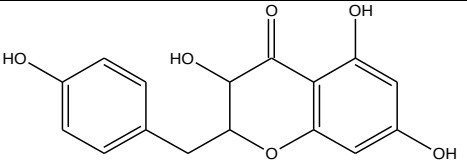
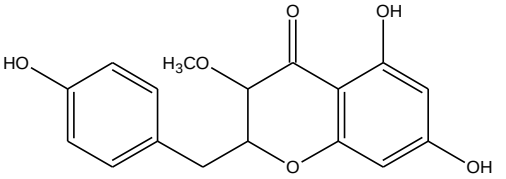
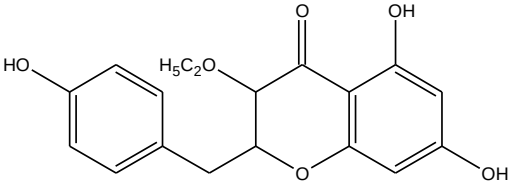
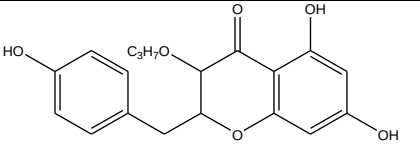


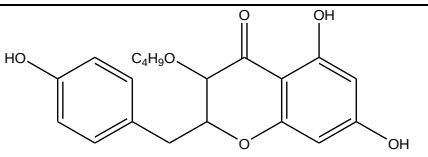
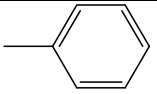
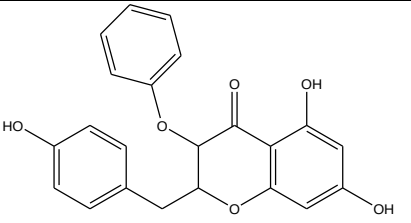
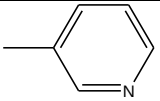
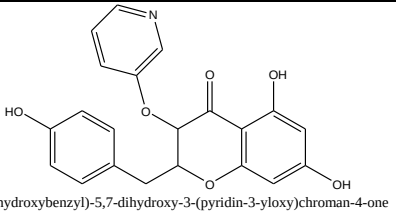
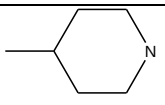
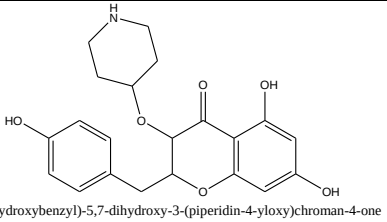
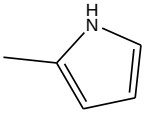
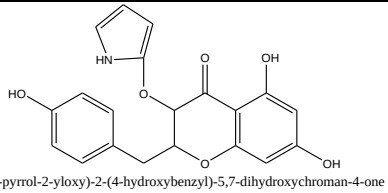
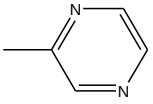
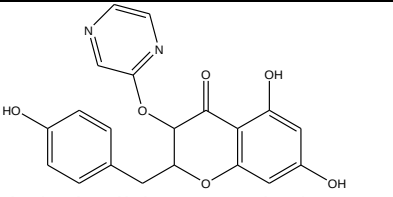
R_1= Chloro derivatives of Meth, Eth, prop, but, phenyl etc [Table 1 SI to SX compounds)

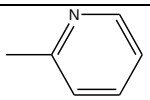
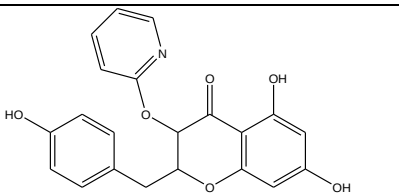


Parent structure

Table 1: List of designed compounds used for docking simulation and synthesis

Compounds	R1(C-3)	R3 (C-7)	R4 (C-4')	R2 (C-5)	Structure with IUPAC name
Parent	H	H	H	H	 2-(4-hydroxybenzyl)-3,5,7-trihydroxychroman-4-one
SI	CH_3	H	H	H	 2-(4-hydroxybenzyl)-5,7-dihydroxy-3-methoxychroman-4-one
SII	C_2H_5	H	H	H	 2-(4-hydroxybenzyl)-3-ethoxy-5,7-dihydroxychroman-4-one
SIII	C_3H_7	H	H	H	 2-(4-hydroxybenzyl)-5,7-dihydroxy-3-propoxychroman-4-one

SIV	C_4H_9	H	H	H	 2-(4-hydroxybenzyl)-3-butoxy-5,7-dihydroxychroman-4-one
SV		H	H	H	 2-(4-hydroxybenzyl)-5,7-dihydroxy-3-phenoxychroman-4-one
SVI		H	H	H	 2-(4-hydroxybenzyl)-5,7-dihydroxy-3-(pyridin-3-yloxy)chroman-4-one
SVII		H	H	H	 2-(4-hydroxybenzyl)-5,7-dihydroxy-3-(piperidin-4-yloxy)chroman-4-one
SVIII		H	H	H	 3-(1*H*-pyrrol-2-yloxy)-2-(4-hydroxybenzyl)-5,7-dihydroxychroman-4-one
SIX		H	H	H	 2-(4-hydroxybenzyl)-5,7-dihydroxy-3-(pyrazin-2-yloxy)chroman-4-one

SX		H	H	H	 2-(4-hydroxybenzyl)-5,7-dihydroxy-3-(pyridin-2-yloxy)chroman-4-one

Result and Discussion

Characterization of compounds

2-(4-hydroxybenzyl) 3,5,7-trihydroxychroma-4-one: Yield: 71%; M.Pt. 282-289 ℃
IR (cm^{-1}): 3273.40 (O-H str, broad), 3180 (C=C str.), 1095.00 (C-O str), 1190.62(C-O-C), 1027.38(C=O),1587.10 and 1523.36(C=C-C str.) 1474.32(C-H)
^{1}HNMR (DMSO-$d_{6,\ 400}$ MHz): δ 2.09 (s, 3H, O*H*), 2.57 (s, 2H, CH_2), 3.36 (s, 1H, O*H*-CH), 3.36 (s, 2H, C*H*), 7.22 (m, 1H, Ar*H*), 7.38 (m, 1H, aromatic), 7.66 (m, 1H, aromatic), 7.88 (m, 1H, aromatic), 7.92 (m, 1H, aromatic) and 8.22 ppm (m, 1H, aromatic).
^{13}C NMR (DMSO-$d_{6,}$ 100 MHz): δ 24.49 (*C*H_2), 86.71 (*C*H), 90.47 (Ar*C* attached to OH), 125.64 (Ar*C*), 129.45 (Ar*C*), 132.67 (Ar*C*), 135.46 (Ar*C*), 137.60 (quaternary aromatic carbon attached to OH), 140.94 (quaternary aromatic carbon), 161.91 (quaternary aromatic carbon attached to OH), 176.91 (Ar*C* attached to OH) and 196.91 ppm (*C*=O).
MS (ESI): m/z rel. int. % [M + 2] calculated for $C_{16}H_{14}O_6$: 304.

SI (2-(4-hydroxybenzyl)-5,7-dihydroxy-3-methoxychroman-4-one)

Yield: 61%; M.Pt. 263-268 ℃ IR (cm^{-1}): 3125.47 (O-H str.), 3097.40 (C=C str.), 3063.71 (C-H str Aromatic), 2981.46(C-H str), 1627.55(C=O),1525.62, 1572.06 (C=C-C str.),1524.58 1464.89(C-H)
^{1}H NMR: (DMSO-$d_{6,}$ 400 MHz): δ 2.39 (s, 3H, O*H*), 2.39 (m, 2H, CH_2), 3.36 (s, 3H, CH_3), 3.86 (s, 2H, 2 x C*H*), 7.21 (m, 1H, aromatic), 7.38 (m, 1H, aromatic), 7.66 (m, 1H, aromatic), 7.88 (m, 1H, aromatic), 7.92 (m, 1H, aromatic) and 8.22 ppm (m, 1H, aromatic).
^{13}C NMR: (DMSO-$d_{6,}$ 100 MHz): δ 24.49 (*C*H_3), 60.09 (*C*H_2), 86.71 (*C*H), 90.49 (Ar*C*), 125.00 (2 x Ar*C*), 125.68 (Ar*C*), 129.45 (3 x Ar*C*), 132.67 (Ar*C*), 135.46 (Ar*C*), 135.46 (Ar*C*), 137.60 (Ar*C*), 140.94 (*C*-OH), 161.91 (*C*-OH), 176.91 (*C*-OH) and 196.91 ppm (*C*=O).

SII (2-(4-hydroxybenzyl)-3-ethoxy-5,7-dihydroxychroman-4-one)

Yield: 58%; M.Pt. 272-274 ℃ IR (cm^{-1}): 2928.33(O-H str.), 2849.20(C=C str.), 2797.42(C-H str Aromatic), 1949.55(C-H str), 1599.76(C=O), 1488.10(C-H), 1376.84(C-H), 1340.30(C-C), 1266.49(C-H str)
^{1}H NMR :(DMSO-$d_{6,}$ 400 MHz): δ 2.09 (s, 1H, CH_2), 2.39 (s, 1H, CH_2), 3.35 (s, 3H, O*H*), 7.43 (m, 1H, aromatic), 7.57 (m, 1H, aromatic), 7.67 (m, 1H, aromatic), 8.07 (m, 1H, aromatic), 8.32 (m, 1H, aromatic) and 8.97 ppm (m, 3H, aromatic).

^{13}C NMR:(DMSO-*d*$_6$, 100 MHz): δ 18.48 (*C*H$_3$), 25.49 (*C*H$_2$), 60.09 (*C*H$_2$), 86.71 (*C*H), 90.49 (Ar*C*), 125.00 (Ar*C*), 125.68 (Ar*C*), 129.45 (Ar*C*), 132.46 (Ar*C*), 137.60 (Ar*C*), 140.94 (*C*-OH), 161.91 (*C*-OH), 176.91 (*C*-OH) and 196.91 ppm (*C*=O).

SIII (2-(4-hydroxybenzyl)-3-propoxy-5,7-dihydroxychroman-4-one)

Yield: 52%; M.Pt. 277-280 ℃ IR (cm^{-1}): 3127.85(O-H str.), 2983.51(C=C str.), 2833.80(C-H str),1596.84(C=O), 1573.42(C-H), 1526.10(C-H), 1440.75(C-H), 1236.49(C-H str)
^{1}H NMR: (DMSO-*d*$_{6,\ 400}$ MHz): δ 1.58 (m, 5H, C$_2$*H*$_5$), 2.76 (m, 4H, 2 x C*H*$_2$), 3.15 (m, 2H, 2 x C*H*), 4.56 (m, 3H, 3 x O*H*), 7.20 (m, 1H, Ar*H*), 7.30 (m, 1H, aromatic), 7.52 (m, 1H, aromatic), 7.66 (m, 2H, aromatic) and 7.79 ppm (m, 1H, aromatic).
^{13}C NMR: (DMSO-*d*$_6$, 100 MHz): δ 13.43 (C*H*$_3$), 24.49 (*C*H$_2$), 60.09 (*C*H$_2$), 70.07 (*C*H$_2$), 86.71 (*C*H), 90.49 (Ar*C* attached to OH), 125.00 (Ar*C*), 125.68 (Ar*C*), 129.45 (Ar*C*), 132.67 (Ar*C*), 135.46 (Ar*C*), 137.61 (*C*-OH), 140.94 (*C*-OH), 176.91 (*C*-OH) and 196.91 ppm (*C*=O).

SIV (2-(4-hydroxybenzyl)-3-butoxy-5,7-dihydroxychroman-4-one)

Yield: 28%; M.Pt. 280-286 ℃ IR (cm^{-1}): 3127.85(O-H str.), 2983.51(C=C str.), 2833.80(C-H str),1596.84(C=O), 1573.42(C-H), 1526.10(C-H), 1440.75(C-H), 1236.49(C-H str)
^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 1.48 (m, 6H, 2C*H*$_3$), 2.29 (m, 2H, C*H*$_2$), 2.85 (d, 2H, C*H*$_2$), 3.12 (m, 3H, C*H*), 6.49 (m, 4H, -O*H*, >N*H*), 7.25 (m, 2H, Ar*H*), 7.54 (m, 2H, aromatic), 7.72 (m, 2H, aromatic) and 8.24 ppm (m, 2H, aromatic).
^{13}C NMR(DMSO-*d*$_6$, 100 MHz): δ 18.48 (*C*H$_3$), 25.49 (*C*H$_3$), 60.09 (*C*H$_2$), 86.71 (*C*H$_2$), 90.49 (*C*H), 95.21 (Ar*C*), 98.49 (Ar*C*) , 125.00 (Ar*C*), 125.68 (Ar*C*), 129.45 (3 x Ar*C*), 132.67 (Ar*C*), 135.46 (Ar*C*), 137.60 (Ar*C*), 140.94 (*C*-OH), 161.91 (*C*-OH), 176.91 (*C*-OH) and 196.91 ppm (*C*=O).

SV (2-(4-hydroxybenzyl)-5,7-dihydroxy-3-phenoxychroman-4-one)

Yield: 41%; M.Pt. 302-304 ℃ IR (cm^{-1}): 3127.23 (O-H str.), 2982.45 (C=C str.), 1629.24(C=O),1595.95(C=C str.), 1574.14(C=C str.),1527.23(C-H), 1108.00(-C-O-C)
^{1}H NMR:(DMSO-*d*$_6$, 400 MHz): δ 2.39 (s, 2H, C*H*$_2$), 2.55 (s, 2H, C*H*$_2$), 3.35 (s, 3H, O*H*), 6.97 (m, 4H, Phenyl), 7.43 (m, 1H, aromatic), 7.57 (m, 1H, aromatic), 7.67 (m, 1H, aromatic), 8.07 (m, 1H, aromatic), 8.12 (m, 1H, aromatic) and 8.31 ppm (m, 2H, aromatic).
^{13}C NMR:(DMSO-*d*$_6$, 100 MHz): δ 78.67 (*C*H$_2$), 79.45 (*C*H), 113.43 (2 x Ar*C*), 117.83 (2 x Ar*C*), 119.24 (Ar*C*), 119.56 (Ar*C*), 120.29 (Ar*C*), 124.87 (Ar*C*), 125.23 (3 x Ar*C*), 125.71 (Ar*C*), 125.81 (Ar*C*), 126.74 (Ar*C*), 134.98 (Ar*C*), 143.01 (Ar*C*), 143.30 (Ar*C*), 145.16 (Ar*C*), 147.94 (*C*-OH), 151.20 (*C*-OH), 160.20 (*C*-OH) and 160.59 ppm (*C*=O).

SVI 2-(4-hydroxybenzyl)-5,7-dihydroxy-3-(pyridin-3-yloxy) chroman-4-one

Yield: 38%; M.Pt. 309-312 ℃ IR (cm^{-1}): 3268.71(O-H), 3174.14(C-H), 1630.49(C=O), 1577.81(C=N str ring), 1361.12(C=C), 1168.45(C-O-C), 1086.00 (C-H str)

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 2.39 (s, 2H, C*H*$_2$), 2.55 (s, 2H, 2 x C*H*), 3.35 (s, 3H, 3 x O*H*), 6.97 (m, 4H, Pyridine), 7.57 (m, 1H, aromatic), 7.67 (m, 1H, aromatic), 8.07 (m, 1H, aromatic), 8.12 (m, 1H, aromatic) and 8.31 ppm (m, 1H, aromatic).
^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 78.67 (*C*H$_2$), 79.45 (*C*H), 117.83 (Ar*C*), 119.24 (Ar*C*), 119.54 (Ar*C*), 119.59 (Ar*C*), 120.29 (Ar*C*), 124.87 (Ar*C*), 125.23 (Ar*C*), 125.76 (Ar*C*), 126.74 (Ar*C*), 134.98 (Ar*C*), 143.01 (Ar*C*), 143.30 (3 x Ar*C*), 145.16 (Ar*C*), 147.94 (*C*-OH), 151.20 (*C*-OH), 160.20 (*C*-OH) and 160.59 ppm (*C*=O).

SVII (2-(4-hydroxybenzyl)-5,7-dihydroxy-3-(piperidin-4-yloxy) chroman-4-one)

%Yield: 33%; M.Pt. 298-303 ℃ IR (cm^{-1}):3268.71(O-H), 3174.14(C-H), 1630.49(C=O), 1577.81(C=N str ring), 1361.12(C=C), 1168.45(C-O-C), 1086.00 (C-H str)
^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 2.42 (m, 4H, 2C*H*$_2$), 3.35 (t, 2H, -C*H*$_2$), 3.70 (t, 2H, -C*H*$_2$), 4.91 (s, 3H, O*H*), 7.47 (m, 1H, aromatic), 7.57 (m, 1H, aromatic), 7.69 (s, 1H, aromatic), 7.81 (m, 1H, aromatic), 7.91 (m, 1H, aromatic) and 8.16 ppm (d, 1H, aromatic).
^{13}C NMR(DMSO-*d*$_6$, 100 MHz): δ 20.58 (*C*H$_2$), 55.83 (*C*H$_2$), 68.63 (*C*H$_2$), 76.63 (*C*H$_2$) 116.83 (Ar*C*), 117.64 (Ar*C*), 119.02 (Ar*C*), 120.39 (Ar*C*), 123.03 (Ar*C*), 125.08 (Ar*C*), 130.36 (Ar*C*), 130.92 (Ar*C*), 134.96 (Ar*C*), 138.93 (2 x Ar*C*), 143.16 (quaternary aromatic carbon attached to OH), 143.92 (quaternary aromatic carbon attached to OH), 152.39 (quaternary aromatic carbon attached to OH) and 176.63 ppm (*C*=O).

SVIII (3-(1*H*-pyrrol-2-yloxy)-2-(4-hydroxybenzyl)-5,7-dihydroxychroman-4-one)

% yield= 42 M.Pt. 302-307 ℃ IR (cm^{-1}):3268.71(O-H), 3174.14(C-H), 1630.49(C=O), 1577.81(C=N str ring), 1361.12(C=C), 1168.45(C-O-C), 1086.00 (C-H str)
^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 2.09 (s, 1H, C*H*$_2$), 2.39 (s, 1H, C*H*$_2$), 3.35 (s, 3H, O*H*), 6.97 (m, 3H, aromatic), 7.43 (m, 1H, aromatic), 7.57 (m, 1H, aromatic), 7.67 (m, 1H, aromatic), 8.07 (m, 1H, aromatic), 8.32 (m, 1H, aromatic) and 8.97 ppm (m, 3H, aromatic).
^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 20.58 (*C*H$_2$), 55.83 (*C*H$_2$), 68.63 (*C*H$_2$), 76.63 (*C*H$_2$), 78.88 (Ar*C*), 102.81 (Ar*C*), 116.83 (Ar*C*), 117.64 (Ar*C*), 119.02 (Ar*C*), 120.39 (Ar*C*), 123.03 (Ar*C*), 125.08 (Ar*C*), 130.36 (Ar*C*), 130.92 (Ar*C*), 134.96 (Ar*C*), 138.93 (2 x Ar*C*), 143.16 (quaternary aromatic carbon attached to OH), 143.92 (quaternary aromatic carbon attached to OH), 152.39 (quaternary aromatic carbon attached to OH) and 176.63 ppm (*C*=O).

SIX 2-(4-hydroxybenzyl)-5,7-dihydroxy-3-(pyrazin-2-yloxy) chroman-4-one: Yield

46%; M.Pt. 322-328 ℃
IR (cm^{-1}):3266.71(O-H), 3174.14(C-H), 1628.49(C=O), 1577.81(C=N str ring), 1361.12(C=C), 1168.45(C-O-C), 1085.00 (C-H str)
^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 2.09 (s, 1H, C*H*$_2$), 2.39 (s, 1H, C*H*$_2$), 3.35 (s, 3H, O*H*), 7.43 (m, 1H, aromatic), 7.57 (m, 1H, aromatic), 7.67 (m, 1H, aromatic), 8.07 (m, 1H, aromatic), 8.32 (m, 1H, aromatic) and 8.97 ppm (m, 3H, aromatic).

^{13}C NMR (DMSO-d_6, 100 MHz): δ 27.29 (*C*H_2), 78.67 (*C*H), 117.83 (2 x Ar*C*), 119.24 (Ar*C*), 119.54 (Ar*C*), 120.29 (Ar*C*), 124.87 (Ar*C*), 125.23 (Ar*C*), 125.71 (Ar*C*), 125.81 (Ar*C*), 126.74 (Ar*C*), 134.98 (3 x Ar*C*), 145.16 (Ar*C*), 147.94 (*C*-OH), 151.20 (*C*-OH), 160.20 (*C*-OH) and 160.59 ppm (*C*=O).

SX (2-(4-hydroxybenzyl)-5,7-dihydroxy-3-(pyridin-2-yloxy) chroman-4-one)

Yield: 45%; M.Pt. 306-309 ℃
IR (cm^{-1}):3268.71(O-H), 3174.14(C-H), 1630.49(C=O), 1577.81(C=N str ring), 1361.12(C=C), 1168.45(C-O-C), 1086.00 (C-H str)
^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.39 (s, 2H, CH_2), 3.35 (s, 3H, O*H*), 7.43 (m, 2H, aromatic), 7.57 (m, 2H, aromatic), 7.67 (m, 3H, aromatic), 8.07 (m, 2H, aromatic), 8.32 (m, 1H, aromatic) and 8.97 ppm (m, 2H, aromatic).
^{13}C NMR (DMSO-d_6, 100 MHz): δ 49.29 (*C*H_2), 78.67 (*C*H), 117.83 (2 x Ar*C*), 119.24 (Ar*C*), 119.54 (Ar*C*), 120.29 (Ar*C*), 124.87 (Ar*C*), 125.23 (Ar*C*), 125.71 (Ar*C*), 125.81 (Ar*C*), 126.74 (Ar*C*), 134.98 (3 x Ar*C*), 143.01 (Ar*C*), 143.30 (Ar*C*), 145.16 (Ar*C*), 147.94 (*C*-OH), 151.20 (*C*-OH), 160.20 (*C*-OH) and 160.59 ppm (*C*=O).

Molecular docking

The docking study were performed using MVD 6.0.2 software. The docking results as Mol dock score, rerank score, interaction energies, numbers of H-bond(s) with their energies and interactions of ligand with amino acids of protein 1CX2 are shown in Table 2. The docking pose of the new chromane, SII and SVI molecule are reflected in Figure 1 shows their high number of interactions with annotations. New chromane as parent and ten relevant O-substituted (C-3) compounds (SI to SX) were docked into the active pockets of the enzymes COX-2 (PDB ID: 1CX2) which showed better docking scores in the range of -68.44 to -105.74 with good hydrogen interactions and docking scores so considered as good analgesic and anti-inflammatory agents. From which ligand SII and SVI showed good mol dock score -77.59 and -75.75 with high number of H-interactions (09) w.r.t reference quercetin and diclofenac sodium. (Table 2)

Table 2: The Mol Dock score, rerank score and hydrogen bond interaction energy of the different substituents of COX 2 (PDB id: 1CX2)

Compounds	Moldock score	Rerank score	H-bond	Interactions	Energy	Distance Annotation	Ligand	Protein
Parent (Chromane)	-105.608	-66.28	-9.149	07	-2.5	2.85	[O] C-3	[O]Gly551
					-2.5	2.68	[O] C-3	[O]Cys569
					-2.48	2.81	[O] C-3	[O]Cys569
					-1.05	3.39	[O] C-7	[O]Cys57
					-2.5	3.10	[O] C-7	[O]Asp58
					-2.5	2.80	[O] C-5	[O]Cys56
					-1.41	3.31	[O] C-5	[O]Glu46
S1	-71.73	-62.59	-8.64	05	-2.5	3.06	[O] C-7	[O]Glu346
					-2.5	3.04	[O] C-7	[O]Arg109
					-2.5	2.70	[O] C-7	[O]Arg109
					-2.5	2.71	[O] C-4'	[O]Glu364

					-1.55	2.35	[O] C-5	[O]Asn560
S2	-77.59	-57.91	-10.70	**9**	-2.49	3.10	[O] C-3	[O]Cys360
					-2.5	3.00	[O] C-3	[O]Cys569
					-2.5	2.67	[O] C-4’	[O]Asn104
					-2.5	3.09	[O] C-4’	[O]Asn105
					-1.62	3.28	[O] C-4’	[O]Pro106
					-2.49	2.81	[O] C-5	[O]Cys56
					-2.5	2.92	[O] C-7	[O]Phe361
					-2.5	2.88	[O] C-7	[O]Asn560
					-1.35	3.33	[O] C-7	[O]Asp362
S3	-68.74	-0.69	-7.28	08	-1.96	3.21	[O] C-1	[O]Cys557
					-0.17	2.46	[O] C-4	[O]Trp545
					-2.5	2.67	[O] C-5	[O]Glu364
					-1.03	3.39	[O] C-5	[O]Trp545
					-0.29	3.52	[O] C-5	[O]Arg61
					-1.98	3.20	[O] C-4’	[O]Glu346
					-2.5	3.10	[O] C-4’	[O]Phe361
					-2.25	3.04	[O] C-4’	[O]Lys342
S4	-82.39	-56.98	-7.57	07	-2.5	2.82	[O] C-4’	[O]Asn104
					-2.5	2.83	[O] C-7	[O]Phe361
					-2.5	2.68	[O] C-7	[O]Lys342
					-2.5	2.60	[O] C-7	[O]Glu346
					-0.19	3.54	[O] C-7	[O]Phe361
					-2.15	3.90	[O] C-5	[O]Asn560
					-2.15	3.90	[O] C-5	[O]Asn560
S5	-75.50	-56.70	-8.81	06	-2.5	2.60	[O] C-4’	[O]Cys57
					-0.56	3.49	[O] C-4’	[O]Asp58
					-2.5	3.03	[O] C-5	[N]Lys557
					-2.2	2.94	[O] C-7	[N]Arg61
					-2.48	2.60	[O] C-7	[O]Trp545
					-2.5	3.02	[O] C-7	[O]Glu364
S6	-75.75	-44.86	-9.32	9	-0.57	3.84	[O] C-1	[N]Lys36
					-2.47	2.60	[O] C-4’	[O]Asn105
					-2.49	2.60	[O] C-4’	[O]Asn104
					-2.5	3.02	[N] C-2’	[O]Lys358
					-2.5	2.88	[N] C-2’	[N]Arg109
					-1.31	3.34	[O] C-5	[O]Asn560
					-2.5	2.97	[O] C-7	[O]Asp362
					-1.65	3.27	[O] C-7	[O]Asn560
					-1.84	3.23	[O] C-7	[O]Phe361
S7	-91.08	-65.04	-4.47	05	-2.5	2.62	[O] C-7	[O]Glu339
					-2.5	3.00	[O] C-1	[O]Ser566
					-1.49	3.38	[O] C-4’	[O]Gln270
					-0.78	3.16	[O] C-4’	[N]Gln270
					-1.16	3.37	[O] C-4’	[O]Lys243
S8	-105.74	-72.51	-2.51	04	-2.5	2.93	[O] C-4’	[O]Ser566
					-2.5	2.67	[O] C-4’	[O]Asp268
					-0.80	3.44	[O] C-4’	[O]Asp268
					-0.18	3.04	[O] C-7	[O]Asp239
S9	-68.44	-59.11	-6.63	07	-2.48	3.08	[O] C-5	[O]Asp362

					-2.5 -2.14 -2.5 -2.5 -1.17 -0.99	2.75 3.17 2.80 3.10 3.37 3.40	[O] C-5 [O] C-5 [N] [O] C-4' [O] C-4' [O] C-7	[O]Phe361 [O]Asn560 [N]Lys342 [O]Pro106 [O]Ser110 [O]Glu364
S10	-80.00	-61.53	-8.07	07	-1.30 -2.5 -1.69 -1.37 -0.27 -2.5 -2.5	2.97 2.63 2.96 3.32 3.48 2.60 3.10	[O] C-4' [O] C-5 [O] C-5 [O] C-5 [O] C-4 [O] C-7 [O] C-7	[O]Asp239 [O]Gln270 [N]Gln270 [O]Asp268 [N]Gln270 [O]Arg253 [N]Lys253
Diclofenac sodium	-64.15	-52.29	-2.43	4	-2.47 -2.49 -2.43 -2.14	3.10 2.60 2.59 3.17	O-H C=O O-H N-H	[O] Glu 346 [N] Lys 342 [O] Phe 361 [O]Glu 346
Quercetin	-79.02	-63.83	-12.76	9	-2.48 -2.49 -2.5 -0.38 -2.5 -1.29 -1.60 -2.5 -0.82	2.60 2.60 3.01 3.41 2.60 3.34 2.61 2.80 2.80	[O] C-3' [O] C-3' [O] C-4' [O] C-4' [O] C-3 [O] C-3 [O] C-4 [O] C-5 [O] C-5	[O]Lys342 [O]Glu346 [N]Arg109 [N]Arg109 [O]Phe361 [O]Asn560 [O]Trp545 [O]Trp545 [O]Glu364

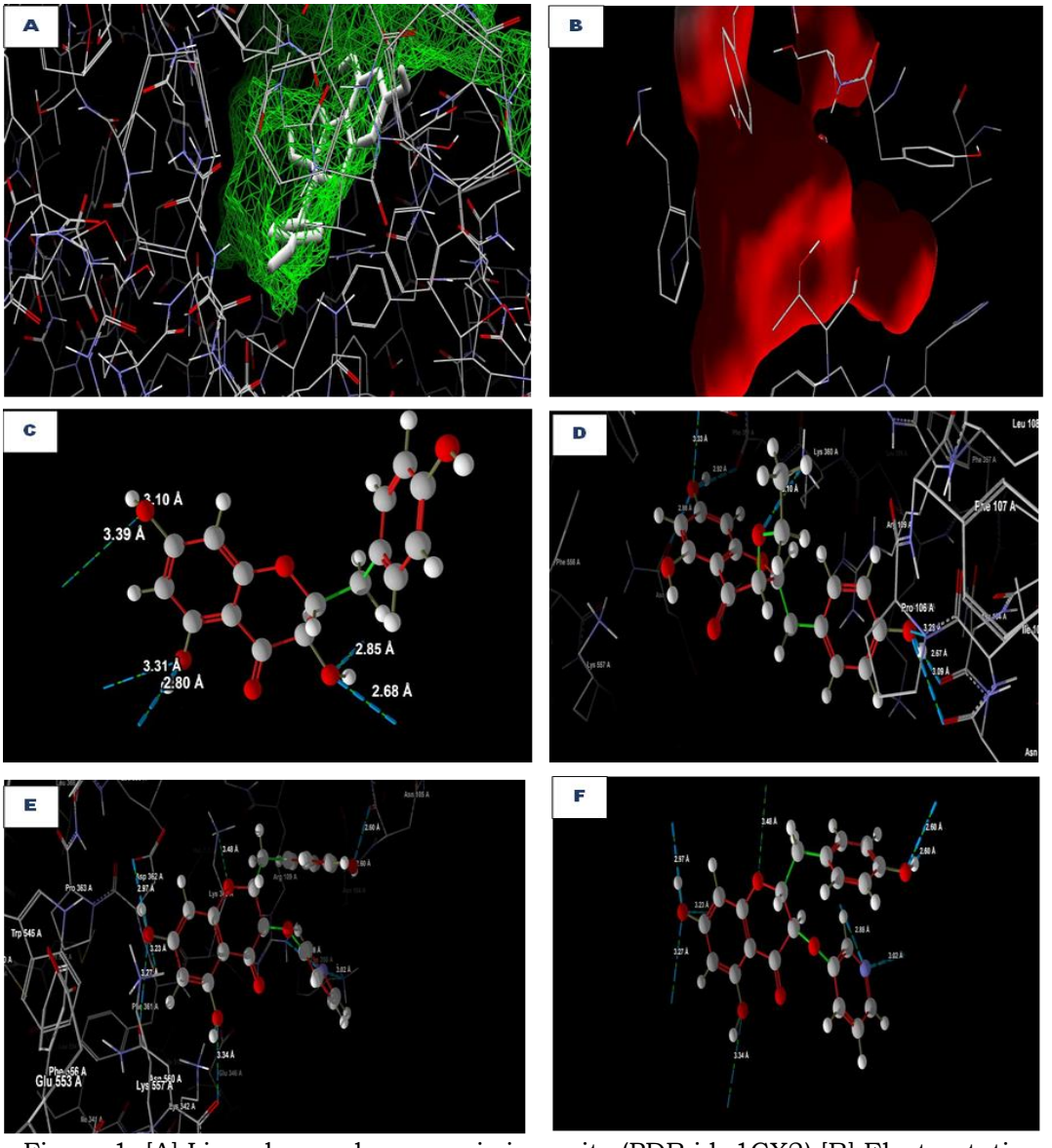
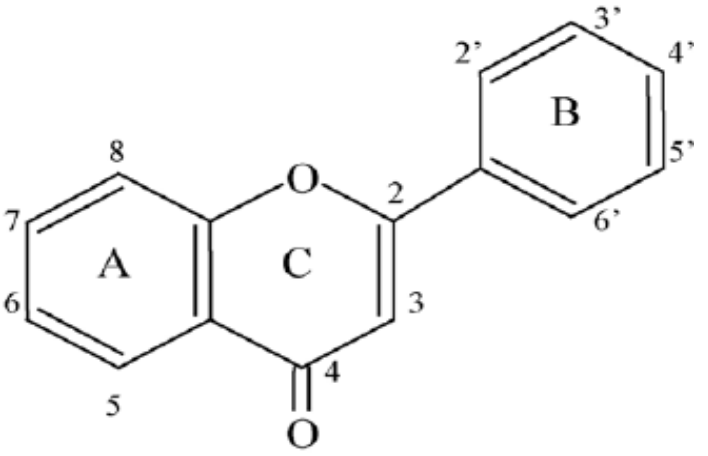


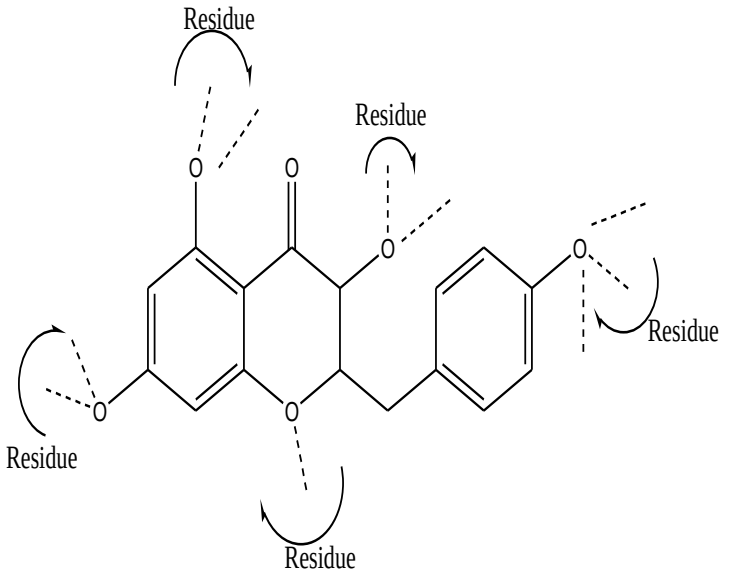
Figure 1: [A] Ligand new chromane is in cavity (PDB id: 1CX2) [B] Electrostatic interactions [C] H-interactions of parent with annotations [D] Hydrogen interactions of ligand SII with 1CX2 [E] Hydrogen interactions of ligand SVI with 1CX2 [F] Annotations of ligand SVI with 1CX2

Structure Activity Relationship (SAR)

- Hydroxylation at C-3 of C-ring, C-5,7 of A-ring and C-4' of B-ring as well as 2,3 C=C in C-ring are important for the inhibitory activity of flavonoids.



- All Oxygen atoms at C3, C5, C7 and C4' of ligand SII shows high number of hydrogen interactions (9) with a good Mol dock score, re-rank score and H-bond energy -77.59, -57.91 and -10.70. It means -O- is mostly required and plays an important role in bindings of ligand with protein COX2(cyclooxygenase-2 or (prostaglandin synthase-2)



Ligand new chromane with 1CX2 protein

- By following relevant literature of flavonoid synthesis and ligand protein interactions, substitutions should be preferred as -O substitution with alkyl, aromatic and hetero groups resulting mono (C-3) substituted compounds (Table 1).
- C-3 position of saturated C-ring substitutions shows feasibility and efficacy so this position considered for synthesis. [20-21]

- As docked compounds show good interactions with 1CX2 protein so suggestive mechanism of action of new chromane and derivatives is COX2 inhibition which signs for anti-inflammatory and analgesic activity.

Conclusion

In conclusion, we have developed an efficient methodology to synthesize the derivatives of the title compounds, namely 2-(4-hydroxybenzyl) 3,5,7-trihydroxychroman-4-one. The spectroscopic data confirmed the composition and the structure of the newly synthesized compounds. The investigated molecules are displayed in similar manner of protein binding to the active site of COX2 (PDB ID: ICX2) on molecular docking studies. The calculated docking energies indicated that interaction of SI to SX with selected protein is more favourable but only to a limited extent. Structure–activity relationship studies of the 3–(O-R) substituted chroman-4-one have confirmed that the conformational structure plays an important role in the potency inhibitory activity displayed by SII and SVI. This study has revealed potent COX2 inhibitors with good predicted moldock, rerank score and good interactions with that are useful for further investigation for their in vitro as well as in vivo activity towards COX2 receptor.

The study thus serves an attempt to progress towards the discovery of novel anti-inflammatory and analgesic drugs. Additional derivatives may be prepared and further extended for in-depth investigations so as to establish more points in SAR based on rational study. In conclusion, the designed new chromane scaffold is an interesting anti-inflammatory and analgesic pharmacophore and considered as novel lead scaffold of chromane family for any future optimization.

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