Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(4):1125-1135

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and docking studies of novel series of 7-(*o*-triazolo pyridazin-3yl)-4-methyl coumarins

Rama K.*, Sumakanth M. and Anupama S. P.

Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad, Telangana-500007

ABSTRACT

Coumarin containing heterocycles attract major attention due to their wide range of therapeutic activities. In our present work, a novel series of 4-methyl-7-[(6- substituted phenyl-7,8-dihydro-[1,2,4]triazolo[4,3,b]pyridazin-3yl)methoxy)-2H-chromen-2-one and 4-methyl-7-[(6- substituted phenyl-[1,2,4]triazolo[4,3,b]pyridazin-3yl)methoxy)-2H-chromen-2-one were synthesized in a convenient synthetic route. Docking studies of these compounds were performed to evaluate the binding affinities of the compounds with cyclooxygenase enzyme (cox-2 with PDB ID: 3LN1). The results showed good correlation in enzyme inhibitory activity with celecoxib (co-crystallized ligand).

Key words: Coumarin, Triazolo Pyridazine, Cyclooxygenase-2, Docking studies

---

INTRODUCTION

Compounds possessing the coumarin moiety (2*H*-1-benzopyran-2-one) constitute an important class of heterocycles, many examples of which are found in nature [1]. Coumarins can be divided into four sub-types[2]: i) simple Coumarins which are hydroxylated, alkoxylated or alkylated on the benzene ring (ii) Furanocoumarins, which contain a five-membered furan ring attached to the coumarin moiety and which are sub-divided into the linear furanocoumarins and the angular furanocoumarins (iii) pyranocoumarins, containing a six-membered ring attached to the coumarin moiety and (iv) Coumarins with substituent in the pyrone ring. From the literature, it was evident that among the various coumarin derivatives, 7-hydroxy-4-methyl coumarin is an important synthetic building block due to its ease of functionalisation at various ring positions and also exhibits various activities like anti-inflammatory, anti-microbial[2],anti-HIV[3]etc. It was also found that O-acylation of coumarin enhanced the biological activity.

In this context, it has been planned to synthesize O-substituted Coumarins wherein a triazolo ring is to be substituted by exploiting the reactivity of methylene group of 0-acylated coumarin and reactivity of -NH and oxo group of pyridazine-3[2H]-one. To estimate the cox-2 antagonistic activity of synthesized compounds, docking is to be performed using 'GLIDE' module of Schrodinger software.[4]

EXPERIMENTAL SECTION

All the chemicals (starting materials, solvents, reagents) used in this work were laboratory grade. Proton Nuclear Magnetic Resonance Spectra was recorded on AVANCE 300 MHz spectrophotometer using Tri Methyl

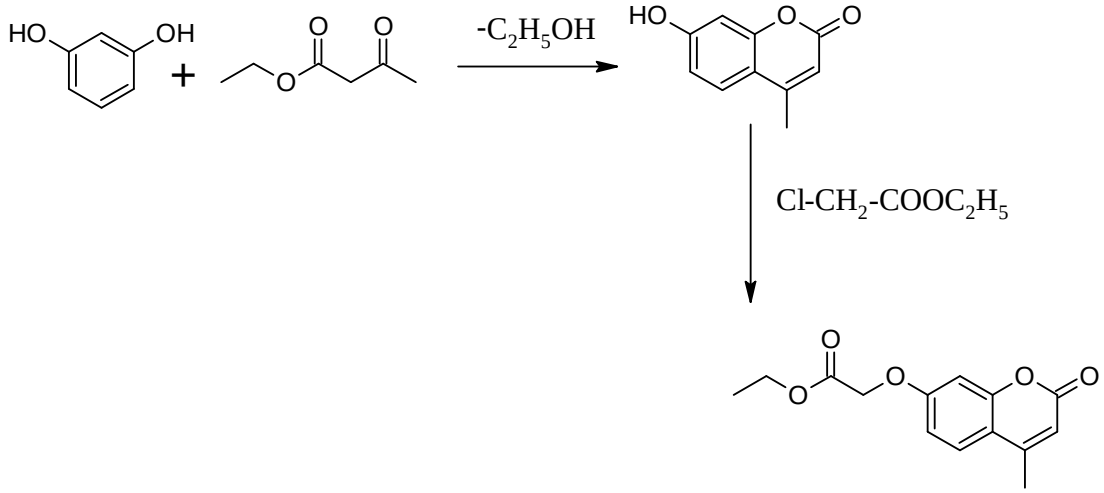
Silane(TMS) as internal standard. Chemical shifts were expressed in ppm units downfield from TMS. Melting points were recorded on BTI melting point apparatus, using one-end open capillary. IR spectra were scanned on SCHIMADZU spectrometer with Potassium Bromide Disc .All the reactions were monitored by analytical Thin Layer Chromatography (TLC) using E-merc 0.25 Silica gel plate. Visualization was accomplished with UV light (256nm) and Iodine chamber.

PROCEDURE FOR THE SYNTHESIS OF 7-HYDROXY-4-METHYL COUMARIN:

In a cleaned three necked flask, equipped with thermometer and mechanical stirrer, concentrated sulphuric acid(30ml,0.3mol) was taken and cooled to 5^0C within an ice-bath. Resorcinol (0.03)mol was dissolved in Ethyl acetoacetate(0.05)mol and was taken in a dropping funnel. Then the mixture was added drop wise into the flask with continuous stirring for 30min and progress of the reaction was monitored by TLC plated on silica gel using Ethylacetate: Chloroform(6:4). After completion of the reaction, the mixture was poured into 1kg of crushed ice with vigorous stirring. Precipitate was collected by suction pump and washed with cold water. Solid was dissolved in 5% NaOH solution and 2M Sulphuric acid was added with vigorous stirring until the solution was acid to litmus. Finally coumarin was collected, filtered with the help of suction pump and washed with cold water. It was then re-crystallized by using ethanol.M.P-180-190^0 C. Yield(90%)

GENERAL PROCEDURE FOR THE SYNTHESIS OF ETHYL 2-(4-METHYL-2-OXO-2H-CHROMEN-7-YLOXY) ACETATE:

To the solution of 7-hydroxy-4-methyl coumarin (0.1mole) in ethanol, small quantity of potassium carbonate solution was added and stirred until a clear solution is obtained. Ethylchloroacetate(0.2moles)was added drop wise and refluxed for 4-5 hours and progress of the reaction was monitored by TLC using ethylacetate:chloroform(6:4).Thus obtained solution was transferred into ice and precipitate obtained was filtered, dried and was re-crystallized from ethanol.M.P.170-171 ^{0}C.



SYNTHESIS OF 6-ARYL-4, 5-DIHYDROPYRIDAZIN-3(2H)-ONES (IIIa-e)

Step-1: SYNTHESIS OF β-AROYL PROPIONIC ACIDS (IIa-e)

To a 1 liter round bottomed flask with a mechanical stirrer and condenser carrying a gas outlet connected to a scrubbing system, aromatic hydrocarbon (2.25moles) and succinic anhydride (0.34moles) were added. The reaction mixture was stirred and powdered anhydrous aluminium chloride (6.75moles) was added in installments. After the addition, the reaction mixture was heated under reflux in an oil bath for one hour. The reaction mixture was cooled and the contents were slowly added to a mixture of ice 150gm and concentrated hydrochloric acid 50ml. The separated organic layer was subjected to steam distillation. The residue after steam distillation was cooled, separated solid was filtered off and washed with 100ml of cold dilute HCl followed by cold water. The crude product was taken in aqueous Na_2CO_3 solution (prepared by dissolving 40gm of Na_2CO_3 in 250ml of water) and heated under reflux for 10-15 minutes to get a clear solution, which was treated with charcoal (2g) and filtered. The filtrate was cooled to room temperature and acidified with 60-70-ml of concentrated HCl and the mass was cooled at 10℃ for

1hour. The separated solid was filtered and washed with cold water (100ml) and dried to yield β-aroyl propionic acid.

Step 2: A mixture of appropriate β-aroyl propionic acid (0.1moles) and hydrazine hydrate (0.21moles) in ethanol (100ml) placed in a round bottomed flask was heated under reflux for 3 hours on a water bath. The reaction mixture was concentrated under vacuum and cooled to room temperature to afford a crystalline product. (IIIa-e)

PROCEDURE FOR SYNTHESIS OF 6-ARYL-PYRIDAZIN-3(2H)ONES (IVa-e)

To a solution of 6-(substituted phenyl)-4,5-dihydro-pyridazin-3(2H)ones(0.05moles) of DimethylSulphoxide (DMSO) ,0.05moles of chloranil was added and heated at 80^oC for 3 hours with continuous stirring. The completion of the reaction was confirmed from TLC using solvent system, Ethyl acetate: Chloroform(6:4).The reaction mixture was poured into crushed ice and the solution was filtered off under vacuum to obtain 6-aryl-pyridazin-3(2H)ones (IVa-e)

PROCEDURE FOR THE SYNTHESIS OF 2-(2-(4-METHYL-2-OXO-2H-CHROMEN-7-YLOXY)ACETYL)-6-(SUBSTITUTEDPHENYL)-4,5-DIHYDROPYRADIZINE3(2H)-ONE (Va-e)AND 2-(2-(4-METHYL-2-OXO-2H-CHROMEN-7-YLOXY)ACETYL)-6-(SUBSTITUTEDPHENYL)PYRADIZINE3(2H)-ONE(VIIa-e):

The ethyl -2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetate (0.1mole) on treatment with 6-aryl 4,5-dihydropyridazinones/6-aryl Pyridazin-3(2H)ones(0.2) in the presence of sodium metal in ethanol and reflux it for 6hrs and progress of the reaction was monitored by TLC plated on silica gel using ethylacetate:chloroform(6:4) formed the intermediates Va-e and VIIa-e

GENERAL PROCEDURE FOR THE SYNTHESIS OF 4-METHYL-7-[(6- SUBSTITUTED PHENYL-7,8-DIHYDRO[1,2,4]TRIAZOLO[4,3,b]PYRIDAZIN-3-YL)METHOXY)-2H-CHROMEN-2-ONE (VIa-e) AND 4-METHYL-7-[(6- SUBSTITUTED PHENYL)[1,2,4]TRIAZOLO[4,3,b]PYRIDAZIN-3-YL)METHOXY)-2H-CHROMEN-2-ONE :(VIIIa-e)

The intermediate (Va-e and VIIa-e)(0.1mol) were subjected to cyclization with hydrazine hydrate(0.15) in ethanol and refluxed it for 6hrs and progress of the reaction was monitored by TLC plated on silica gel using ethylacetate:chloroform(6:4) to form the cyclized product on distilling of ethanol to form title compounds **.**The final compounds were recrystallized from ethanol. Adopting this procedure, the following ten compounds were synthesized:

(a)4-methyl-7-[(6-phenyl-7,8-dihydro[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one(VIa): Recrystallized from ethanol to get colorless crystals. Yield=4.0gm(70.13.%);M.P.139-141^0C.IR(KBr,ν,cm-1):1751(C=O),1570,1610,1590cm^{-1} (C=N) ,2937,2757 (CH_2) 767,624,562(C-H)$_{def}$

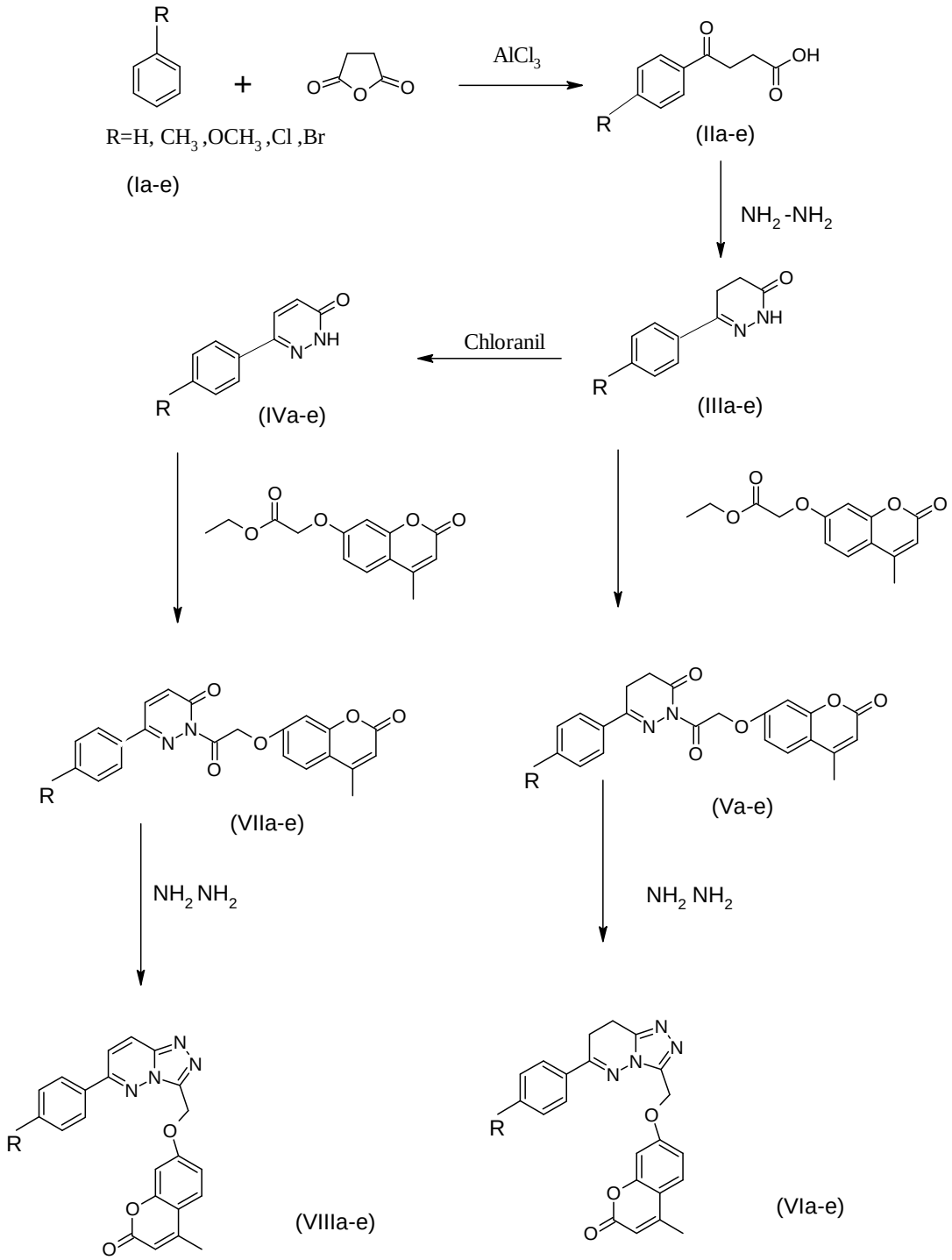
(b)4-methyl-7-[(6- methyl phenyl-7,8-dihydro[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one(VIb)::Recrystallized from ethanol to get colourless crystals,Yield=4.5gm(76.96%);. M.P.142-145^0C. IR(KBr,ν,cm-1)1751(C=O),1570,1610, 1590 (C=N), 2945,2740 (CH_2) 764,618,534(C-H)$_{def}$

(c)**4-methyl-7-[(6-methoxyphenyl-7,8-dihydro[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one((VIc):**Recrystallized from ethanol to get colourless crystals.Yield:5.5gm(75%); M.P.139-140^0C. IR(KBr,ν,cm-1)1721(C=O),1560,1630,1570 (C=N), 2945,2740 (CH_2) 764,618,534(C-H)$_{def}$,1247,1058 (OCH_3 **)**

(d)4-methyl-7-[(6-chlorophenyl-7,8-dihydro[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one(VId): Recrystallized from ethanol to get colourless crystals.Yield:5.8gm(75.92%);M.P.169-170^0C.IR(KBr,ν,cm-1)1740(C=O),1540,1610,1550 (C=N), 2945,2740 (CH_2) 764,618,534(C-H)$_{def}$,623(C-Cl)

(e)4-methyl-7-[(6-bromophenyl-7,8-dihydro[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one(VIe)::Recrystallized from ethanol to get colourless crystals,yield:5.8gm(75.23%); M.P .150-155^0 C. IR(KBr,ν,cm-1)1730(C=O),1530,1620,1530 (C=N), 2745,2840 (CH_2) 750,650,524(C-H)$_{def}$,550(C-Br)

(f)4-methyl-7-[(6-phenyl[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one(VIIIa):
Recrystallized from methanol to get colourless crystals,yield:7.3gm(82.12%); M.P 143-144^0.IR(KBr, ν,cm^{-1})1735cm^- (C= O)$_{str}$,1591,1570, 1558 (C=N) , 767,624,562, cm^{-1}(C-H)$_{def}$



SCHEME

(g)4-methyl-7-[(6-methyl phenyl[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one(VIIIb): Recrystallized from methanol to get colourless crystals,yield:7.1gm(83.33%); M.P.147-148^{0}C. .IR(KBr, ν,cm^{-1})1789(C= O),1591,1570,1555(C=N) (C-H)$_{def}$ 767,624,562, cm$^-$

(h)4-methyl-7-[(6- methoxy phenyl[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one(VIIIc):): Recrystallized from methanol to get colourless crystals.Yield:7.35gm(86.33%);M.P.142-143^{0}C.IR(KBr,ν,cm-^{1})1755(C=O)1591,1570,1558 (C=N), 768,654,582(C-H)$_{def}$ 1247 (OCH_3 **)**

(i)4-methyl-7-[(6- chloro phenyl[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one(VIIId): Recrystallized from methanol to get colourless crystals.Yield:7.5gm(75.52%); M.P.172-173^{0}C. IR(KBr,ν,cm-^{1})1735cm^{-1}(C=O)1591,1570, 1558 (C=N))670(C-Cl)

(j) 4-methyl-7-[(6- bromo phenyl[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)-2H-chromen-2-one(VIIIe): Recrystallized from methanol to get colourless crystals,yield:8.9gm(80.45%);M.P.150-155^{0}C.IR(KBr,ν,cm-^{1})1790(C=O),1591,1570,1558(C=N) 642,562,346(C-H)$_{def}$588,(C-Br)$_{def}$

Molecular docking studies:
Docking is the formation of non-covalent protein-ligand complexes. Binding events between ligands and their receptors in biological system form the basis of physiological activity and pharmacological effects of chemical compounds [5]. Docking is the computational tool to investigate the binding affinity between macromolecular targets and the potential ligands. The process involves the prediction of ligand conformation and orientation(or posing) within a targeted binding site[6] .

Docking methodology:
Docking was executed by using the module 'GLIDE' of 'Schrodinger' software in maestro 9.3.

Protein preparation:
The Crystalline COX-2 enzyme (PDB ID 3LN1) was retrieved from protein data bank. The protein was prepared to ensure its structural correctness using Protein Preparation wizard in maestro 9.3 of Schrodinger software. In executing this application, of the four chains of tetramer protein, chain A was selected .The waters beyond 5 A^0 from heterogeneous group were deleted, those waters within 5 A^0 having significant interaction with protein, ligand and metal ion Zn^{+2} are retained. Finally the protein was minimized by converging heavy atoms to RMSD 0.3 A^0 using force field OPLS 2005.

Grid generation:
The binding site of the prepared protein was determined by generating the grid (rectangular box) around the ligand in the protein using 'Receptor grid generation' module of 'Glide (of Schrodinger software).

Ligand preparation:
The possible tautomers (at $p^H \pm 7$ 2) of the synthesized compounds were generated by retaining specified chiralities using 'ligprep' module.

Ligand docking:
Flexible docking was accomplished using OPLS 2005 force field. The output format was set to pose viewer file so as to view the output of the resulting docking studies from pose viewer.

The entire process was validated by performing 'Enrichment calculations' .The docking results were analyzed by examining the glide scores and the ligand interactions with the protein along with MMGBSA values.

RESULTS AND DISCUSSION

CHEMISTRY:
6-aryl - 4, 5 dihydro Pyridazin -3(2H)-ones (IIIa-e) were synthesized by a known convenient method.Ethyl-2-(4-methyl-2-oxo-2h-chromen-7-yloxy)acetate was synthesized from 7-hydroxy-4-methyl coumarin by the action of ethylacetoacetate. Intermediates (Va-e) were synthesized by the reaction of III(a-e) with the Ethyl-2-(4-methyl-2-oxo-2h-chromen-7-yloxy)acetate and cyclization of the same was brought about by the action of hydrazine hydrate to form the title compounds 4-methyl-7-[(6- substituted phenyl-7,8-dihydro[1,2,4]triazolo[4,3,b]pyridazin-3-yl)methoxy)-2H-chromen-2-one.(VIa-e)

By the action of chloranil, dehydrogenation of 6- aryl -4,5 dihydropyridazin 3-ones (IIIa-e) was achieved in presence of aprotic solvent such as DMSO resulting in the formation of compounds(IV a-e) i.e 6- aryl Pyridazin

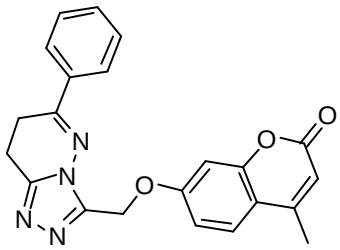
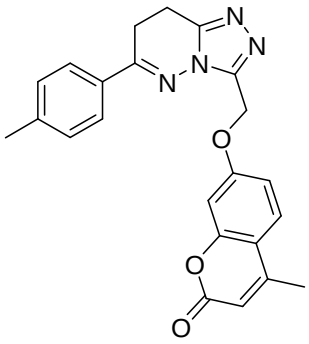
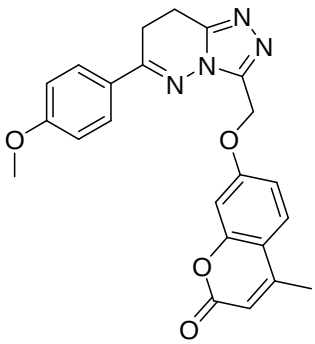
3(2H)-ones . By taking 6- aryl –Pyridazin 3(2H)-ones, another series of 4-methyl-7-[(6- substituted phenyl-[1,2,4]triazolo[4,3,b]pyridazin-3-yl)methoxy)-2H-chromen-2- one (VIIIa-e) were synthesized as per scheme in a similar way . The compounds were characterized by IR and ^{1}H NMR. Thus our main objective of synthesizing 7-(O-Triazolopyridazin-3yl)-4-methyl Coumarins in a simpler way has been accomplished.

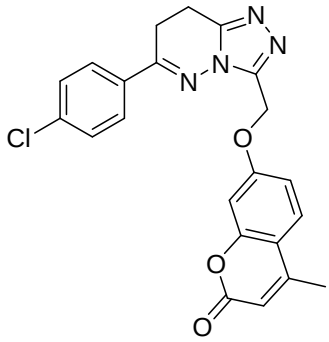
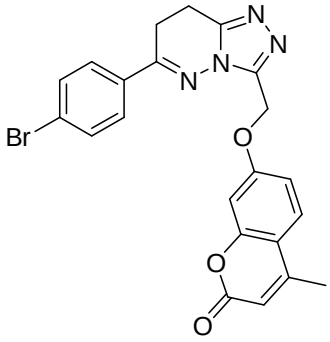
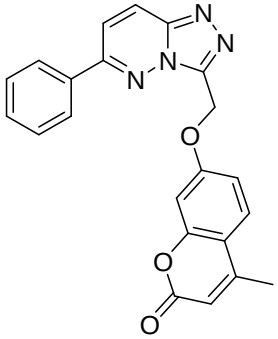
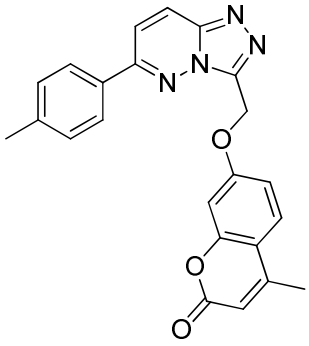
DOCKING RESULTS

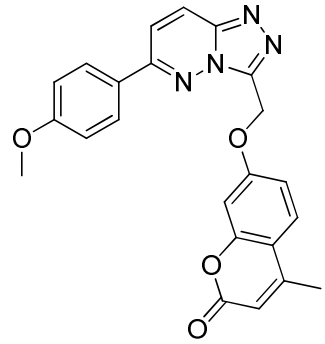
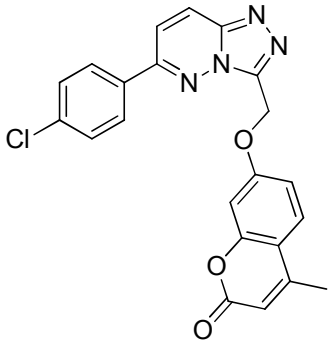
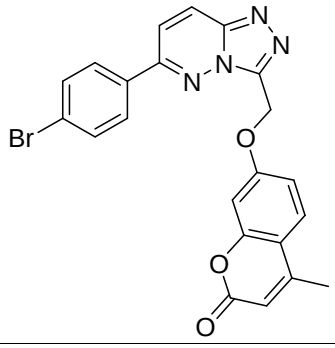
The molecular docking of title compounds into the active site of cox-2 (PDB ID: 3LN1) was carried out using Glide and the evaluation was made on the Glide Score.(table 1).The G-score values were ranging between -8.0 to -10.0. Compounds VIb and VIa showed the best G-Score value of -10.0 and -9.8 respectively. More negative Glide Score indicates the better interaction of the inhibitor with the target protein

The compounds were positioned in a similar orientation to that of celecoxib and showed strong hydrogen bonding interactions between the oxygen atom of the coumarin ring present at 2nd position and the amino acid residues such as Arg 499 and Tyr 371 of protein which was similar to that of celecoxib. The binding site of the protein is the hydrophobic pocket comprising of amino acids Ala 502, Leu 345, Tyr 373, Met 508, Phe 504, and Val 509.

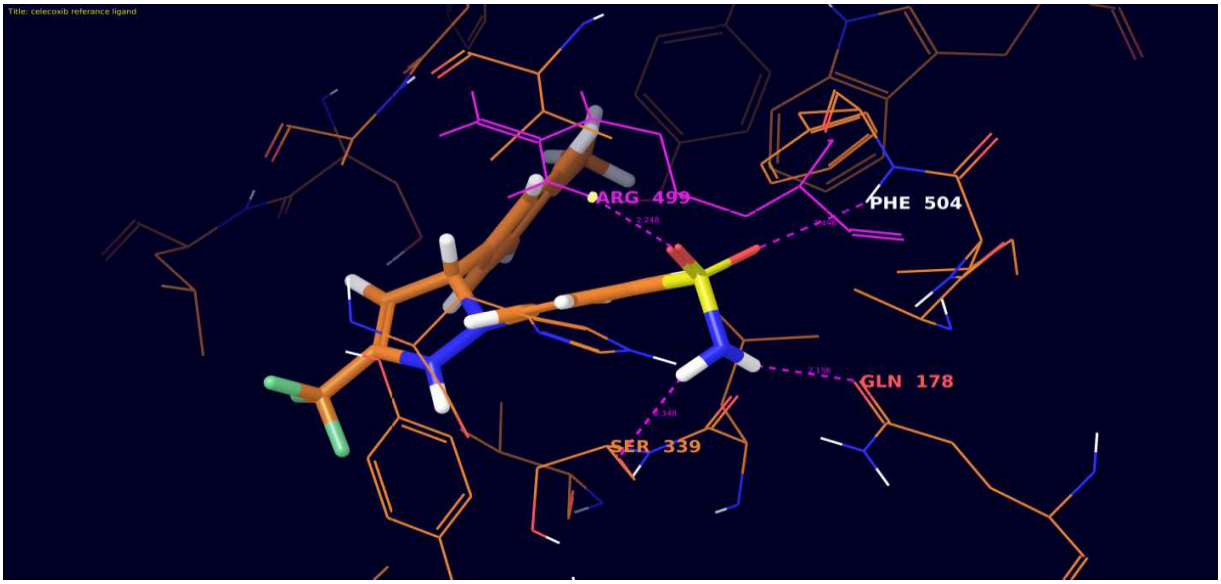
XP DOCKING RESULTS OF THE TITLE COMPOUNDS:(Table 1)

NAME OF THE COMPOUND	STRUCTURE OF THE COMPOUND	G-SCORE	MMGBSA	HYDROGEN BONDING FORMING RESIDUES
VIa		-9.8	-64.771	Arg 499
VIb		-10.0	-59.720	Arg 499
VIc		-8.8	-42.147	Arg 499

VId		-9.2	-52.902	Tyr 371
VIe		-8.8	-67.7	Tyr 371
VIIIa		-8.9	-49.168	Arg 499
VIIIb		-9.0	-46.864	Arg 499

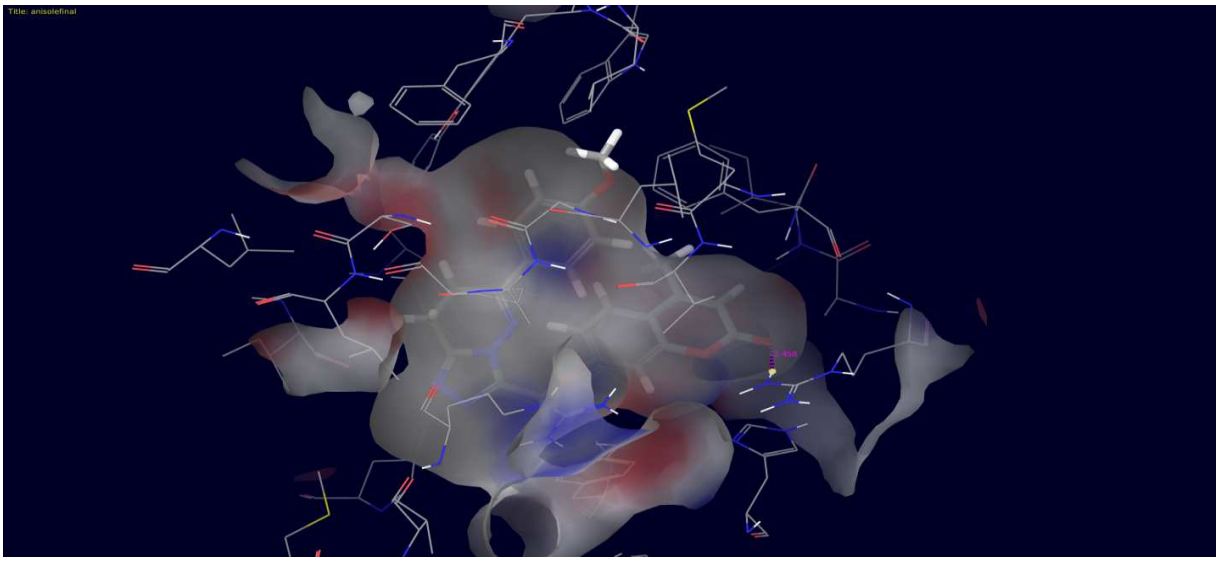
VIIIc		-8.2	-40.156	Arg 499
VIIId		-8.2	-53.882	Arg 499
VIIIe		-8.2	-51.419	No HB

The prepared protein COX-2(PDB ID:3LN1)with its co-crystallized ligand celecoxib showing hydrogen bonding interaction with amino acid residues such as Arg 499,Gln 178,Phe 504,Ser 339 (Figure 1)



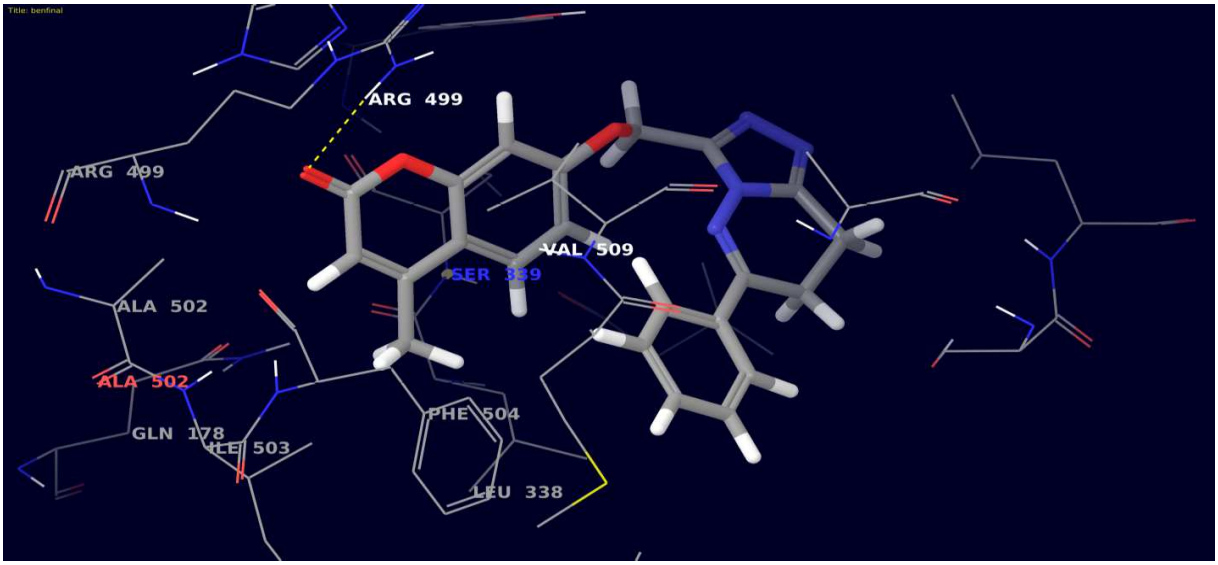
(Figure 1)

The receptor surface generated on the target indicated that the protein binding site showed good fit to the compound (VIc) and the pose of the ligand is in the binding site of the receptor. (figure 2)



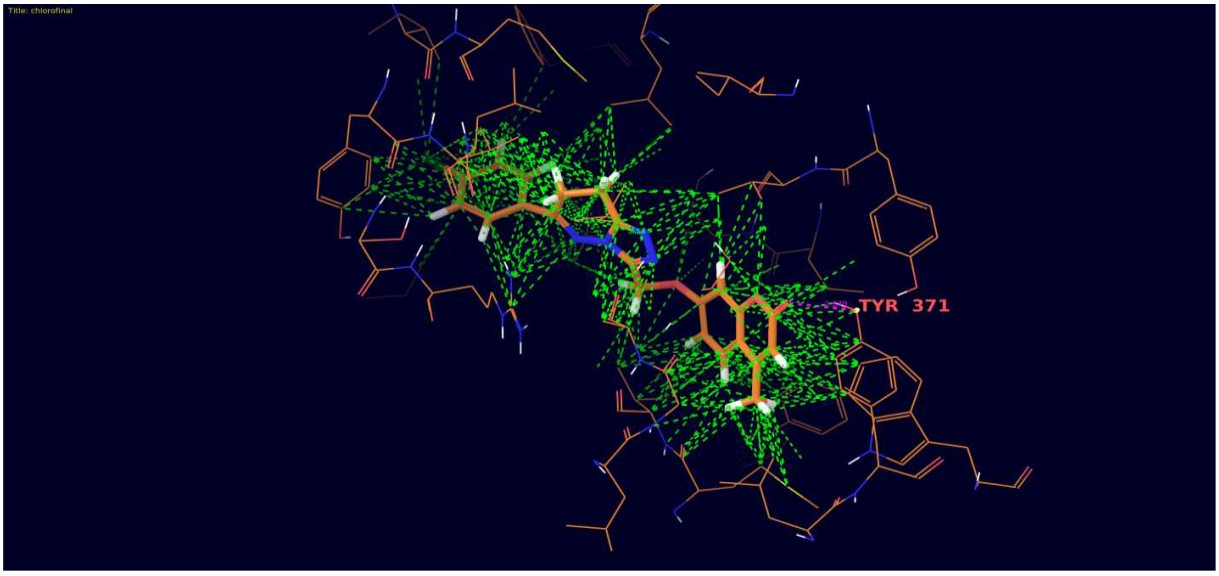
(Figure 2)

The binding mode of the compound(VIc)shows hydrogen bonding interactions with amino acid residue ARG 499 and it has surrounding interactions with amino acid residues such as PHE 504 ,LEU 338,VAL 509,SER 339. (Figure 3)



(Figure 3)

The binding mode of the compound(VId) hydrogen bonding interactions with amino acid residue TYR 3711.It also exhibits hydrophobic interactions(indicated in green colour) (Figure 4)



(Figure 4)

CONCLUSION

A novel series of O-substituted Coumarins were synthesized in a convenient synthetic route. Docking studies allowed us to estimate the cyclooygenase antagonist activity of title compounds in a rapid and efficient way. It suggested that most of the synthesized compounds are as potent as Celecoxib, in inhibiting the COX-2 enzyme (based on glide score and hydrogen bonding interactions), opening avenues for developing them further as analgesic and anti-inflammatory agents. Docking methodology represents a promising tool for the discovery of active compounds as drugs.

REFERENCES

[1] C Gleye ; G Lewin ; A Laurens, *J. Nat. Prod.*, **2003**, 66, 690-692.
[2] DA Mutlu.; DE Dilek, *Turk. J. Chem.* **2003,** 27, 757 -764.
[3] C Kontogiorgis; D Hadjipavlou, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **2003**, *18(1)*, 63–69.
[4] Glide, version 9.3,Schrodinger, llc,newyork,NY,2012.
[5] I Harperin; H Wolfson; R Nussinov, *Proteins*, **2002**,*47*, 409-443,
[6] N Brooijmans; ID Kuntz, *Annu. Rev. Biophys. Biomol. Struct*, **2003**, *32*, 335-373,