STRUCTURE-BASED VIRTUAL SCREENING, DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF COUMARIN-BENZOTHIAZOLE HYBRID DERIVATIVES AS INHA (ENOYL ACYL CARRIER PROTEIN REDUCTASE) INHIBITORS IN THE TREATMENT OF TUBERCULOSIS.

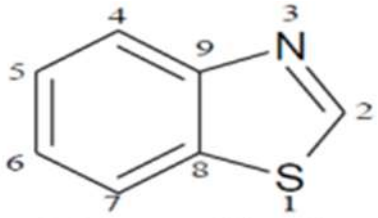
Polamoni Manisha, Thota Shirsha, Dr. M.Vijaya Bhargavi, Dr. M Sumakanth

RBVRR Women's College of Pharmacy

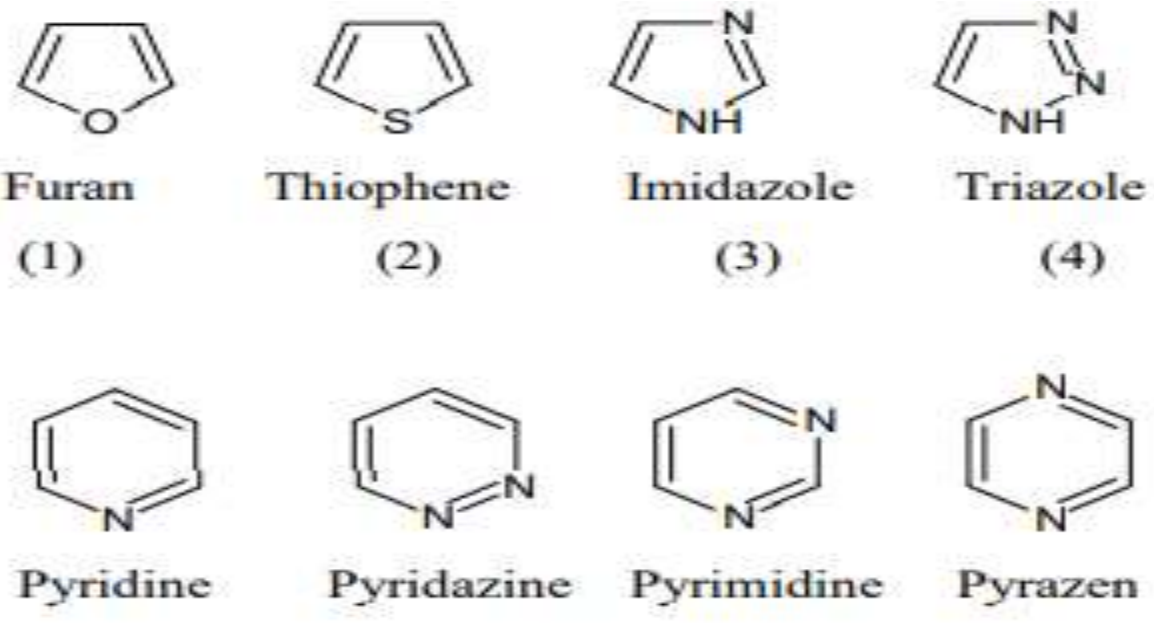
Abstract: Benzothiazole is a privileged fused heterocyclic moiety, that possesses diverse biological activities, such as antimicrobial, antitubercular, antitumor, antimalarial, anticonvulsant, anthelmintic, analgesic, and anti-inflammatory activity, and other miscellaneous activities which makes benzothiazole an interesting molecule for the researchers to work on. In the present work, the benzothiazoles and coumarins were subjected to structure-based virtual screening against InhA inhibitor (PDB ID:4QXM). Top lead molecules were identified as HITS and subjected to molecular docking studies. By understanding their important pharmacophoric features and incorporating them into a single molecule, a series of novel compounds such as coumarin-benzothiazole hybrid derivatives were designed and their molecular properties and toxicity prediction studies were carried out to know the safety and efficacy of the title compounds by using molinspiration, OSIRIS property explorer. Six derivatives of coumarin-benzothiazole hybrid derivatives were subjected to in silico docking studies with the PDB ID:4QXM using AutoDock Vina 1.5.6 software. The newly synthesized derivatives are characterized by using IR, 1H NMR, and Mass spectral data. From the docking results, the compounds showed moderate to the good activity which was comparable to that of the standard drug Isoniazid.

Keywords: Benzothiazole Coumarin Structure-based virtual screening, Molecular docking, Molinspiration, OSIRIS property explorer

Introduction to Heterocyclic Compounds:Heterocyclic compounds are of main interest in medicinal chemistry. The most complex branches of chemistry are normally heterocyclic chemistry. It is equally contributed in interesting for the industrial and physiological significances and for the diversity of its synthetic procedure as well as its theoretical implication. Synthetic heterocyclic chemistry has not only played an important role in every place of human life and also found its application in diverse fields like agriculture, medicine, polymer, and various industries. Most of the synthetic heterocyclic compounds act as anticonvulsants, hypnotics, antineoplastics, antiseptics, antihistaminic, antiviral, anti-tumour, etc [1]. General features of Heterocyclic Compounds: The most common heterocycles are those having five- or six-membered rings and containing heteroatoms of nitrogen (N), oxygen (O), or sulphur (S). The best-known simple heterocyclic compounds are pyridine, pyrrole, furan, and thiophene. A molecule of pyridine contains a ring of six atoms-five carbon atoms and one nitrogen atom. Pyrrole, furan, and thiophene molecules each contain five-membered rings, composed of four atoms of carbon and one atom of nitrogen, oxygen, or sulfur, respectively [2]. A few rings of e eterocyclic compounds are listed below:



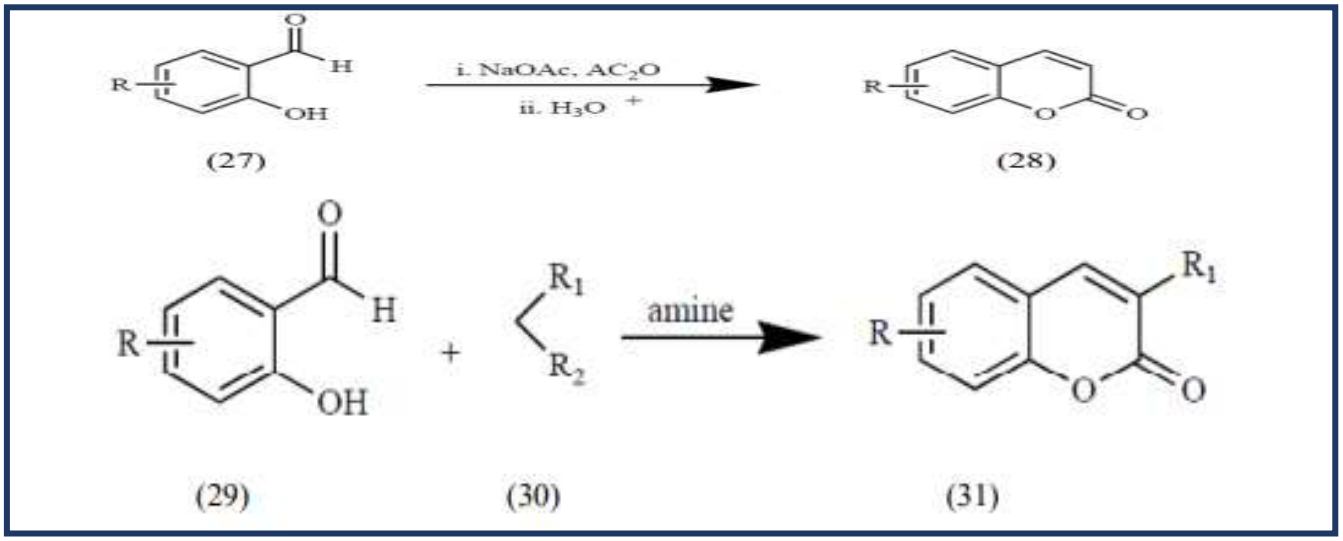
1,3-benzothiazole



Introduction to Benzothiazoles Benzothiazole is the combination of two rings, which contain the heterocycles thiazole and benzene. The core structure of thiazole and its pharmacologically and biologically active compounds are due to the presence of sulfur and nitrogen atoms present in the ring. Various marine or terrestrial natural compounds, which have useful biological activities like- anti-tubercular, antibacterial, antiviral, antidiabetic, anticancer etc. is due to the presence of the benzothiazole ring [3] .

Physical properties of Benzothiazoles: -Benzothiazole is a colourless, slightly viscous liquid with a molecular formula C7H5NS. It is weakly basic in nature with a boiling point of 227–228°C. It is very soluble in ether, acetone, alcohol, carbon disulphide and slightly soluble in water [4].

Knoevenagel reaction Various coumarins have been prepared via Knoevenagel condensation reaction by reacting 2- hydroxy benzaldehydes with activated methylene compounds in the presence of an amine (e.g. piperidine). Two different mechanisms have been proposed for Knoevenagel reaction: 1) formation of an imine or iminium salt, followed by reaction with the enolate of the active methylene compound, then elimination of the amine and intramolecular ring closure to give the coumarin, ii) attack by the carbanion formed from the deprotonation of the active methylene compound by the amine, on the carbonyl group to give the intermediate follow by proton transfer, and ring-closure via acyl substitution, and finally dehydration to give the coumarin.



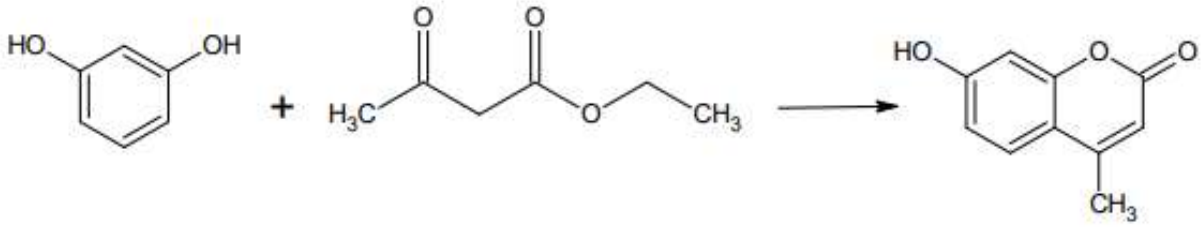
$R_1 = CO_2H, CO_2Et, CONH_2, CN$

$R_2 = CO_2H, CO_2Et, CN$

Marketed Products Many of the synthesized benzothiazole containing commercial drugs is reported as best selling Drugs by US Retail. There is also large number of approved benzothiazole containing drugs in the market like Pramipexole (32), Riluzole (33), Ethoxzolamide (34), Frentizole (35) and Zopolrestat (36) are currently going through different clinical phases [13].

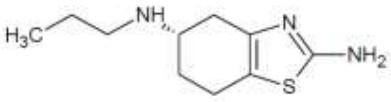
EXPERIMENTAL METHODS

Step 1: Synthesis of 7-hydroxy-4-methyl coumarin: 13.75 ml of concentrated sulphuric acid was transferred in a beaker and cooled on ice bath until the temperature of acid was about 50 C. In another beaker 2.5g (0.022mol) of resorcinol was dissolved in 3.26ml (0.025mol) of ethyl acetoacetate. The mixture was dissolved until a complete solution was added slowly to conc. sulphuric acid, so that the temperature of the mixture doesn't rise about 100 C. Then stirring was continued for 30mins. The mixture was poured into crushed ice with vigorous stirring. The solid 7-hydroxy-4-methyl coumarin separated. Once the solid was precipitated, the solution and reprecipitated it by addition of dilute HCl and then it was recrystallized from ethanol (or) methylated spirit using charcoal.

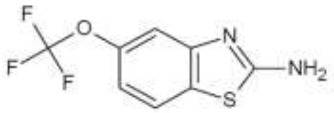


Resorcinol (1) + Ethyl acetoacetate (2) → 7-hydroxy-4-methyl coumarin (3)

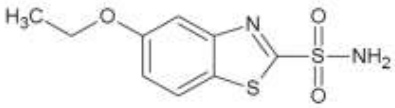
Step 2: Synthesis of 7-chloroacetoxy-4-methyl coumarin: A mixture of 7-hydroxy -4-methyl coumarin (1g, 0.0056 mol) and chloroacetyl chloride (10- 12cc) was reflexed at 1200 C in an oil bath. The contents rapidly assumed cherry red colour and hydrogen chloride was evolved briskly. After heating for about 2 hours, most of the excess of the chloroacetyl chloride was



Pramipexole (40)

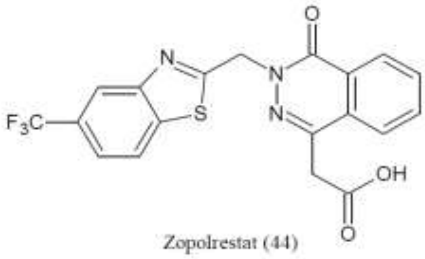


Riluzole (41)



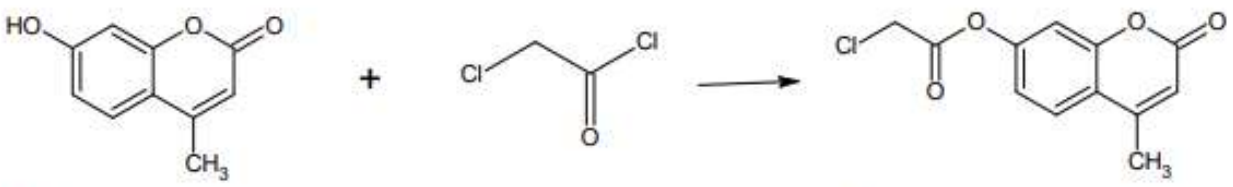
Ethoxzolamide (42)

Frentizole (43)



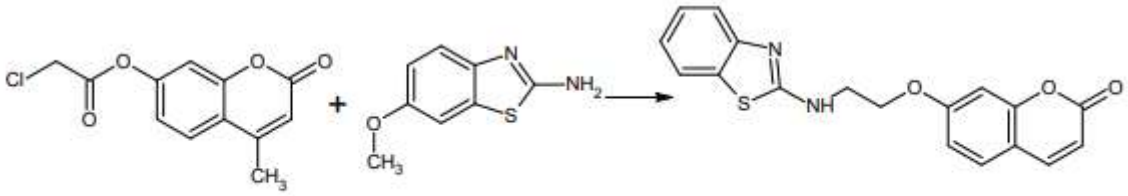
Zopolrestat (44)

recovered by distillation and the remaining quantity was decomposed by pouring residual mixture into ice cold water with stirring. The precipitated solid was filtered, washed with water and recrystallized from alcohol, when 7-chloroacetoxy-4-methyl coumarin was obtained



7-hydroxy-4-methyl coumarin (3) + chloroacetyl chloride (4) → 7-chloroacetoxy-4-methyl coumarin (5)

Step 3: Synthesis of 4-methyl-2-oxo-2H-chromen-7-yl (1,3-benzothiazol-2-yl amino)acetate: 2-amino-6-methoxy benzothiazole (1.80g, 0.010 mol) in dichloro methane (DCM) was placed in a flask and 7-chloroacetoxy-4-methyl coumarin (1.89g, 0.0087 mol) in DCM was added dropwise with stirring in an ice water bath with rock salt (0-5 0 C). After completion of the reaction by TLC reference (Hexane and ethyl acetate in 5:5), then reaction mixture was slowly poured in ice water and solid was collected by filtration and washed with water. The product was recrystallized from ethanol.



Virtual Screening studies on coumarin-benzothiazole derivatives

- Virtual screening of 150 molecules were performed by retrieving the coumarin and benzothiazole molecules from CHEMBL database for anti-tubercular activity.
- From the above results obtained by virtual screening shows coumarins and benzothiazoles have good anti tubercular activity.
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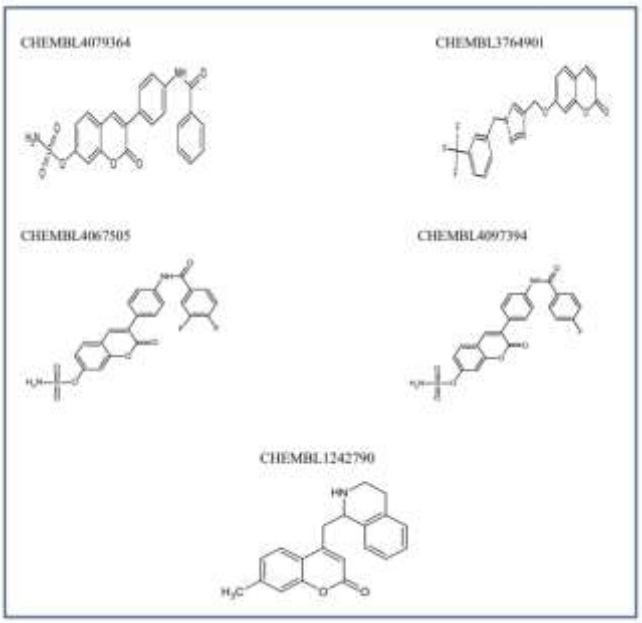


From all these 150 molecules we selected 5 highest score molecules of benzothiazoles and 5 highest score molecules of

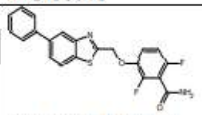
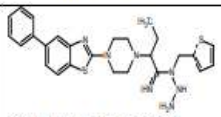
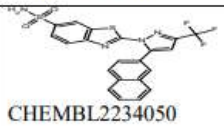
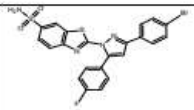
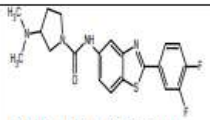
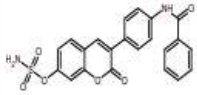
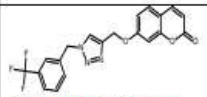
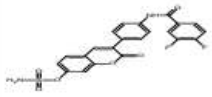
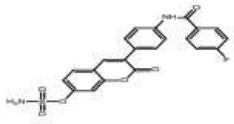
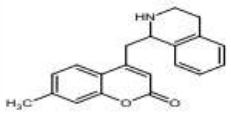
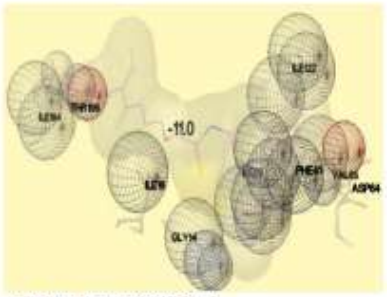
S.No	Structure of the lead molecule	Hydrogen bonds	Other interactions	Docking Scores
1.	 CHEMBL1096127	Hydrogen bonds with GLY104,218	**Hydrophobic** interactions with PHE108	-11.0
2.	 CHEMBL3740692	Hydrogen bonds with GLY96,PHE41	**Hydrophobic** interactions with ILE95,122,16, VAL65	-10.4
3.	 CHEMBL2234050	Hydrogen bonds with ALA22, ILE21, SER94	**Hydrophobic** interactions with PHE97, 41, LUE197	-11.0
4.	 CHEMBL3342145	Hydrogen bonds with LYS132,TYR182	**Hydrophobic** interactions with ALA128	-10.3
5.	 CHEMBL4067241	Hydrogen bonds with GLY14, ILE194,TRY158	**Hydrophobic** interactions with PHE149, MET199	-9.5
6.	 CHEMBL4079364	Hydrogen bond with PHE41	**Hydrophobic** interactions with PHE97	-11.4
7.	 CHEMBL3764901	Hydrogen bonds with GLY14,96, LYS165	**Hydrophobic** interactions with PHE41,ILE21,95	-10.4
8.	 CHEMBL4067505	Hydrogen bonds with GLY14,66,96,LYS118, SER94	**Hydrophobic** interactions with ILE95,VAL65	-11.9
9.	 CHEMBL4097394	Hydrogen bonds with MET98, PHE41	**Hydrophobic** interactions with ILE21	-11.5
10.	 CHEMBL1242790	Hydrogen bonds with ALA22, ILE21, SER20	**Hydrophobic** interactions with PHE149,	-11.2

Figure 1:Structures of five potent choosen from Benzothiazoles hits

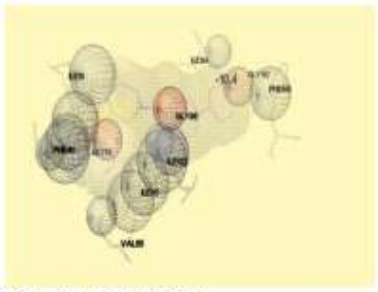
Molecular Docking Results of Potent HITS

As the binding affinity studies between ligands and their receptors form the basis of physiological activity and pharmacological effects of chemical compounds. We carried out docking studies of ten HITS obtained from virtual screening to investigate the correct binding pose of the novel molecules.

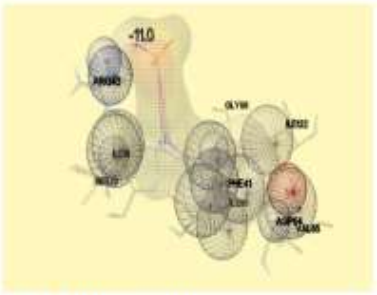
Table 5: Molecular Docking interactions of 10 potent HIT molecules (PDB ID: 4QXM) obtained from virtual screening results



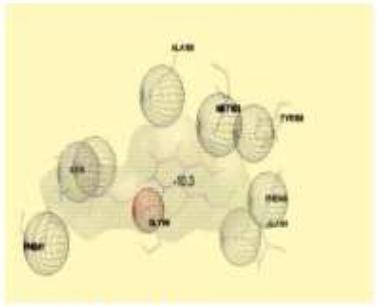
CHEMBL1096127



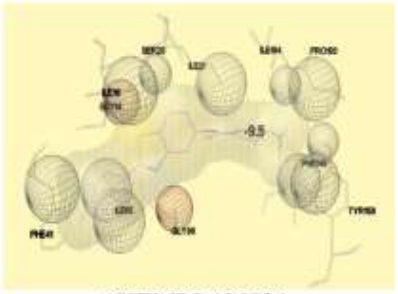
CHEMBL3740692



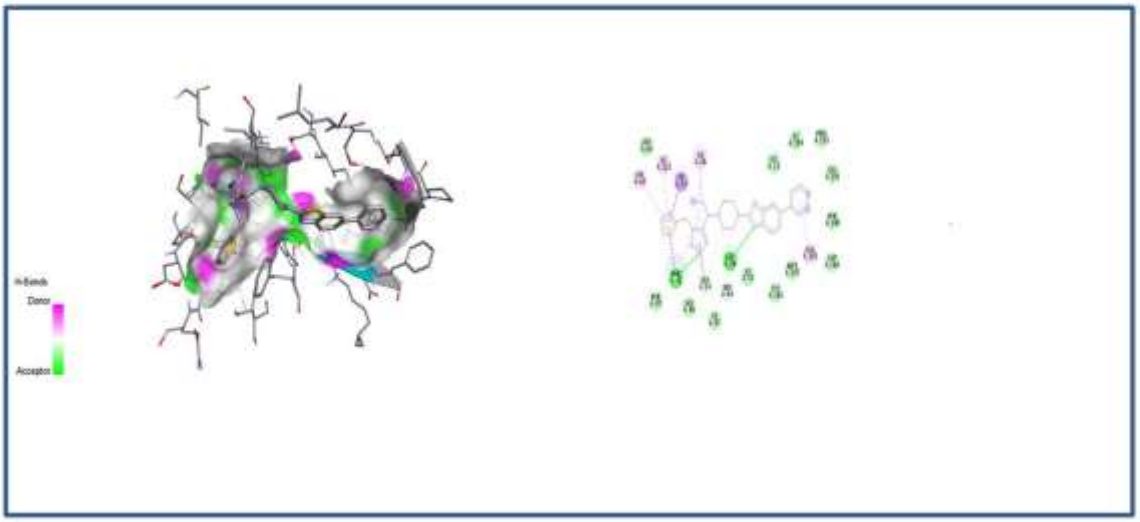
CHEMBL2234050



CHEMBL3342145



CHEMBL406724



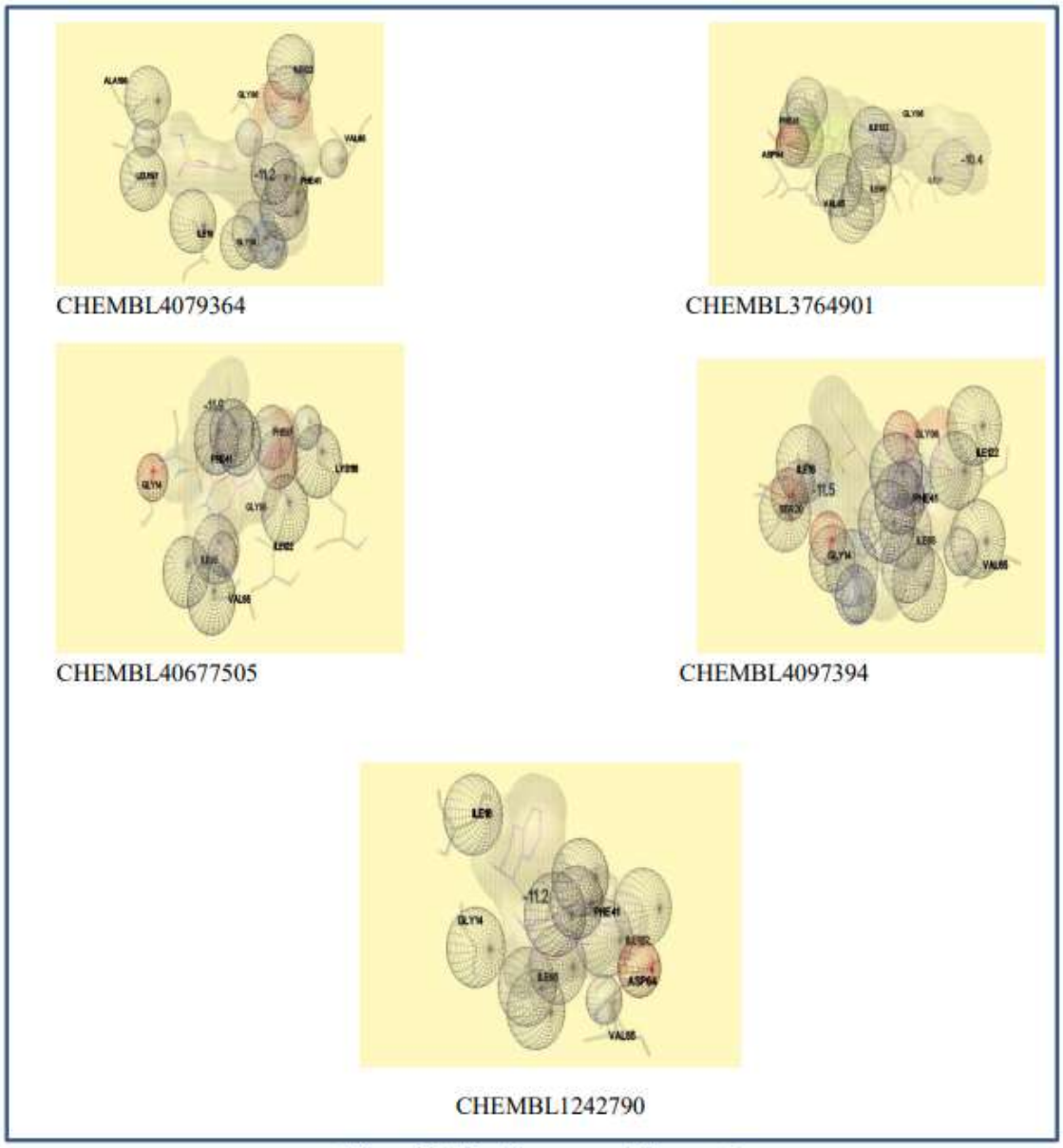


Figure 28: Docking poses of Coumarins

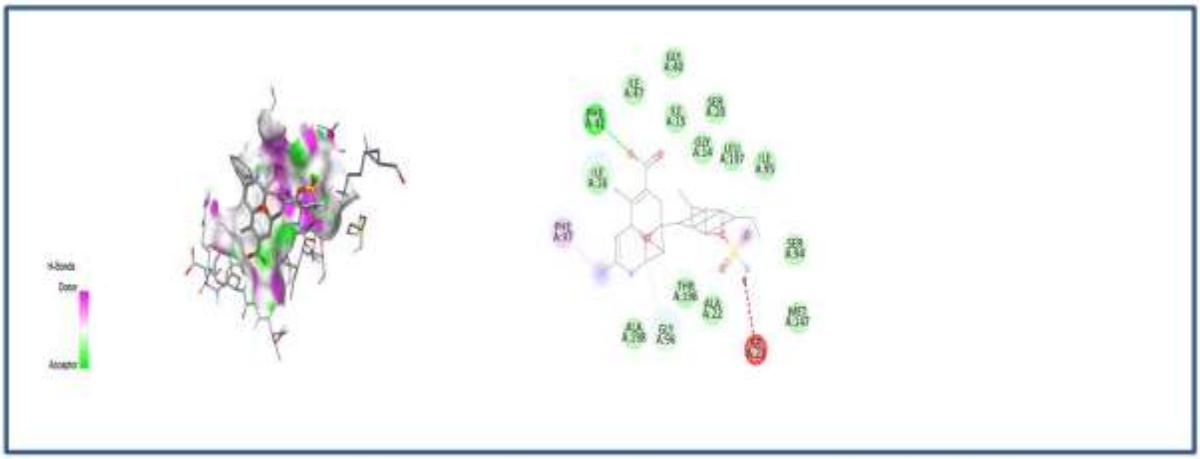


Figure 29:Binding interactions and 2D diagram of CHEMBL4079364

Compound	IUPAC	LogP	TPSA	Mol wt	nON	nOHNH	Nrotb
7 (a)	4-methyl -2-oxo-2H-1-benzopyron-7yl [(6-methoxy-1,3-benzothiazol-2-yl)amino]acetate	3.22	89.80	398.44	7	2	6
7 (b)	4-methyl-2-oxo-2H-1-benzopyran-7-yl[(1,3-benzothiazol-2-yl)amino]acetate	3.19	80.57	368.41	6	2	5
7 (c)	2-oxo-2H-1-benzopyran-7-yl[(6-methoxy-1,3-benzothiazol-2-yl)amino]acetate	2.84	89.80	384.41	7	2	6
7(d)	2-oxo-2H-1-benzopyran-7-yl[(1,3-benzothiazol-2-yl)amino]acetate	2.81	80.57	354.39	6	2	5
7 (e)	4-(chloromethyl)-2-oxo-2H-1-benzopyran-7-yl[(6-methoxy-1,3-benzothiazole-2-yl) amino acetate	3.45	89.80	418.86	7	2	6
7 (f)	4-(chloromethyl)-2-oxo-2H-1-benzopyran-7-yl[(1,3-benzothiazol-2-yl)amino]acetate	3.41	80.57	388.83	6	2	5

DESIGN OF THE LEAD MOLECULE FROM SIMILAR STRUCTURES AND THEIR PHARMACOPHORIC FEATURES

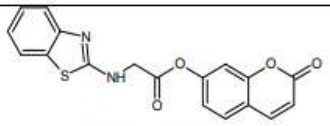
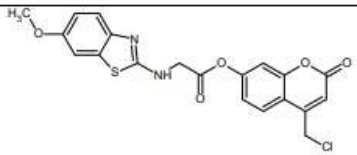
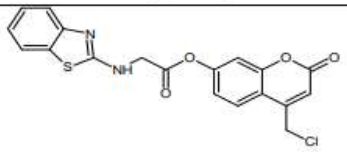
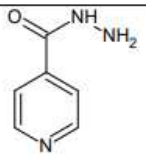
Physical properties of Coumarin-Benzothiazole hybrid derivatives using Molinspiration

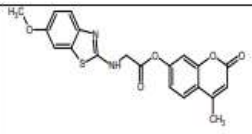
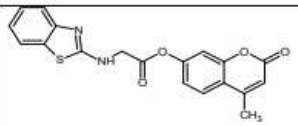
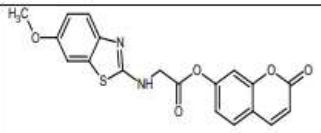
Drug likeness and toxicity calculation of Coumarin-Benzothiazole derivatives using OSIRIS property calculator

Figure 42: IR spectra of 4-methyl -2-oxo-2H-1-benzopyran-7-yl[(6-methoxy-1,3-benzothiazol-2-yl)amino]acetate

Compound	IUPAC	Log S	Drug score	Drug likeness	Toxicity risk
7 (a)	4-methyl-2-oxo-2H-1-benzopyran-7-yl[(6-methoxy-1,3-benzothiazol-2-yl)amino]acetate	-4.38	0.12	-17.62	None
7 (b)	4-methyl-2-oxo-2H-1-benzopyran-7-yl[(1,3-benzothhiazol-2-yl)amino]acetate	-4.36	0.21	-17.35	None
7 (c)	2-oxo-2H-1benzopyran-7-yl[(6-methoxy-1,3-benzothiazol-2-yl)amino]acetate	-4.65	0.13	-3.18	None
7 (d)	2-oxo-2H-1-benzopyran-7-yl[(1,3-benzothiazol-2-yl)amino]acetate	-4.63	0.22	-2.88	None
7 (e)	4-(chloromethyl)-2-oxo-2H-1-benzopyran-7-yl[6-methoxy-1,3-benzothiazol-2-yl)amino]acetate	-4.78	0.07	-7.74	None
7 (f)	4-(chloromethyl)-2-oxo-2H-1-benzopyran-7-yl[(1,3-benzothiazol-2-yl)amino]acetate	-4.76	0.07	-7.45	None

Molecular Docking of title compounds with PDB ID:4QXM • Molecular docking studies of the title compounds were carried out to understand the correct binding interactions of the title compounds with PDB ID: 4QXM

4.	 2-oxo-2H-1-benzopyran-7yl [(1,3-benzothiazol-2-yl)amino]acetate	Hydrogen bonds with GLY14,96, SER94	**Hydrophobic** interactions with PHE41, ILE21,95	**-9.3**
5.	 4 (chloro methyl)-2-oxo-2H-1-benzopyran-7-yl[(6-methoxy-1,3-benzothiazol-2-yl)amino]acetate	Hydrogen bonds with ALA198, LEU197,SER94	**Hydrophobic** interactions with ILE16	**-9.7**
6.	 4-(chloromethyl)-2-oxo-2H-1-benzopyran-7-yl[(1,3-benzothiazol-2-yl)amino]acetate	Hydrogen bonds with GLY14,SER94	**Hydrophobic** interactions with ILE21,95, PHE41	**-9.1**
7.	 ISONIAZID (standard)	Hydrogen bonds with ALA22, GLY14, ILE21, SER94	–	**-5.4**

S.No	Structure of the molecule	Hydrogen bonds	Other interactions	Docking scores
1.	 4-methyl-2-oxo-2H-1 benzopyran-7yl [(6 methoxy-1,3 benzothiazole-2 yl)amino]acetate	Hydrogen bonds with GLY14,96, SER94, VAL65	**Hydrophobic** interactions with PHE41, ILE21,95	**-10.0**
2.	 4-methyl -2-oxo-2H-1-benzopyran-7-yl[(1,3-benzothiazol-2-yl)amino]acetate	Hydrogen bonds with GLY14,ALA22 ILE21,SER94	**Hydrophobic** interactions with	**-8.4**
3.	 2-oxo-2H-1-benzopyran-7-yl[6-methoxy-1,3-benzothiazol-2-yl)amino]acetate	Hydrogen bonds with GLY96, MET98	**Hydrophobic** interactions with PHE149, ALA198, MET161	**-9.4**