Journal of Pharmaceutical and Applied Chemistry

ISSN: 2357-0210 VOLUME 2, ISSUES 2, 2016 www.egyptfuture.org/ojs/

Synthesis, Computational & Docking Studies Of Bis-(4-Hydroxycoumarin-3-Yl) Methanes As Potential Inhibitor For Carbonic Anhydrase, Glyceraldehyde-3-Phosphate Dehydrogenase

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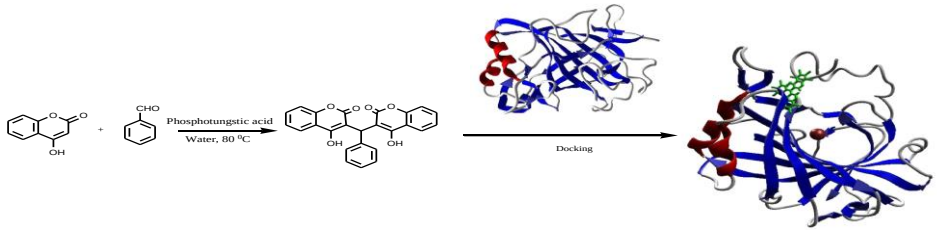
Received: 5 Jan. 2016, Revised: 12 Feb. 2016, Accepted: 15 Feb. 2016.
Published online: 1 May 2016.

Abstract: A general, simple and straight forward approach was used for the aqueous phase synthesis of bis-(4-hydroxycoumarin-3-yl)methanes via phosphotungstic acid as catalyst and provides efficient and environmentally benign route. Knoevenagel-type condensation followed by Michael reaction was carried between 4-hydroxycoumarin and an aldehyde in water as a solvent in shorter duration with high yields. Coumarin is a biological active chemical compound found in many plants, notably in high concentration in the Tonka bean, woodruff, and bison grass. Coumarins have potential in therapeutic application as anticoagulant and sustaining agents, they have results as antibiotics and antitumor drug. We have determined various biological properties of the synthesized compounds on basis of pharmacophores, structures. We docked the above synthesized compounds and evaluated hydrogen bonding, steric interaction with both enzymes (carbonic anhydrase and glyceraldehydes-3-phosphate dehydrogenase). Also, structural activities relationship of the compounds in reference molecular modelling, Lipinski rule of five, drug likeness, toxicity profiles were determined.



Keywords: Bis-(4-hydroxycoumarin-3-yl)methanes, carbonic anhydrase, biological properties, docking.

1 Introduction

In last decade, an enhancement in the number of different chemotypes other than the sulfonamides were reported, who showed as potential inhibitor for carbonic anhydrase (CAs, EC 4.2.1.1). CAs play crucial roles in processes connected with respiration and transport of CO_2/ bicarbonate, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions, bone resorption, calcification, tumori-genicity, and many other physiologic or pathologic processes thoroughly studied in vertebrates [1-5]. The carbonic anhydrase inhibitors (CAIs) belong to four main classes: (i) sulfonamides, (ii) phenols, (iii) the polyamines, and (iv) the recently reported class of effective CAIs, the coumarins. The main problem encountered with this class of CAIs is related either to the low solubility in aqueous media for the potent, structurally complex such compounds, or the low affinity to the enzyme for the simple, water soluble derivatives, which precluded us in obtaining good quality crystals of adducts of these inhibitors with various CA isoforms[6-13].

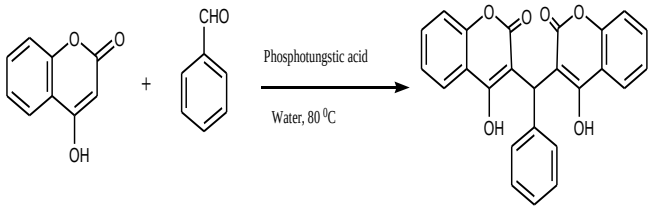
Glyceraldehyde-3-phosphate dehydrogenase (abbreviated as GAPDH) (EC1.2.1.12) is an enzyme of ~37kDa that catalyzes the sixth step of glycolysis and thus serves to break down glucose for energy and carbon molecules. In addition to this long established metabolic function, GAPDH has recently been implicated in several non-

metabolic processes, including transcription activation, initiation of apoptosis, and ER to Golgi vesicle shuttling. It catalyses the conversion of glyceraldehyde 3-phosphate to D-glycerate 1,3-bisphosphate. GAPDH can also be inhibited by arsenate, inhibiting glycolys is in red blood cells and causing hemolytic anemia [6-13]. Coumarins are biological active chemical compound found in many plants, notably in high concentration in the tonka bean, woodruff, and bison grass. A number of coumarins exhibit interesting pharmacological activities and are therefore of therapeutic use. Along with these, coumarins have recently revealed new biological activities with interesting potential in therapeutic application besides their traditional employment as anticoagulant and sustaining agents, they have yielded important results as antibiotics and antitumor drug. Thus, herein we investigated a number of such derivatives by computational and docking studies, allowing us to understand in some detail the inhibition mechanism of bis-(4-hydroxycoumarin-3-yl)methanes for carbonic anhydrase, glyceraldehyde-3-phosphate dehydrogenase [14-21].

2 Materials and Methods

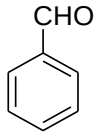
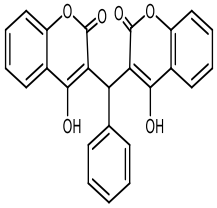
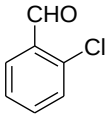
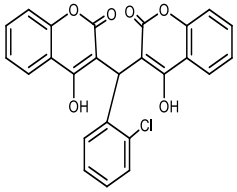
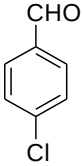
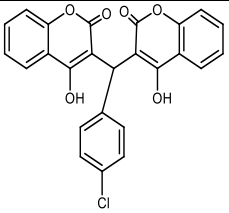
2.1 General Procedure for the Synthesis of Bis-(4-hydroxycoumarin-3-yl)methanes

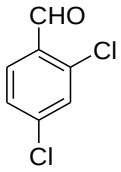
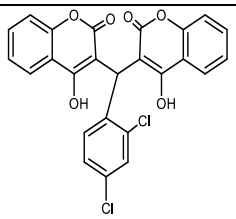
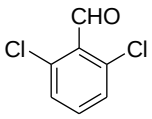
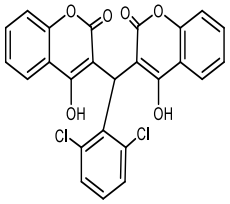
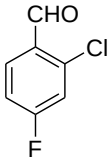
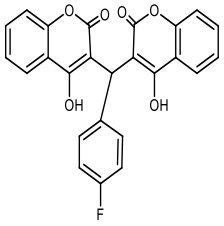
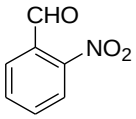
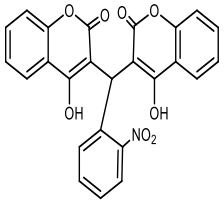
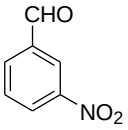
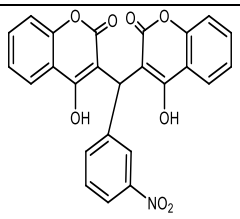
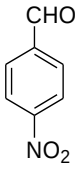
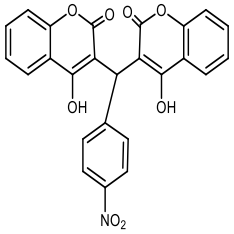
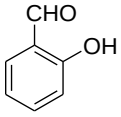
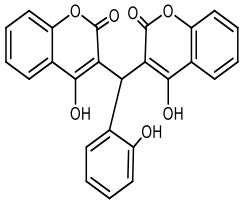
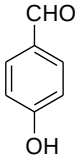
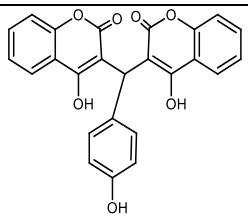
In a 50 mL round-bottomed flask, 4-hydroxycoumarin (20 mmol) and aromatic aldehyde (10 mmol) in water were taken and the resulting mixture was stirred at 80^0 C for 10 min, after then phosphotungstic acid (15 mmol%) was added to the reaction mixture. The progress of the reaction was well monitored by thin layer chromatography (TLC). After the completion of the reaction, the reaction mixture was cooled until the solidification appears and then filtered the solid and washed it water and then the filtrate was centrifuged at 8,000 rpm for 10 min to pellet out the catalyst and washed with absolute ethanol to remove all the organic impurities and then kept at 90 C for 30 min. The phosphotungstic acid was reused for evaluating the performance in the next reaction. The isolated products were subjected to further purification by column chromatography using petroleum ether and ethylacetate with increasing polarity as eluent to yield bis-(4-hydroxy-coumarin-3-yl)methanes as in **Scheme 1** and mentioned in **Table 1**. Structural assignments of the products are based on their ^{1}H-NMR, ^{13}C-NMR, IR and Mass analysis. The analysis of complete spectral and compositional data revealed the formation of bis-(4-hy-droxycoumarin-3-yl) methanes [14].

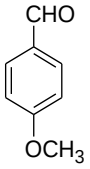
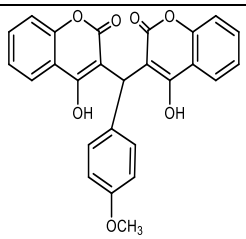
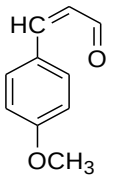
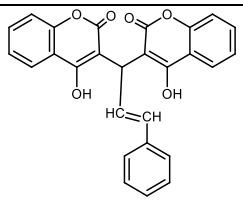
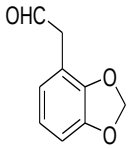
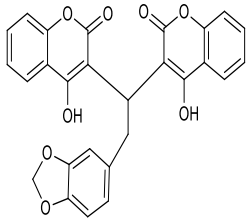
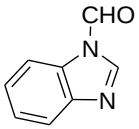
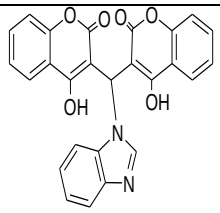
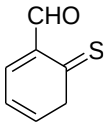
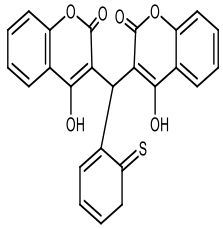
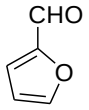
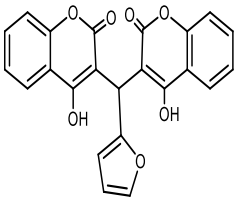
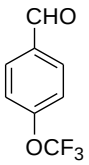
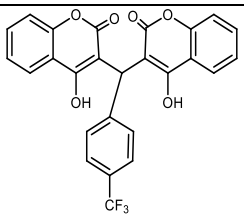


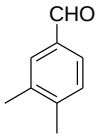
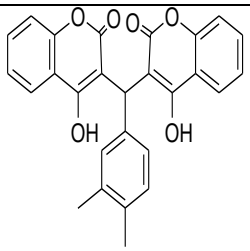
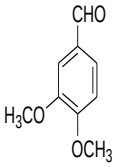
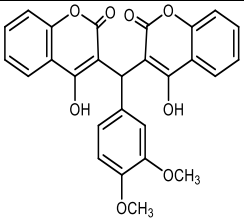
Scheme 1 Aqueous phase synthesis of bis-(4-hydroxycoumarin-3-yl)methanes via reaction of 4-hydroxycoumarin using phosphotungstic acid as catalyst.

Table 1. Aqueous phase synthesis of bis-(4-hydroxycoumarin-3-yl)methanes (3a-3t) by coupling of 4-hydroxycoumarin and aromatic aldehydes.

Compd. No.	Reactant	Product	Product No.	Yield (%)
1			3a	93
2			3b	95
3			3c	93

4			3d	92
5			3e	98
6			3f	98
7			3g	96
8			3h	94
9			3i	95
10			3j	98
11			3k	98

12			3l	99
13			3m	98
14			3n	96
15			3o	95
16			3p	91
17			3q	93
18			3r	98

19			3s	90
20			3t	90

2.2 Software's used

Structures were drawn using ChemDraw12. Docking was carried out using Molegro Molecular Viewer 2.5. Several biological properties (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors) of the coumarins are calculated from the http://www.molinspiration.com/cgi-bin/properties (Molnspiration bioactivity score v2011.06). Molinspirationa is also used to determine log P, polar surface area, number of hydrogen bond donors and acceptors and others. Other biological properties methylenetetrahydrofolate reductase (NADPH) inhibitor, monodehydroascorbate reductase (NADH) inhibitor, nitrate reductase (cytochrome) inhibitor, cholestanetriol 26-monooxygenase inhibitor and membrane integrity agonist were determined from www.pharmaexpert.ru/passonline/predict.php.
Molinspiration and pharmaexperts are free on-line services for calculation of important molecular properties.

2.3 Selection of PDB files

The PDB file having ID 3K34, 1K3T and 3F8E were taken from the RCSB protein data bank (www.rcsb.org/) and these PDB files are of carbonic anhydrase and glyceraldehyde-3-phosphate dehydrogenase.

3 Result and Discussion

3.1 Enzyme inhibition studies

The enzyme inhibition activity (or bioactivity) of compound are taken from pharma expert /pass *(Prediction of Activity Spectra for Substances)* available online. The prediction is based on the structure activity – relationships .The biological activity spectrum represents the "intrinsic" property of a substance depending only on its structure and physical-chemical characteristics. By analyzing the data given in the **Table 2**, it can be seen that all derivatives showed better inhibitory activity and selectivity against enzyme (1 to 5).All compound gives greater *Pa (probability "to be active")* value against enzyme.

Compound (**3a,3j,3k**) have highest inhibitory activity against MTHFR (NADPH) enzyme because compound **3a** are resonance stabilize and compound **3j & 3k** have hydroxyl group at 2nd and 4th position which are stabilize the benzene ring . Compound 3n have less inhibitory activity against MTHFR (NADPH) enzyme because of electron donating group which are destabilize the benzene ring. Compound (**3a, 3g, 3h, 3i**) have better inhibitory activity against MADR (NADH) enzyme because compound **3a** are resonance stabilize and compound **3g, 3h, 3i** have NO_2 group at *-o, -m* & *p-* position of benzene, having electron withdrawing property to make more resonance stabilize ring.

Compound **3a** and 3c have better inhibitory and selectivity against enzyme Nitrate reductase (cytochrome) and compound **3b, 3e & 3d** also gives better results against this enzyme because compound **3c** have –Cl group at *ortho* position, at *ortho* –Cl group withdraw electron more easily and more stabilize the ring than *para*-position. The compound **3a** have better activity and selectivity against Cholestanetriol 26-monooxygenase enzyme than compound **3n** because of more resonance stabilize than compound **3n** .Compound **3n** have more antagonistic property with Membrane integrity enzyme than all derivatives because of

electron donating group . We are conclude that compound have electron withdrawing groups or electronegative atoms (F, O & N) increase the hydrophilic property & gives better steric interaction and hydrogen bonding with enzymes/proteins and compound have electron donating groups increase the hydrophobicsity so more easily penetrate the membrane so this type of compound gives better antagonistic property [21-29].

Table 2 Bioactivity of compounds 1 to 20 from pharma expert website

Compound No.	Pa Value				
	MTHFR (NADPH) inhibitor	**MADR (NADH) inhibitor**	**NR (cytochrome) inhibitor**	**CM inhibitor**	**Membrane integrity agonist**
3a	0.929	0.918	0.901	0.894	0.892
3b	0.861	0.712	0.889	0.789	0.881
3c	0.861	0.712	0.905	0.789	0.903
3d	0.812	-	0.871	0.708	0.868
3e	0.857	0722	0.890	0.790	0.881
3f	0.861	0.762	0.789	0.789	0.841
3g	0.852	0.954	0.809	0.805	-
3h	0.828	0.945	0.778	0.773	-
3i	0.852	0.953	0.809	0.805	-
3j	0.914	0.896	0.876	0.862	0.881
3k	0.914	0.896	0.876	0.862	0.907
3l	0.878	0.761	0.868	0.753	0.879
3m	0.880	0.778	0.819	0.818	0.914
3n	0.782	-	-	0.707	0.935
3o	0.812	-	0.774	0.730	-
3p	0.861	0.836	0.789	0.789	0.733
3q	0.877	0.766	0.755	0.753	0.761
3r	0.861	0.836	0.789	0.789	-
3s	0.810	-	0.713	0.810	-
3t	0.859	-	0.826	0.708	0.864

* MTHFR - Methylenetetrahydrofolate reductase
** MDAR - Monodehydroascorbate reductase
*** NR - Nitrate reductase
**** CM - Cholestanetriol 26-monooxygenase

3.2 Physiochemical parameter studies

Preliminary QSAR study of compound given in Table 1

The QSAR study and drug likeness score by using molinspiration software .Preliminary studies including log P value , TPSA (Topological polar surface area) , molecular volume.

Log P = (octanol/water partition coefficient)

The physiochemical parameter are determined by using various parameter such as miLog P , TPSA , n atoms , nON , Nohnh , MW , Volume , all these parameters are given in the **Table 3** used for the determination of bioactivity and physiochemical property of coumarins. By analyzing the data given in the **Table 3**, firstly we discussed the miLog P {molinspiration Log P (octanol-water partition)} value of all coumarins. Log P is one criterion used in medicinal chemistry to assess the drug likeness of a given molecule, and used to calculate lipophilic efficiency, a function of potency and LogP that evaluate the quality of research compounds. Octanol-water partition coefficient log P is used in QSAR studies and rational drug design as a measure of molecular hydrophobicity. It affects drug absorption, bioavailability, hydrophobic drug-receptor interactions, metabolism of molecules, as well as their toxicity. Log P has become also a key parameter in studies of the environmental fate of chemicals. The logarithm of the ratio of the concentrations of the un-ionized solute in the solvents is called log P: The log P value is also known as a measure of lipophilicity [27-36]. Method for log P prediction developed by Molinspiration, based on group contributions. The following formula used to calculate the log P value of twenty compounds given in the **Table 3**.

Log P OCTANOL/WATER = log ([solute]octanol / [solute]water un-ionized)

By analysis of data given in the **Table 3**, we seen that compound 3d shows greater log P value so compound are highly lipophilic in nature showed greater hydrophobicity (affect drug absorption, bioavailability, hydrophobic drug-receptor interactions, metabolism of molecules, as well as their toxicity) . Some compound also have better log P value such as compound **3e, 3c, 3b, 3m, 3q , 3r** and **3s** than other derivatives . The reference compound gives negative log P value so it measure of the effectiveness of a compound in inhibiting biological or biochemical function and because of less lipophilic in nature [21-29].

Table 3 Parameter evaluation of the compounds 3a-3t as in Table 1 using molinspiration.

Compound No.	Physico-chemical paprameters						
	miLogP	TPSA	n atoms	MW	nON	nOHNH	volume
3a	4.523	100.878	31.0	412.397	6	2	348.81
3b	5.153	100.878	32.0	446.842	6	2	362.346
3c	5.201	100.878	32.0	446.842	6	2	362.346
3d	5.807	100.878	33.0	481.287	6	2	375.882
3e	5.783	100.878	33.0	481.287	6	2	375.882
3f	4.687	100.878	32.0	430.387	6	2	353.742
3g	4.434	146.702	34.0	457.394	9	2	372.145
3h	4.458	146.702	34.0	457.394	9	2	372.145
3i	4.482	146.702	34.0	457.394	9	2	372.145
3j	4.463	121.106	32.0	428.396	7	3	356.828
3k	4.044	121.106	32.0	428.396	7	3	356.828
3l	4.58	110.112	33.0	442.423	7	2	374.356
3m	5.279	100.878	33.0	438.435	6	2	376.227
3n	4.622	119.346	35.0	470.433	8	2	389.542
3o	4.673	116.669	34.0	451.434	7	3	377.788
3p	4.468	100.878	32.0	444.464	6	2	366.057
3q	5.412	114.018	36.0	478.456	7	2	401.787
3r	5.418	100.878	35.0	480.394	6	2	380.108
3s	5.195	100.878	33.0	442.467	6	2	387.821
3t	4.169	119.346	35.0	472.449	8	2	399.902

Molecular Polar Surface Area TPSA (Topological polar surface area)

Another parameter TPSA molecular polar surface area (PSA) is a very useful parameter for prediction of drug transport properties. Polar surface area is defined as a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. This parameter has been shown to correlate very well with the human intestinal absorption, Caco-2 monolayer permeability, and blood-brain barrier penetration.

The methodology for the calculation of TPSA is based on the summation of tabulated surface contributions of polar fragments (atoms regarding also their environment).So we that discussed the transport property of all compound given in **Table 3**.The compound 3g, 3h and 3i have highest TPSA values so have high transport property to penetrate the blood – brain barrier and good intestinal absorption. The compounds 3j and 3k also high transport property. This property are comes from highly electronegative atom O- and N- centered polar fragments. The compound **3g,3h, 3i** have – NO_2 group at *ortho* , *meta* and *para* position of the benzene ring and compound **3j &3k** also have – OH group are polar fragment at *ortho* and *para* position of the benzene ring, are this type of property increase the transport, penetration and absorption property of the compound [30-39]

Molecular Volume

Molecular volume determines transport characteristics of molecules, such as intestinal absorption or blood-brain barrier penetration. Volume is therefore often used in QSAR studies to model molecular properties and biological activity. Various methods may be used to calculate molecular volume, including methods requiring generation of 3D molecular geometries, or fragment contribution methods such as McGowan volume approximation. Method for calculation of molecule volume developed at Molinspiration is based on group contributions. By analyzing the **Table 3**, we seen that the compound **3q , 3t , 3n & 3s** have greater molecular volume than other compounds so have high transport characteristics such as intestinal absorption or blood–brain barrier penetration but standard drug have very low molecular volume so this is not shows greater transport characteristics such as intestinal absorption or blood–brain barrier penetration [35, 40-45].

Drug likeness score of compounds (3a-3t) as in Table 4

Drug likeness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and of course presence of various pharmacophoric features influence the behaviour of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity,

Table 4 Bioactivity prediction of the compounds 3a-3t as in Table 1 using molinspiration.

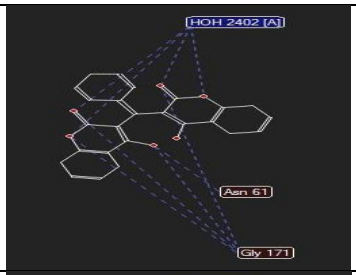
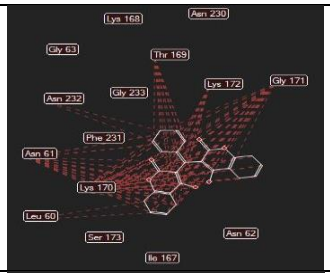
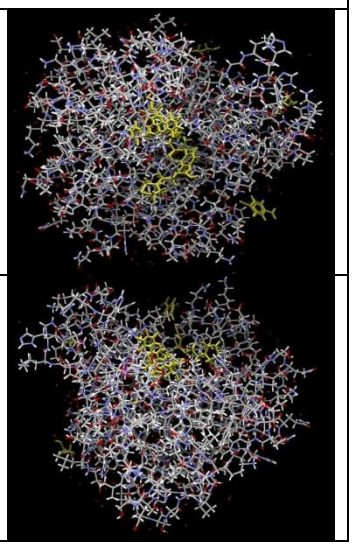
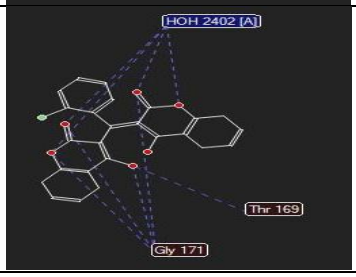
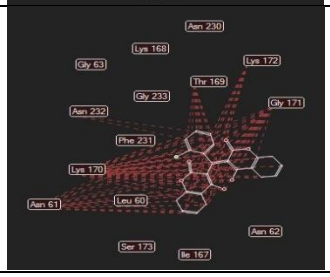
Compound No.	Bioactivity					
	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
3a	-0.27	-0.33	-0.52	-0.07	-0.20	0.09
3b	-0.31	-0.35	-0.48	-0.13	-0.25	-0.01
3c	-0.26	-0.33	-0.52	-0.09	-0.23	0.05
3d	-0.29	-0.33	-0.48	-0.14	-0.25	-0.02
3e	-0.27	-0.31	-0.44	-0.10	-0.21	0.03
3f	-0.25	-0.33	-0.48	-0.05	-0.21	0.07
3g	-0.38	-0.33	-0.53	-0.16	-0.29	-0.04
3h	-0.37	-0.36	-0.58	-0.15	-0.30	-0.02
3i	-0.36	-0.35	-0.58	-0.15	-0.30	-0.01
3j	-0.33	-0.38	-0.49	-0.06	-0.23	0.08
3k	-0.25	-0.31	-0.49	-0.02	-0.19	0.09
3l	-0.29	-0.37	-0.52	-0.08	-0.23	0.04
3m	-0.19	-0.25	-0.30	-0.06	-0.03	0.05
3n	-0.16	-0.22	-0.36	-0.05	-0.00	0.15
3o	-0.16	-0.24	-0.29	-0.09	-0.17	0.10
3p	-0.41	-0.41	-0.48	-0.13	-0.16	-0.03
3q	-0.24	-0.44	-0.42	-0.13	-0.25	0.01
3r	-0.20	-0.25	-0.42	0.04	-0.16	0.07
3s	-0.26	-0.34	-0.47	0.15	-0.17	0.10
3t	-0.28	-0.37	-0.49	-0.10	-0.25	0.03

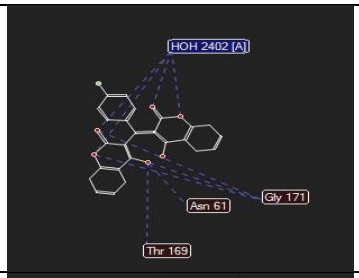
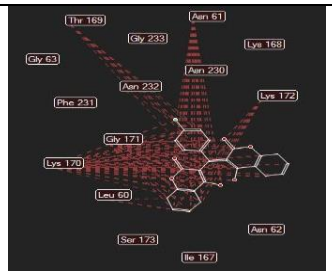
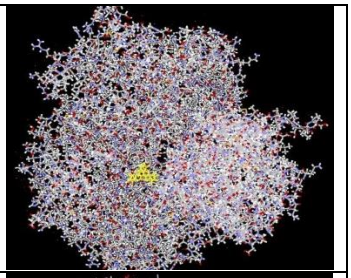
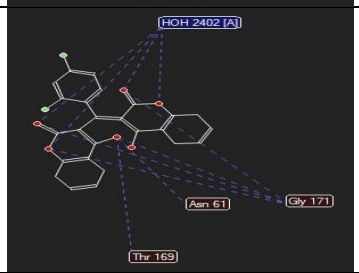
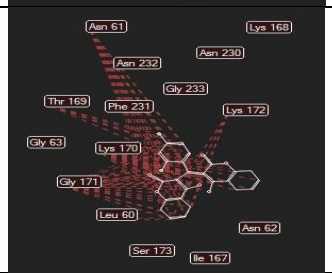
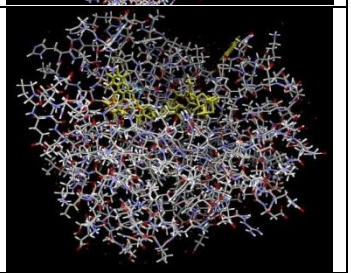
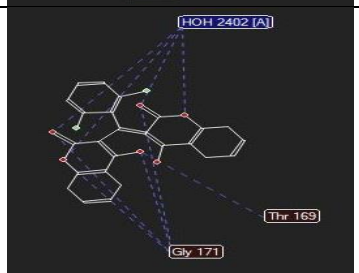
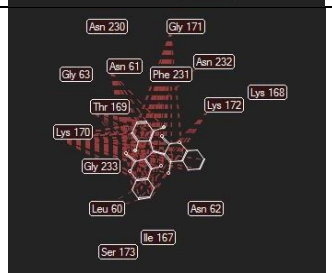
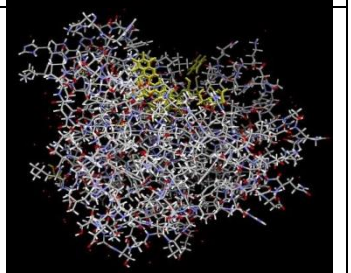
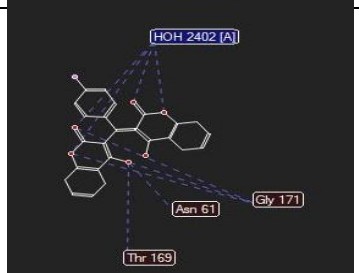
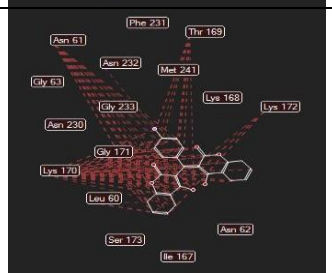
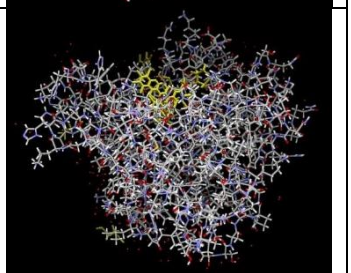
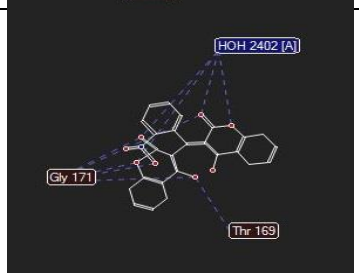
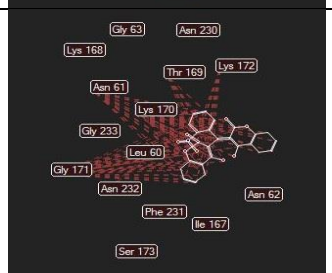
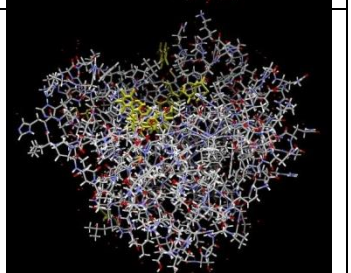
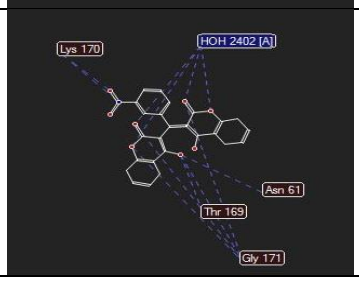
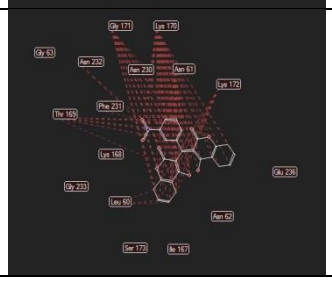
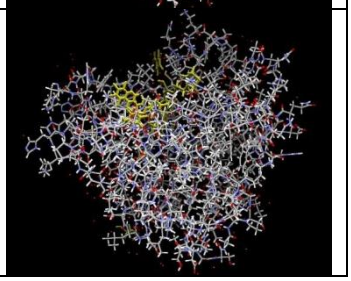
Table 5 Docking Studies of the synthesized bis-(4-hydroxycoumarin-3-yl)methanes (3a-3t) as in Table 1 as potent inhibitor for carbonic anhydrase, Glyceraldehyde-3-phosphate dehydrogenase

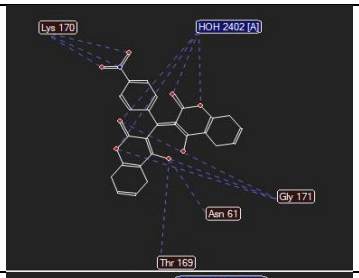
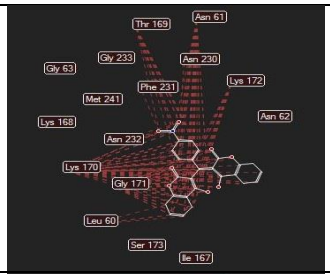
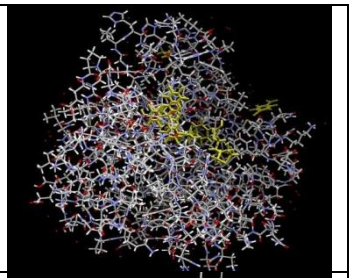
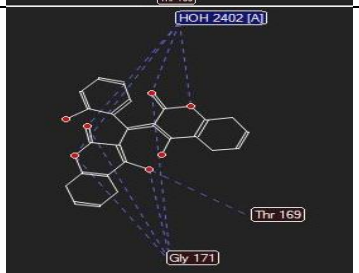
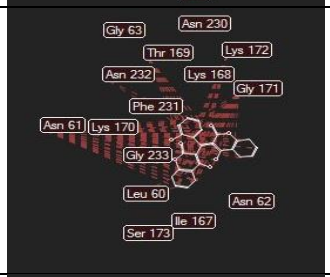
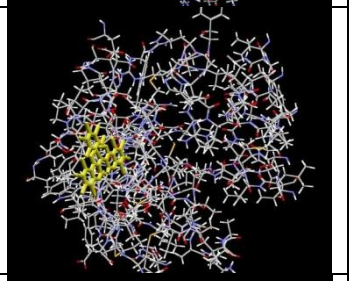
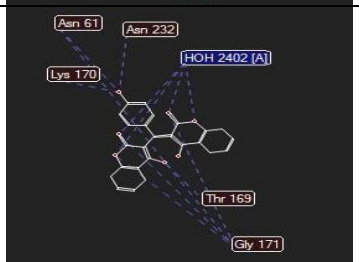
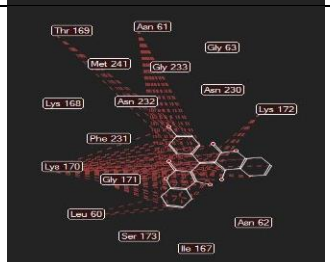
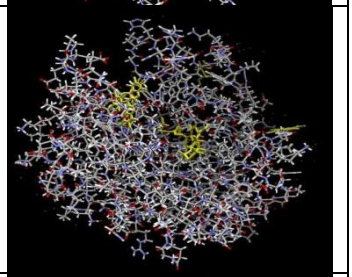
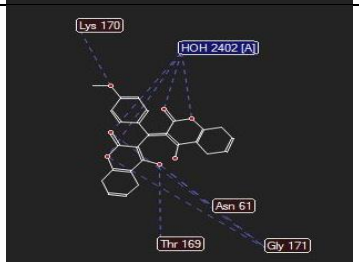
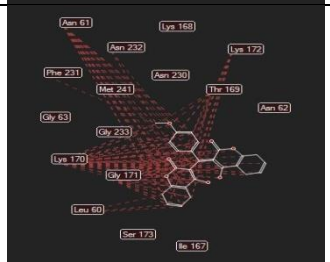
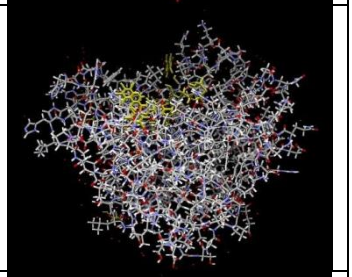
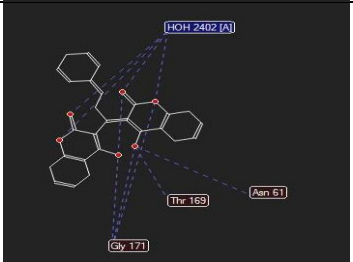
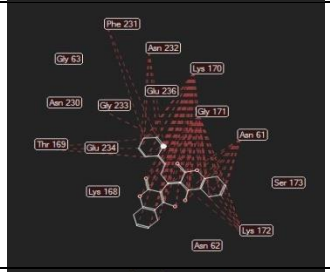
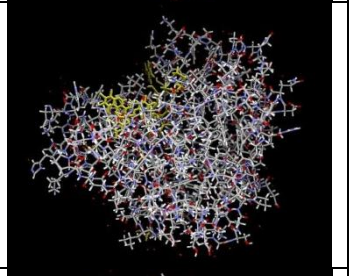
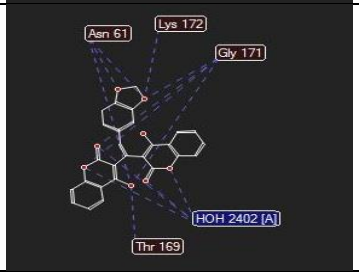
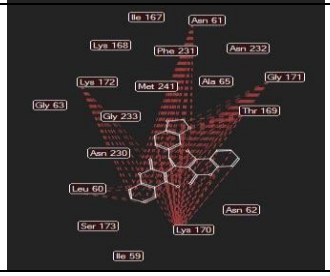
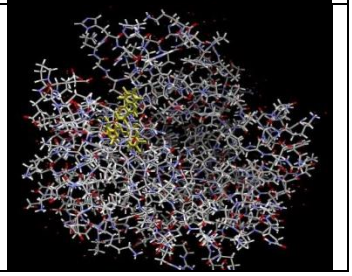
PDB ID	Compound No.	Total energy		External Ligand interactions		Protein - Ligand interactions		Water - Ligand interactions	
		Mol dock score	Rerank score	Mol dock score	Rerank score	Mol dock score	Rerank score	Mol dock score	Rerank score
3K34	3a	48624.32	6785.501	1045.161	6038.4	1069.196	6062.147	-24.036	-23.747
	3b	52570.6	6436.625	1039.898	5823.289	1062.542	5845.661	-22.644	-22.372
	3c	46418.06	5774.726	919.98	5167.001	934.222	5181.072	-14.242	-14.071
	3d	52521.94	5820.159	974.195	5203.892	989.509	5219.022	-15.314	-15.13
	3e	58664.13	6477.789	1092.389	5858.536	1115.522	5881.392	-23.133	-22.855
	3f	46429.13	5825.933	929.605	5219.131	944.784	5234.128	-15.18	-14.998
	3g	64772.9	7251.011	1122.407	6491.047	1144.119	6512.498	-21.712	-21.451
	3h	49543.05	6361.858	996.75	5688.676	1011.21	5702.962	-14.46	-14.287
	3i	48474.99	6004.982	951.518	5344.789	961.039	5354.196	-9.521	-9.407
	3j	52563.08	6670.74	1029.707	6029.048	1051.418	6050.499	-21.712	-21.451
	3k	46420.08	5954.854	921.064	5329.484	935.658	5343.903	-14.594	-14.419
	3l	47424.72	5912.156	913.635	5263.699	923.438	5273.384	-9.803	-9.685
	3m	45365	5760.392	887.873	5154.754	899.999	5166.735	-12.126	-11.981
	3n	28539.22	6638.242	1046.473	5958.746	1065.923	5977.963	-19.45	-19.216
	3o	41461.64	6559.805	987.615	5921.891	999.651	5933.782	-12.036	-11.891
	3p	41356.06	5877.51	910.201	5286.836	925.704	5302.153	-15.503	-15.317
	3q	47588.73	6620.196	1035.785	5944.237	1059.323	5967.493	-23.538	-23.256
	3r	50491.93	5599.849	953.14	4952.698	958.164	4957.66	-5.023	-4.963
	3s	50670.59	6867.707	1060.668	6053.539	1083.391	6075.989	-22.723	-22.45
	3t	54731.57	6810.839	1045.264	5911.058	1062.251	5927.842	-16.987	-16.783
1K3T	3a	47615.27	787.078	36.118	39.977	-25.109	-20.515	61.227	60.492
	3b	51590.78	682.081	60.081	68.745	-26.813	-17.106	86.893	85.851
	3c	45620.72	794.79	122.638	187.065	-27.607	38.623	150.245	148.442
	3d	51668.7	801.679	120.954	185.411	-27.642	38.599	148.596	146.813
	3e	57631.27	687.458	59.528	68.206	-26.837	-17.123	86.365	85.329
	3f	45616.59	778.969	117.069	172.167	-27.753	29.084	144.821	143.083
	3g	63710.27	828.231	59.778	68.268	-26.813	-17.284	86.591	85.552
	3h	48666.1	853.383	119.801	180.2	-27.684	34.486	147.485	145.715
	3i	47674.09	888.946	150.621	228.754	-26.047	54.206	176.668	174.548

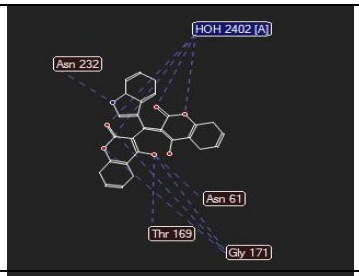
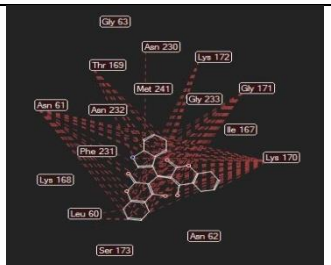
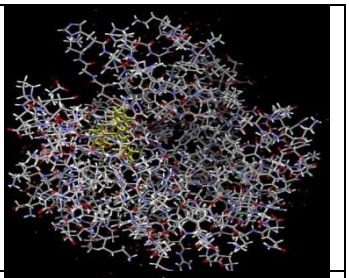
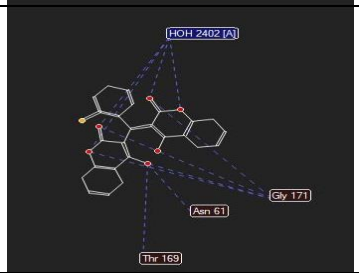
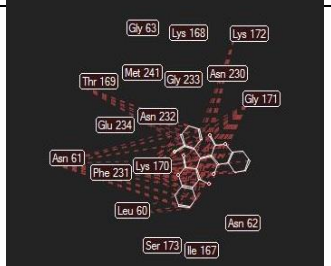
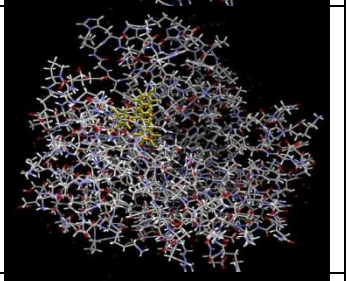
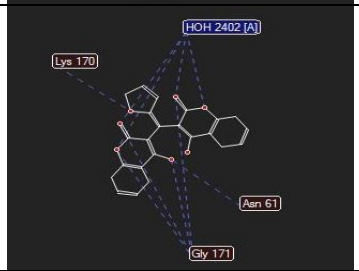
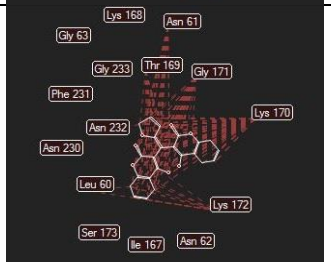
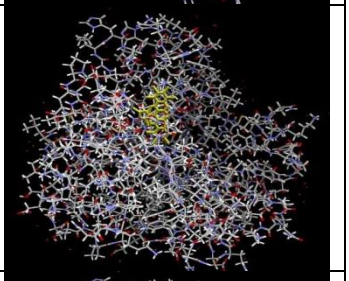
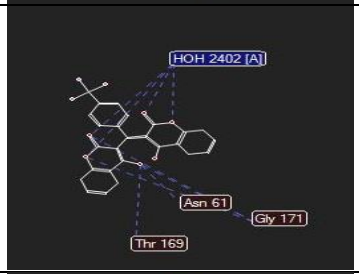
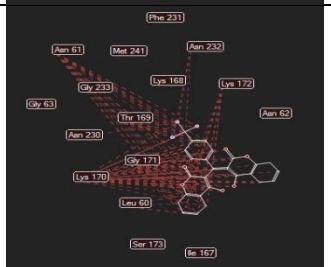
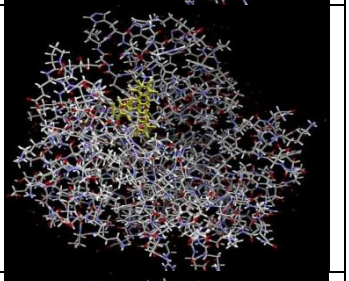
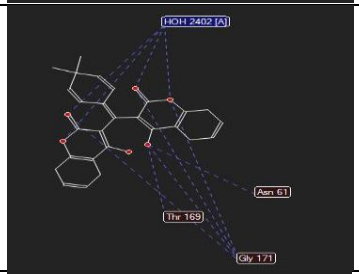
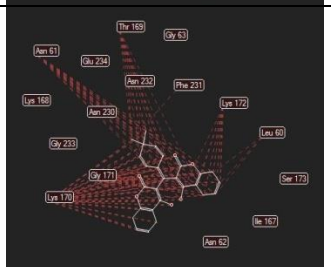
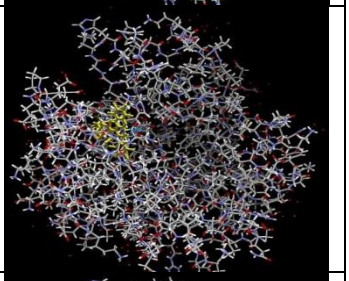
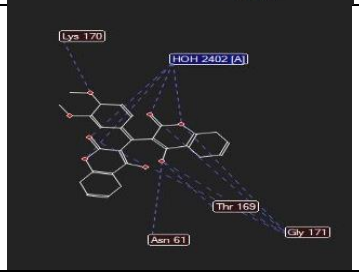
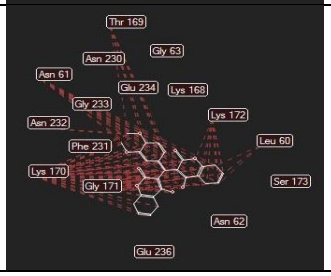
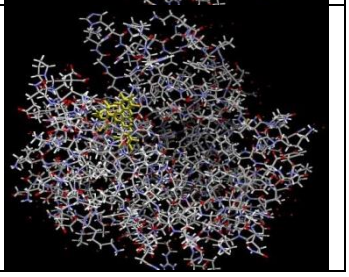
	3j	51594.79	711.709	61.416	70.018	-26.813	-17.152	88.228	87.17
	3k	45618.02	803.156	119.003	177.786	-27.704	32.84	146.707	144.946
	3l	46661.69	877.203	150.612	228.746	-26.047	54.207	176.659	174.539
	3m	44618.59	819.246	141.469	213.607	-26.117	48.033	167.586	165.575
	3n	27569.41	835.657	76.66	156.161	-16.575	64.045	93.235	92.116
	3o	40602.59	833.392	128.568	195.478	-27.414	41.368	155.982	154.11
	3p	40556.46	747.551	110.599	156.877	-27.856	20.083	138.456	136.794
	3q	46569.94	694.945	16.997	18.987	-22.843	-20.375	39.84	39.361
	3r	49722.74	951.173	183.942	304.022	-22.165	-22.165	206.108	203.634
	3s	49682.05	898.596	72.13	84.428	-27.153	-13.664	99.283	98.092
	3t	53804.71	1077.427	118.402	177.646	-27.905	33.095	146.307	144.551
3F8E	3a	48648.92	6815.056	1069.762	6067.955	1045.82	6044.301	23.941	23.654
	3b	52591.17	6402.326	1060.473	5788.99	1037.758	5766.548	22.715	22.442
	3c	46419.33	5711.373	921.248	5103.647	904.669	5087.266	16.58	16.381
	3d	52523.8	5757.915	976.052	5141.648	958.131	5123.941	17.921	17.706
	3e	58683.19	6442.147	1111.453	5822.895	1089.086	5800.797	22.366	22.098
	3f	46432.24	5765.574	932.712	5158.771	915.658	5141.922	17.054	16.849
	3g	64787.57	7228.095	1137.081	6468.132	1121.603	6452.839	15.478	15.292
	3h	49552.63	6330.94	1006.331	5657.758	990.691	5642.306	15.64	15.452
	3i	48468.12	5896.289	944.653	5236.096	929.94	5221.559	14.713	14.537
	3j	52581.25	6634.002	1047.876	5992.311	1027.428	5972.108	20.448	20.203
	3k	46422.98	5903.291	923.968	5277.921	906.573	5260.735	17.394	17.186
	3l	47416.84	5795.434	905.76	5146.977	891.593	5132.979	14.167	13.997
	3m	45378.29	5787.072	901.166	5181.434	884.95	5165.412	16.216	16.022
	3n	28583.45	6688.452	1090.703	6008.956	1068.889	5987.404	21.814	21.552
	3o	41469 .0	6609.039	994.975	5971.125	978.091	5954.444	16.883	16.681
	3p	41371.089	5953.015	925.232	5362.342	906.658	5343.991	18.574	18.351
	3q	47617.14	6750.008	1064.191	6074.049	1039.268	6049.425	24.924	24.624
	3r	50478.41	5480.761	5480.761	4833.609	930.405	4824.512	9.208	9.098
	3s	50688.9	6869.682	1078.971	6055.514	1057.424	6034.225	21.547	21.289
	3t	54744.67	6808.118	1058.36	5908.337	1038.814	5889.025	19.546	19.312

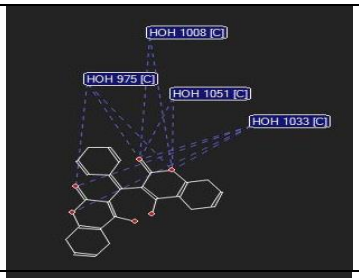
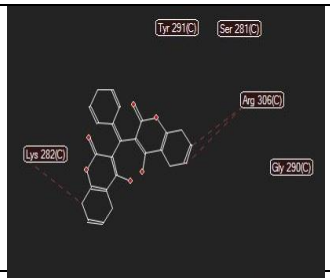
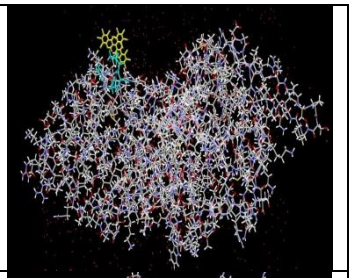
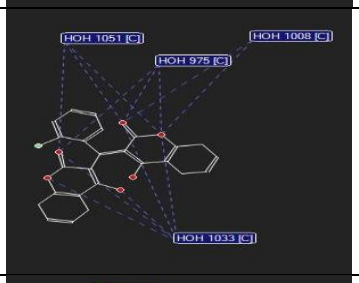
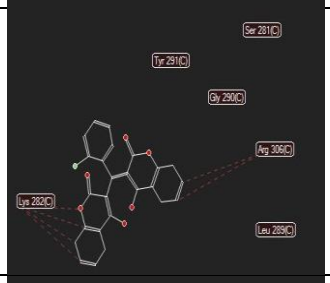
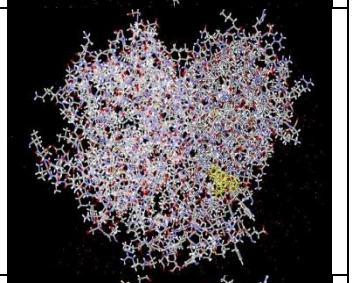
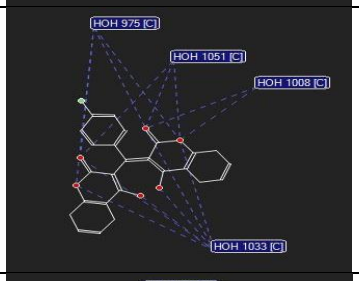
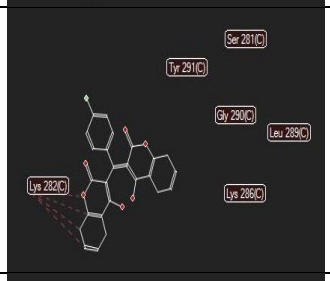
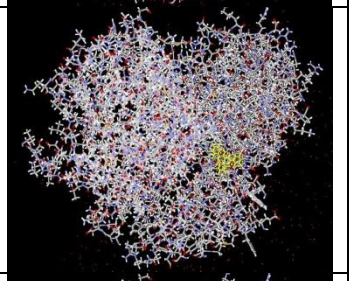
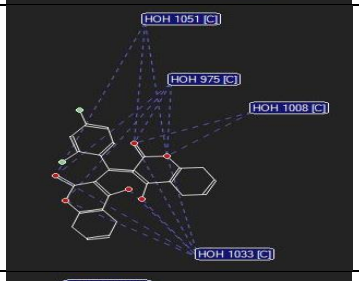
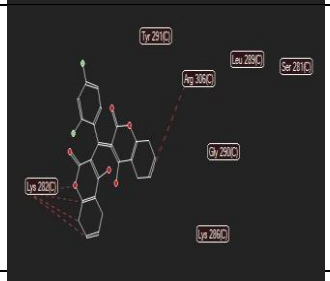
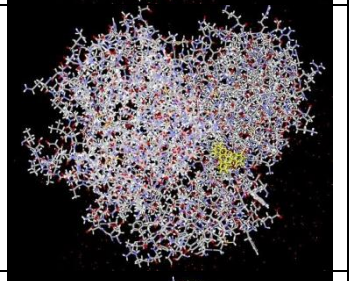
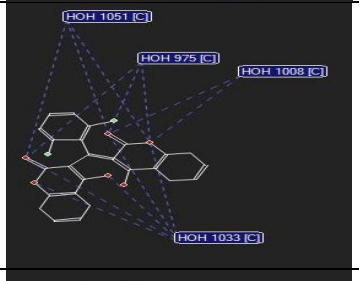
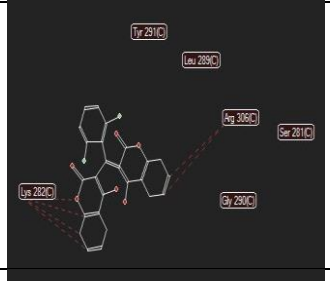
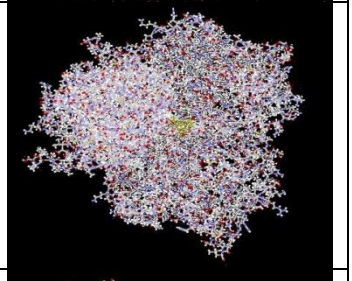
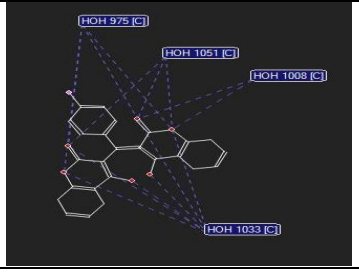
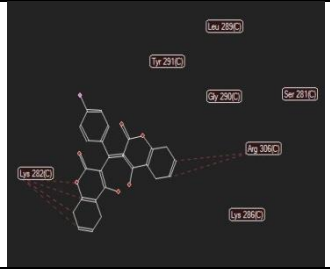
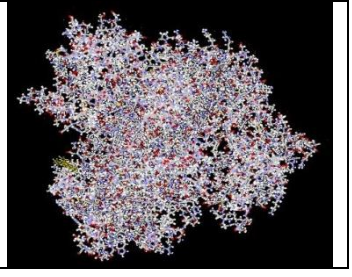
Table 6 Hydrogen bond interaction, steric interaction and Docking views of the synthesized bis-(4-hydroxycoumarin-3-yl)methanes (3a-3t) as in Table 1 as potent inhibitor for carbonic anhydrase, Glyceraldehyde-3-phosphate dehydrogenase

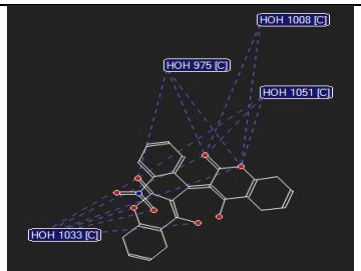
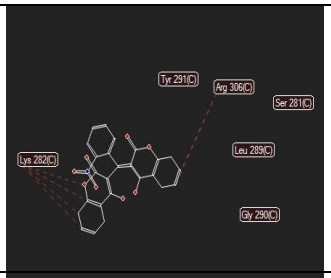
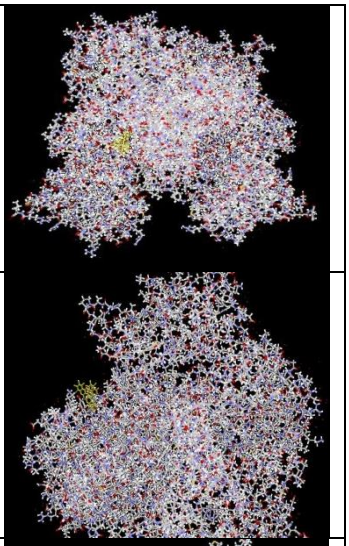
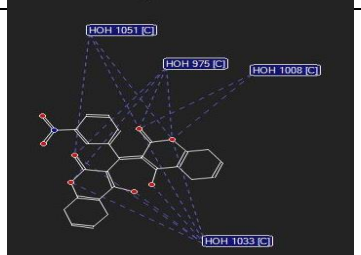
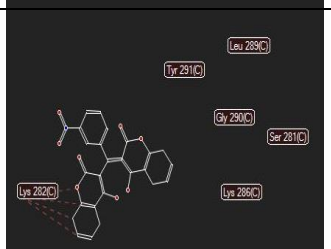
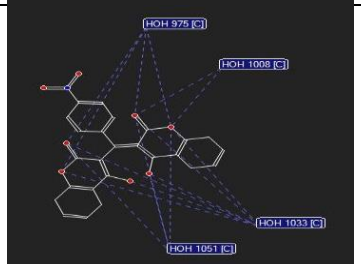
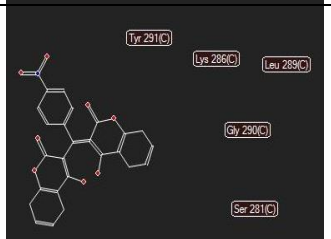
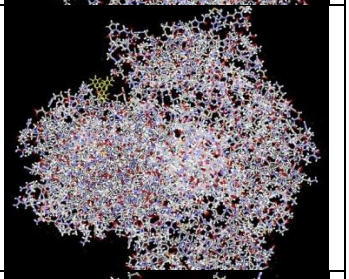
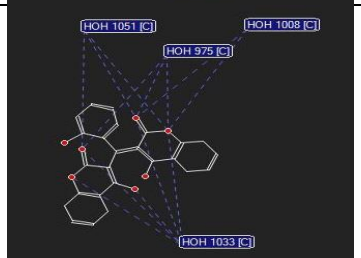
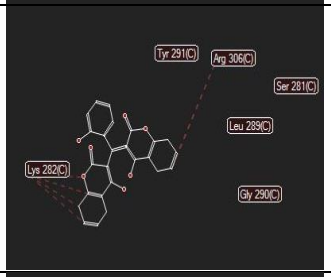
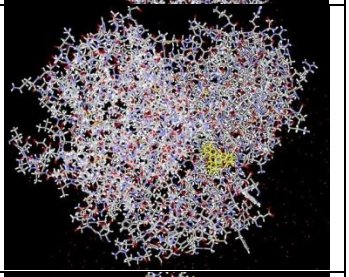
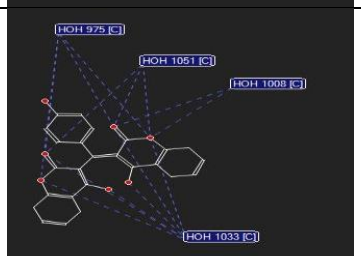
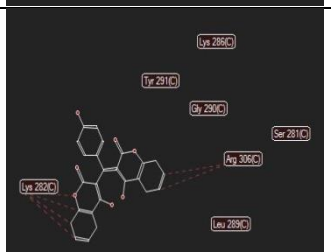
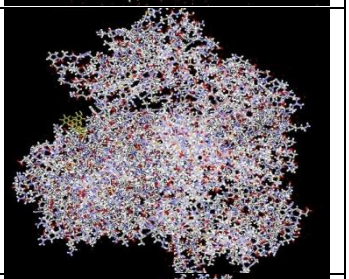
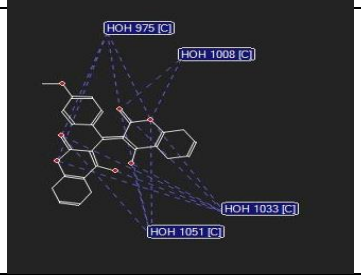
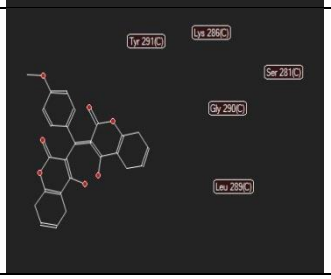
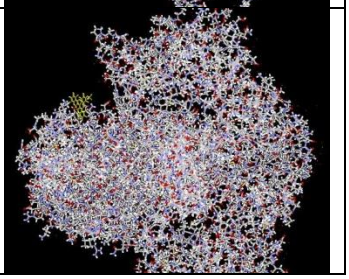
PDB ID	Compound No.	Hydrogen- Bond Interaction	Steric Interaction	Docking View
3K34	3a			
	3b			

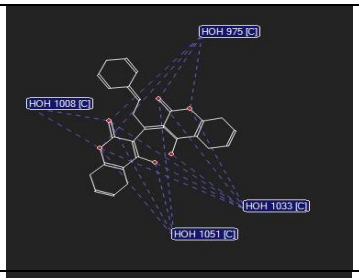
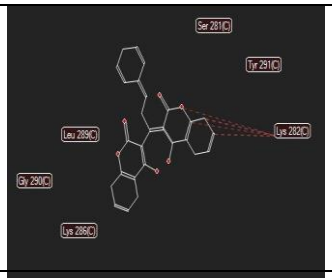
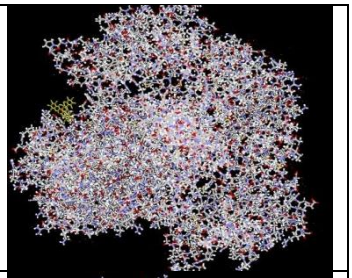
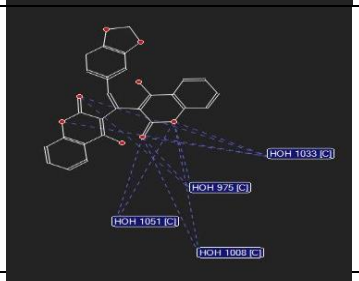
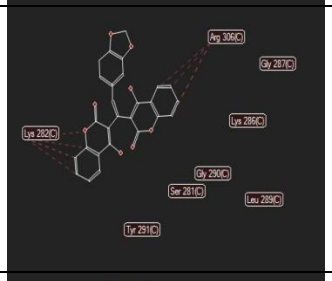
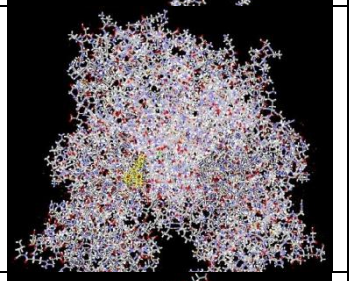
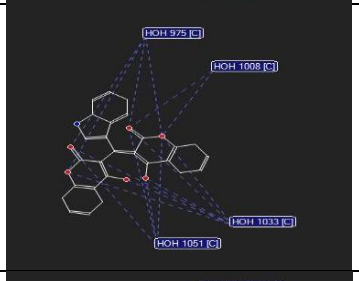
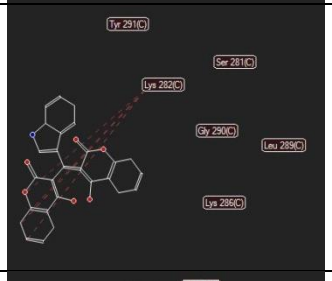
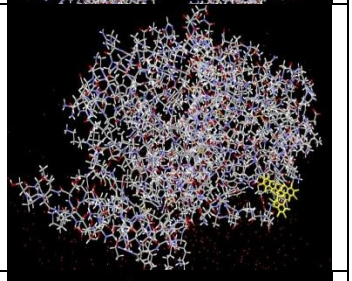
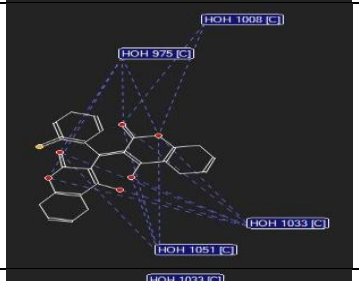
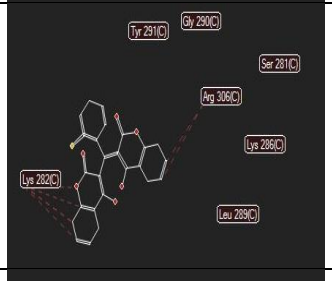
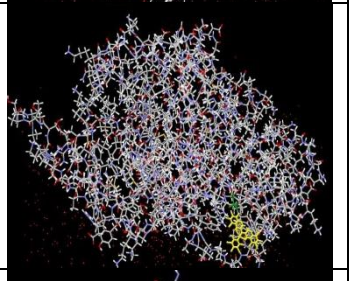
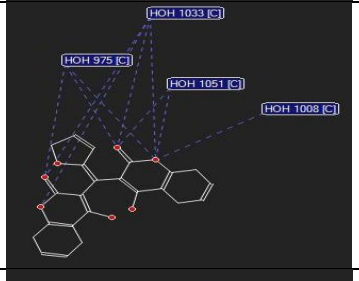
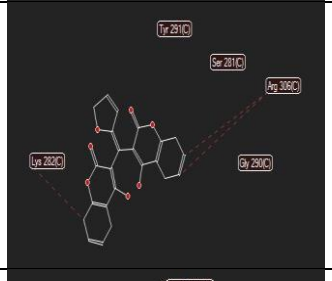
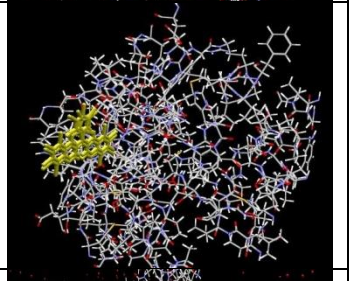
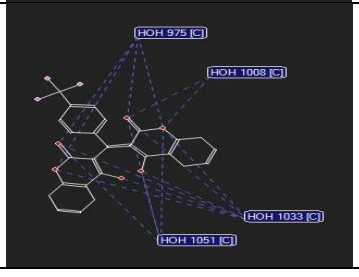
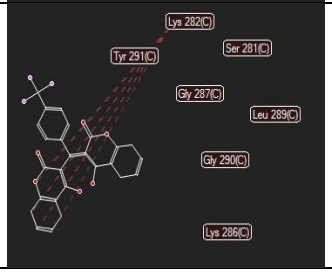
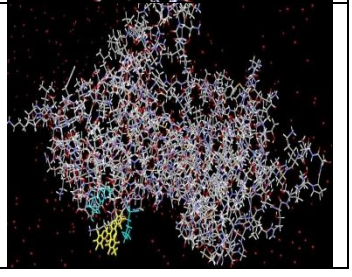
	3c			
	3d			
	3e			
	3f			
	3g			
	3h			

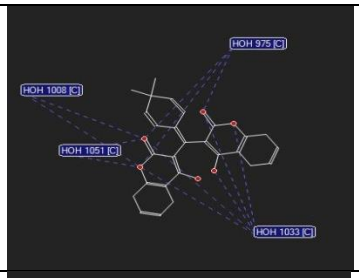
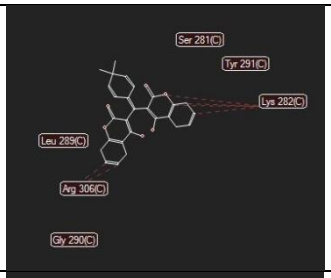
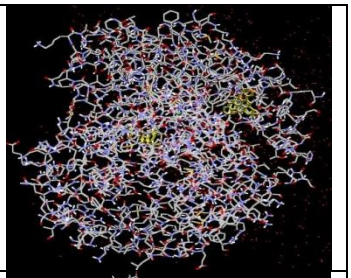
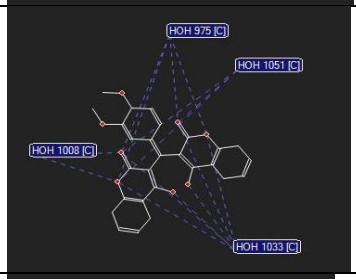
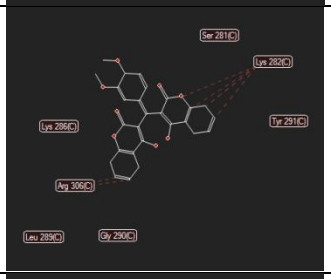
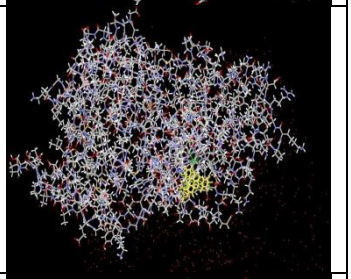
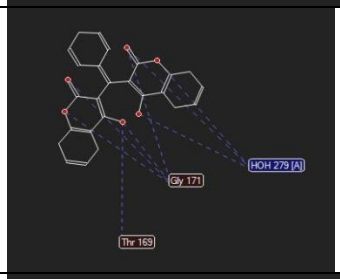
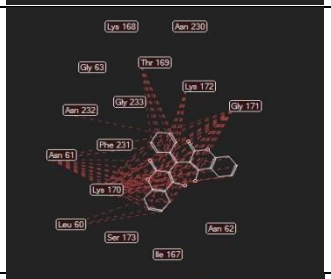
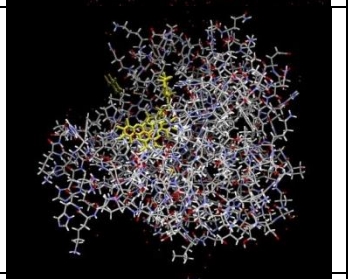
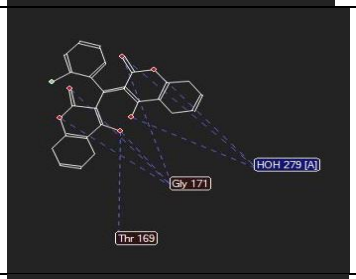
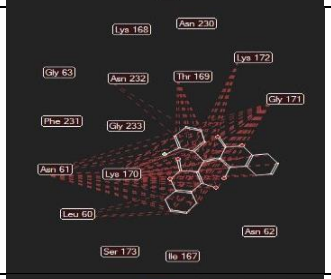
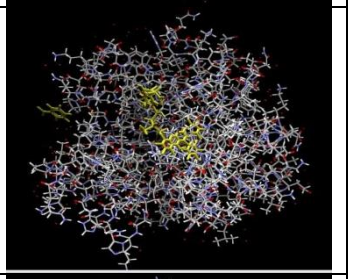
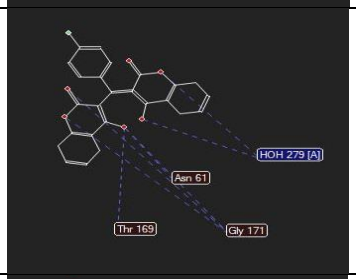
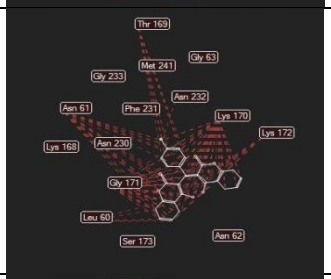
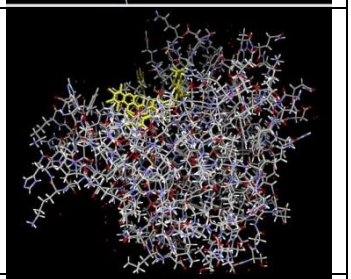
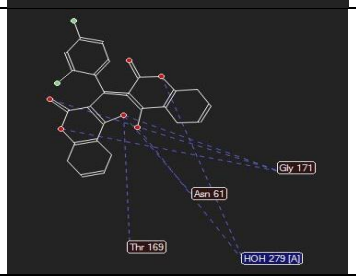
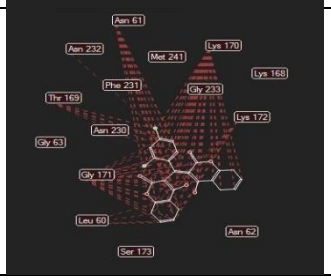
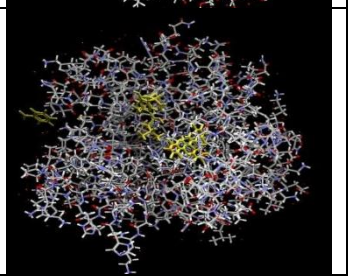
	3i			
	3j			
	3k			
	3l			
	3m			
	3n			

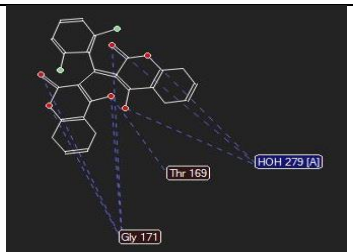
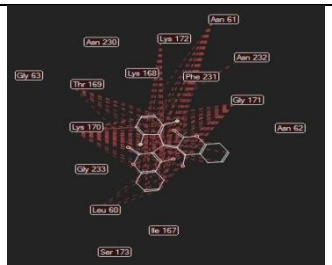
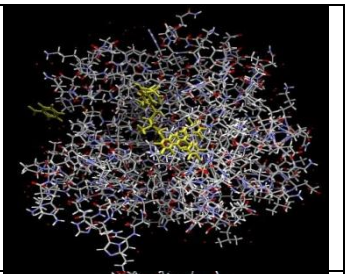
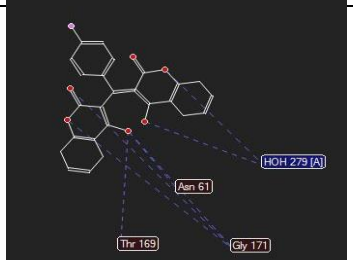
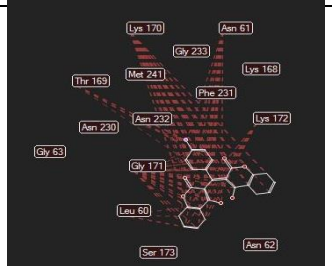
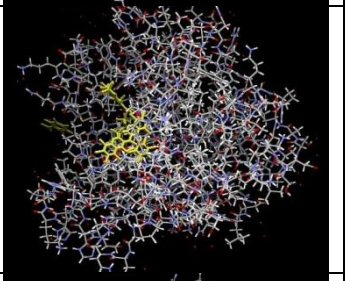
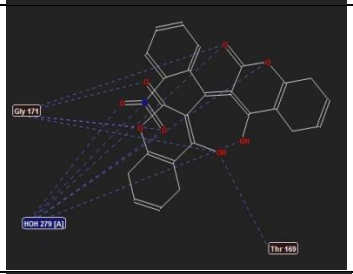
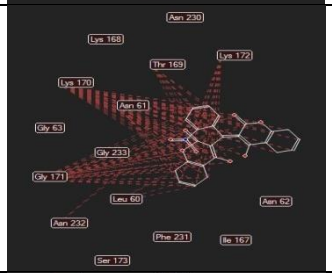
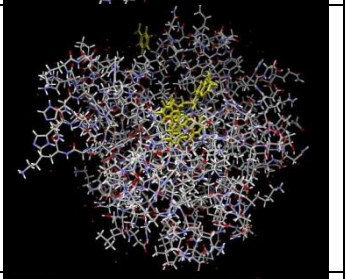
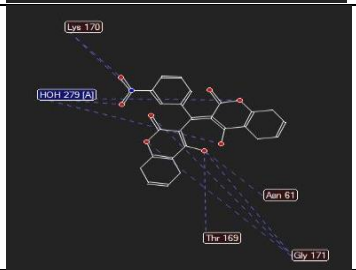
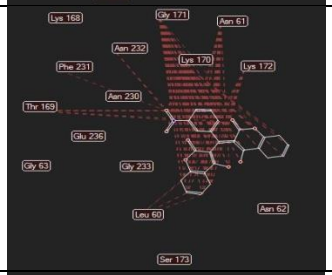
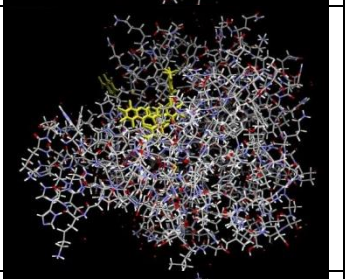
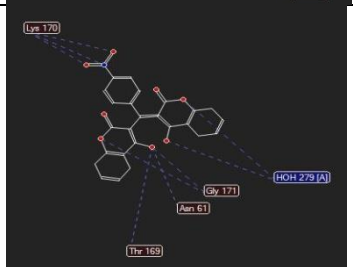
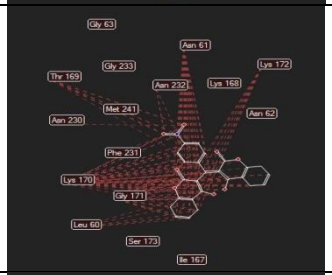
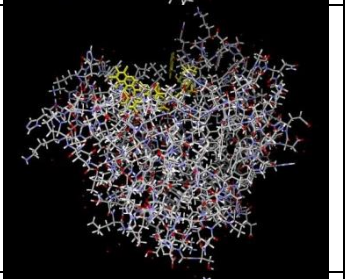
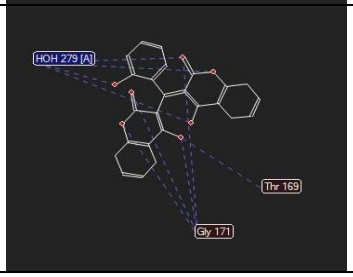
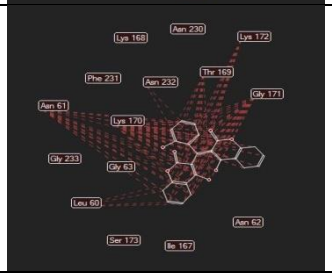
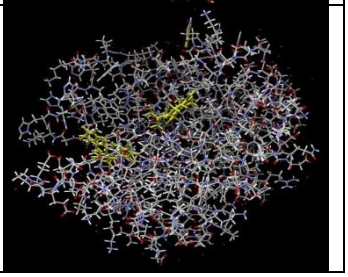
	3o			
	3p			
	3q			
	3r			
	3s			
	3t			

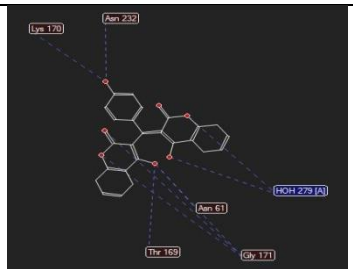
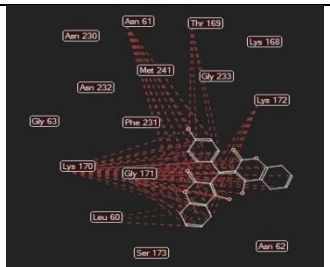
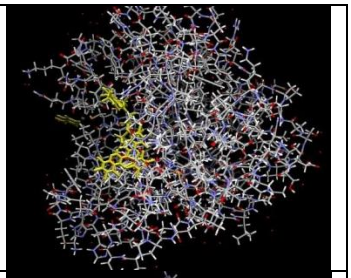
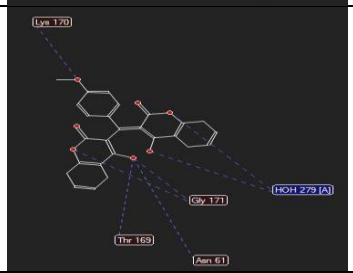
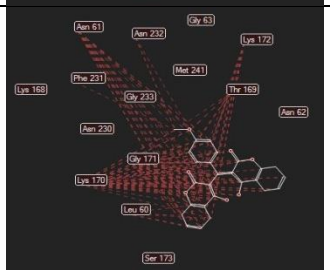
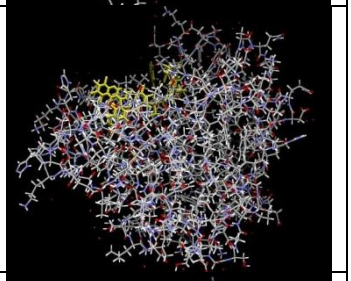
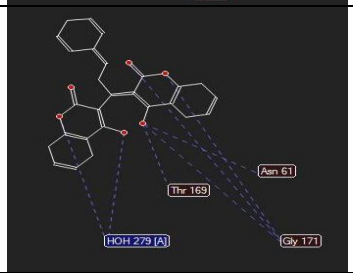
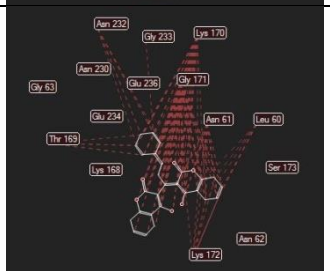
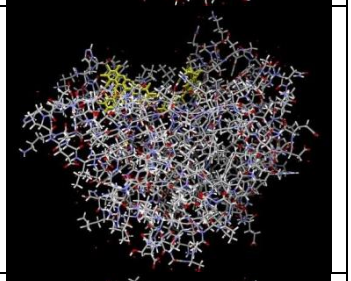
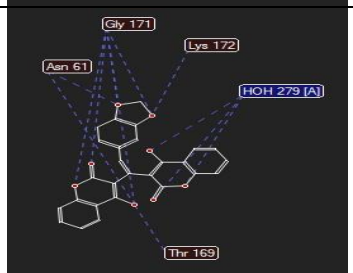
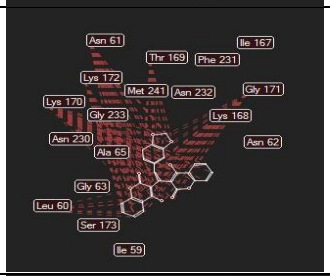
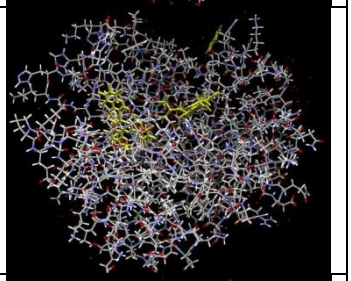
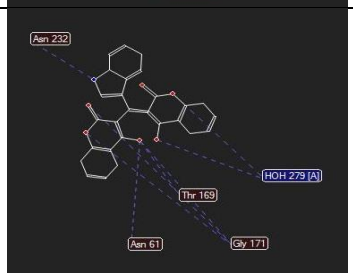
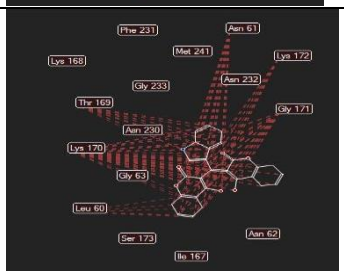
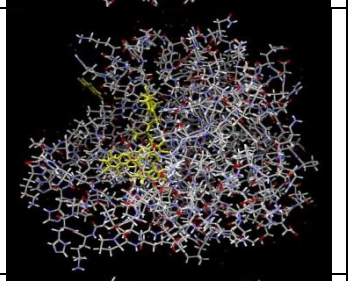
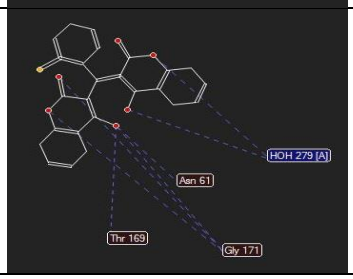
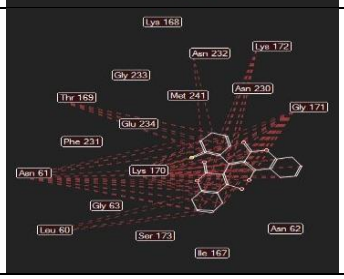
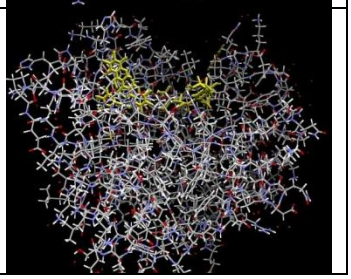
1K3T	3a			
	3b			
	3c			
	3d			
	3e			
	3f			

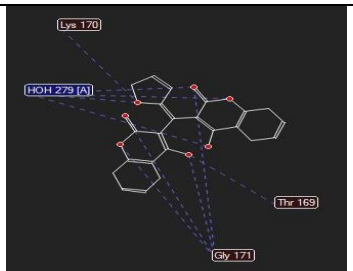
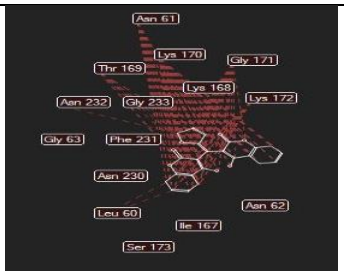
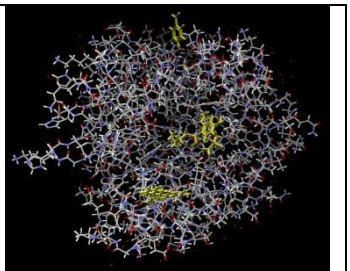
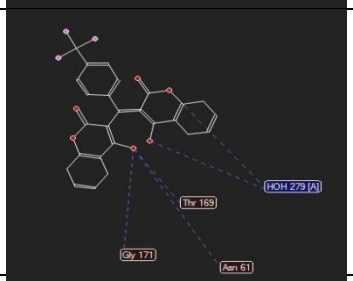
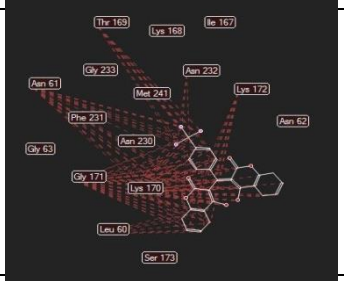
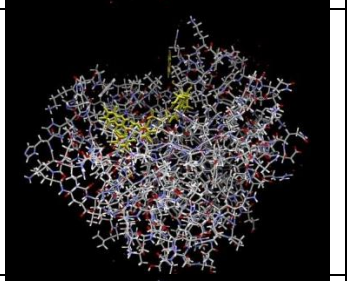
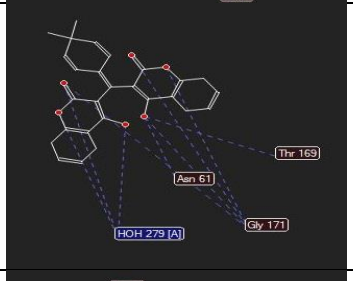
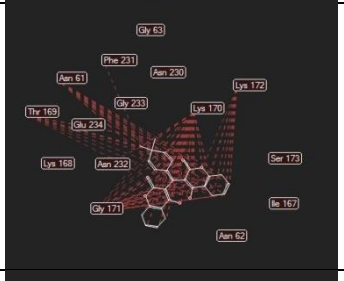
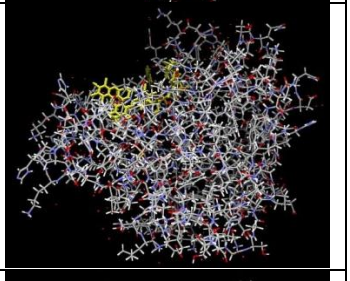
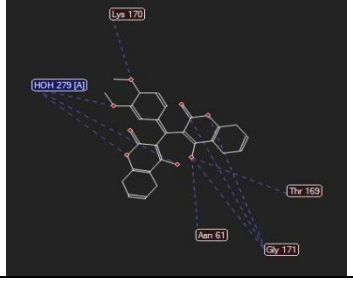
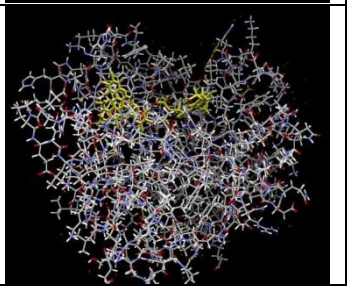
	3g			
	3h			
	3i			
	3j			
	3k			
	3l			

	3m			
	3n			
	3o			
	3p			
	3q			
	3r			

	3s			
	3t			
3F8E	3a			
	3b			
	3c			
	3d			

	3e			
	3f			
	3g			
	3h			
	3i			
	3j			

	3k			
	3l			
	3m			
	3n			
	3o			
	3p			

	3q			
	3r			
	3s			
	3t			

metabolic stability and many others. The diversity of possible drug targets (of which each requires a different combination of matching molecular characteristics) is so enormous, that it is possible to find a common denominator for all of them and to express molecule drug-likeness by a single "magic number". Simple count criteria (like limits for molecular weight, log P, or number of hydrogen bond donors or acceptors) have also relatively limited applicability and are useful only to discard obvious non-drugs [34-45].

At Molinspiration we believe that the strategy which leads to success is not a universal drug-likeness score, but focus on particular drug classes and development of specific activity score for each of these classes. The method implemented uses sophisticated Bayesian statistics to compare structures of representative ligands active on the particular target with structures of inactive molecules and to identify substructure features (which in turn determine physicochemical properties) typical for active molecules [34-45].

By analyzing the data from **Table 4**,we seen that compound 3a to 3t are act as ligand for various receptors like G-Protein coupled receptor (GPCR), Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor, Enzyme inhibitor .The synthesized compound obey the Lipinski rule of five. So the synthesized compound **3a** to 3t may useful as lead compound for various diseases.

The biological properties of selected compound are mentioned in **Table 1** was determined using Molinspiration. Parameters evaluated are helpful in the prediction of biological potent compounds. The globular protein coupled receptors (GPCR) provides correlation data between GPCRs and their ligands, along with chemical information on the ligands, as well as access information to the various web databases regarding GPCRs. These data are connected with each other in a relational database, allowing users in the field of GPCR-related drug discovery to easily retrieve such information from either biological or chemical starting points. GPCR value of compound no.**3p** is -0.41, therefore, found to be most potent ligand according GPCR value. The ion channel modulator (ICM)] activity for **3p & 3q** was found to be -0.41 & -0.44 and said to be more biological potent. Protein kinase inhibitors (PKI) are a type

of enzyme inhibitor that specifically blocks the action of one or more protein kinases. The protein kinase inhibition value for **3h & 3i** were found to be -0.58 & -0.58. Therefore above said are more biological potent. Nuclear receptor (NR) ligand plays a role in every aspect of development, physiology and disease in humans. Value for **3h & 3i** was found to be -0.15. This indicates that Compound no. **3h & 3i** are biological potent. Protease inhibitors (PIs) are a class of drugs used to treat or prevent infection by viruses, including HIV and Hepatitis C. PIs value for 3h & 3i were found to be -0.30. Therefore, compound no. **3h & 3i** will be more biologically potent. Enzyme inhibitors (EIs) are molecules that interact in some way with the enzyme to prevent it from working in the normal manner. Enzyme inhibition value for **3g & 3h** were found to be -0.04 & -0.02. Therefore, compound no. **3h & 3i** will be more potent.

After further studies (**Table 5 & 6**), Molecular docking of the molecules has been performed using Molegero Molecular Viewer 2.5 and we came to know that the mol doc score of interaction between the molecules (**3a-3t**) and PDB ID 3K34, 1K3T & 3F8Eand mol dock score in positive, which clearly indicates the inhibition of the enzymes means higher the mol dock score, lesser the stability of the complex. Amongst all the ligands, **3g** gave highest mol dock score with all the PDB mentioned above and indicates 3g as the most potent inhibitor. Compound 3g showed good inhibition for carbonic anhydrase and the Glyceraldehyde-3-phosphate dehydrogenase. It means 3g can control the activity of inter-conversion of CO_2 and H_2O into bicarbonates and H^+ or vice versa. Further, it can control the phosphorylation of a Monosaccharide [34-45]. Further, it came to know that most of the compounds binds with ASN at 61 position of the carbonic anhydrase PDB and can be one of the reason for its inhibition.

Acknowledgement

The corresponding author acknowledges this work to late Dr. N. N. Ghosh and thankful to Department of Science & Technology (DST) for financial support.

References

[1] Centers for Disease Control and Prevention. National Diabetes fact sheet: general information and national estimates on diabetes in the United States, 2007. Atlanta, GA: Department of Health and Human Services. Centers for Disease Control and Prevention, 2008.

[2] American Diabetes Association 2007. Standards of medical care in diabetes. Diabetes Care. J. 30 Suppl. 1:S4-S41.

[3] K. Schoonjans and J Auwerx. Thiazolidinediones: an update. Lancet; 355,1008-1010(2000).

[4] Rubenstein, Lester A, Lanzara and G Richard. Activation of G protein-coupled receptors entails cysteine modulation of agonist binding. Journal of Molecular Structure (Theochem);430, 57–71(1998).

[5] V Raymond and DB Sattelle. Novel animal-health drug targets from ligand-gated chloride channels.Nature Reviews: Drug Discovery,;1:427-436(2002).

[6] MJ Doenhoff et al. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis.Curr Opin Infect Dis.,;21:659-667(2008).

[7] P. Weissman. Reappraisal of the pharmacologic approach to treatment of type 2 diabetes mellitus. Am J Cardiol,; 90: 42-50(2002).

[8] T. Anastassiadis, et al. Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. Nature Biotech.;29:1039–1045(2011).

[9] M. I. Davis, et al. Comprehensive analysis of kinase inhibitor selectivity. Nature Biotech.;29,1046–1051(2011).

[10] AM Brzozowski, AC Pike, Z Dauter, RE Hubbard, T Bonn, O Engström, L Öhman, GL Greene, J-Å Gustafsson and M Carlquist. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature.;389:753–8(1997).

[12] T Tenson, M Lovmar and M Ehrenberg. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. J. Mol. Biol.;330,1005–1014(2003).

[13] T Kuzuya and A Matsuda. Classification of diabetes on the basis of etiologies versus degree of insulin deficiency. Diabetes Care; 20, 219-20(1997).

[14] AH Katz and CE Caufield. Structure-based design approaches to cell wall biosynthesis inhibitors. Current pharma. Design.;9, 857–66(2003).

[15] S Prashant, K Pradeep, K Anju, K Rashmi, KD Sujata, P Satya and C Ramesh. Phosphotungstic Acid: An efficient catalyst for the synthesis of bis-(4-hydroxycoumarin-3-yl)methanes in water. Cat. Lett., 134, 303-308(2010)

[16] S Prashant, K Kamlesh, K Gaurav and KM Gopal. Efficient One-Pot Four-Component Synthesis of fused Thiazolopyridin-2-ones in Ionic Liquid. J. Chem. Sci. 125(6), 1471–1480(2013)

[17] S Prashant, K Kamlesh, D Monica, KV Vijay, DP Narendera, C Ramesh and KM Gopal. Ionic Liquid catalyzed Synthesis of 7-Phenyl-1,4,6,7-tetrahydro-thiazolo[5,4-d] pyrimidine-2,5-diones. Competes rendus-Chimie,;15,504-510(2012)

[18] K Kamlesh, S Prashant, D Monica, DP Narendera, C Ramesh and KM, Gopal. A catalyst free convenient one pot synthesis of multisubstituted chromeno-thiazolones. Competes rendus-Chimie,;15,267-272(2012).

[19] S Prashant, K Kamlesh, T Vartika, S Mohd., P Rajan, KM Gopal, D Monica, DP Narendra and K Anju. A novel method to chemically bind the thiazolidine-2,4-dione through cross linked chitosan nanoparticles using malanodialdehyde as a cross linker. Can. J. Chem.;89, 1-11(2011).

[20] K Pradip, K Pankaj, S Jagvir, S Prashant and C Ramesh. Synthesis and evaluation of antimicrobial activity of 8-Phenyl-

3,4,7,8-tetrahydrobisthiazolopyridine-2,6-dione derivatives. J Chemistry Asia, 2, 101-114(2011)

[21] S Prashant, K Pradeep, K Anju, K Rashmi, KD Sujata, P Satya and C Ramesh. Synthesis and electrochemical studies of charge transfer complexes of thiazolidine-2, 4-dione with sigma and pi acceptors. Spect. Acta A: Mol. & Biomol. Spect.,75,983-991(2010)

[22] S Prashant, K Kamlesh, K Anju, K Rashmi and C Ramesh. Copper Nanoparticles in Ionic Liquid: An Easy and Efficient Catalyst for Selective Cara-Michael Addition Reaction. Cat. Lett. ;127,119-125(2009).

[23] S Prashant, K Kamlesh, K Anju, K Rashmi and C Ramesh. Cu Nanoparticles in Ionic Liquid: An Easy and Efficient Catalyst for Addition–Elimination Reaction between Active Methylene Compounds and Imines in an Ionic Liquid. Cat. Lett.;130, 648-654(2009)

[24] S Prashant, K Anju, K Rashmi and C Ramesh. Copper nanoparticles in ionic liquid: An easy and efficient catalyst for the coupling of thiazolidine-2,4-dione, aromatic aldehyde and ammonium acetate., Cat. Comm.;9,1618-1623(2008).

[25] S Prashant, K Anju, K Rashmi and C Ramesh. Copper nanoparticles in an ionic liquid: an efficient catalyst for the synthesis of bis-(4-hydroxy-2-oxothiazolyl)methanes. Tet. Lett.;49,727-730(2008).

[26] K Ajeet, S Prashant, S Amit, D Arnab, C Ramesh and M Subho. Nano-sized copper as an efficient catalyst for one pot three component synthesis of thiazolidine-2, 4-dione derivatives. Cat. Comm.;10,17-22(2008).

[27] A Ibrahimi, L Teboul, D Gaillard, E.Z Amri, G Ailhaud, P Young, MA Cawthorne and PA Grimaldi. Evidence for a common mechanism of action for fatty acids and thiazolidinedione antidiabetic agents on gene expression in pre-adipose cells. Mol. Pharmacol.;46,1070-76(1994).

[28] RF Kletzien and SD Clarke, , Ulrich RG.Enhancement ofadipocyte differentiation by an insulin-sensitizing agent. Mol. Pharmacol.; 41, 393-398(1992).

[29] JM Lehmann, LB Moore, TA Smith-Oliver, WO Wilkison, TM Willson, and SA Kliewer. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferatoractivated receptor gamma (PPAR gamma). J. Biol. Chem.; 270, 12953-12956(1995).

[30] R Komers and A Vrana. Thiazolidinediones – tools for the research of metabolic syndrome X. Physiol. Res.; 47, 215-225(1998).

[31] JM Lehmann, LB Moore, TA Smith-Oliver, WO Wilkison, TM Willson and SA Kliewer. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J. Biol. Chem.;270,12953-6(1995).

[32] RM Evans, GD Barish and Y-X Wang. PPARs and the complex journey to obesity. Nat. Med.,10, 355-61(2004).

[33] M, Willson TM, Brown PJ, Sternbach DD and Henke BR. The PPARs: From Orphan Receptors to Drug Discovery. J. Med. Chem.43,527-50(2000).

[34] J Berger and DE Moller. The mechanisms of action of PPARs. Annu. Rev. Med., 53,409-35(2002).

[35] C Hansch and A Leo. Substituent constants for correlation analysis in chemistry and biology. New York: John Wiley & Sons, 1979.

[36] L Rathi, SK Kashaw, A Dixit, G Pandey and A.K Saxena. Pharmacophore identification and 3D-QSAR studies in N-(2-benzoyl phenyl)-l-tyrosines as PPAR gamma agonists. Bioorg. Med. Chem. 12, 63–69(2004).

[37] C Liao, A Xie, L Shi, J Zhou and X Lu. Eigenvalue analysis of peroxisome proliferator-activated receptor gamma agonists. J. Chem. Inf. Comput. Sci.44, 230–238(2004).

[38] KH Hyun, DY Lee, B Lee and CK Kim. Receptor-based 3D QSAR studies on PPARgamma agonists using CoMFA and CoMSIA approaches. QSAR Comb. Sci.; 23, 637–649(2004).

[39] Y Kurogi. Three-dimensional quantitative structure–activity relationships (3D-QSAR) of antidiabeticthiazolidinediones. Drug Des. Discov., 16, 109–118(1999).

[40] C Rucker, M Scarsi and M Meringer. 2D QSAR of PPARgamma agonist binding and transactivation. Bioorg. Med. Chem., 14, 5178–5195(2006).

[41] C Liao, A Xie, L Shi, J Zhou and X Lu. Eigenvalue Analysis of Peroxisome Proliferator-Activated Receptor ç Agonists. J. Chem. Inf. Comput. Sci.;44, 230-238(2004).

[42] L Rathi, SK Kashaw, A Dixit, G Pandey and AK Saxena. .Pharmacophore identification and 3D-QSAR studies in N-(2-benzoylphenyl)-L-tyrosines as PPAR-gamma agonists. Bioorg. Med. Chem.;12, 63-69(2004).

[43] SS Kulkarni, LK Gediya and VM Kulkarni. Three-dimensional quantitative structure activity relationships (3-D-QSAR) of antihyperglycemic agents. Bioorg. Med.Chem.;7, 1475-1485 (1999) .

[44] CA Sotriffer, RH Winger, KR Liedl, M Rode Bernd and JM. Varga Comparative docking studies on ligand binding to the multispecific antibodies IgE-La2 and IgE-Lb4. J. Comp Aided Mol.Des.;10, 305-320(1996).

[45] Y. Kurogi. Three-dimensional quantitative structure-activity relationships (3D-QSAR) of antidiabetic thiazolidinediones. Drug Des Discov.;16, 109–118(1999).

[46] LF. Brass, Thrombin and platelet activation. 2003 Chest 124 (3 Suppl): 18S–25S