International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.08 pp 194-201, 2016

Docking Studies of Some 2-(Benzo[d] isoxazole-3-yl)-N-(oxothiazolidine) Derivative with COX-II and Thromboxane as Target Protein and Evaulation of its Anti Inflammatory Activty

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Abstract: A scaffold of ten thiazolidinone derivatives of 1,2-Benzisoxazole [5a-5j] were synthesized using 4-Hydroxy-2H-chromen-2-one as the starting material. The synthesis was carried out by conventional technique and the synthesized compounds were identified by physical methods and their structures characterized by spectral analysis. The identified compounds were further subjected to docking studies using two proteins, COX-2 and thromboxane which are known as mediators of inflammation. All the compounds showed good docking scores indicating that they are potent inhibitors of COX-2 and thromboxane. In line with the above result, invitro anti-inflammatory studies were carried out on the moieties by HRBC membrane stabilization method using diclofenac as standard. Significant anti-inflammatory activity was observed in compounds 5a, 5b, 5c, 5f, 5g and 5i.
Keywords: Thiazolidinone, 1, 2-Benzisoxazole, anti-inflammatory, docking, Thromboxane.

Introduction:

In the last decade, 3-substituted 1,2-benzisoxazole has evoked a growing interest among other heterocycles, because of their diverse pharmacological properties and their versatility in the development of novel scaffold's with unique properties. Compounds containing this heterocycle are reported to find wide use as anticonvulsant,[1] antipsychotic,[2] anticancer,[3] antimicrobial,[4] analgesic[5] and plant growth regulator.[6] Similarly, thiazolidine-4-ones are also an important class of heterocyclic compounds which has been subjected to extensive study in the recent past. Pharmacological properties like antitubercular,[7] antifungal,[8] antibacterial,[9] anticonvulsant[10] and antithyroid[11] action have been found to be associated with derivatives of thiazolidin-4-one. Keeping the above said wide span of biological properties in mind, a valuable attempt was sought to synthesize a new series of compounds, possessing both 1,2-benzisoxazole and Thiazolidin-4-one heterocycles. The new derivatives were characterized and evaluated for invitro anti-inflammatory properties.[12] The new analogues were subjected to preliminary docking studies to identify the anti-inflammatory potency using the protein sequences of COX-II [13]and thromboxane[14].

We herein report the synthesis of a new series of thiazolidin-4-one derivatives of 1,2-benzisoxazole and the results of their biological evaluation for invitro anti-inflammatory properties along with the results of the docking studies for anti-inflammatory action.

Materials and Methods:

Synthesis:

Ten different thiazolidinone derivatives (5a-5j) were synthesized[15-18]. Hydroxycoumarin was converted into oxime to form 1,2-Benzisoxazole-3-acetic acid. 1,2-Benzisoxazole-3-acetic acid was converted to Ethyl 2-(benzo[d]isoxazol-3-yl)acetate by esterification.This esterified product undergoes hydrazinolysis to give the resultant 2-(Benzo[d]isoxazol-3-yl)acetohydrazide derivatives which undergoes cyclisation reaction to yield thiazolidinone derivatives of 1,2-benzisoxazole. The procedure afforded various thiazolidinone derivatives in 59-82% yield.

Docking Studies:

In the present investigation, the enzymes COX-2 and thromboxane was identified with PDB ID from RCSB Protein Data Bank [PDB ID: 3NTG and PDB ID: 3NGV][19-20]. The location of the binding sites on the protein was identified using Q-site finder. Q-site finder uses the interaction energy between the protein and a simple Vander waal's probe to locate energetically favorable binding sites. Energetically favorable probe sites are clustered according to their spatial proximity and clusters are then ranked according to their sum of interaction energies for sites within each cluster 2D &3 D. Structures of all the synthesized compounds were drawn in ACD labs CHEMSKETCH (a structure drawing program). Geometry optimization of 3 D structures and docking simulation was carried out in Argus lab 4.0.1 (from Thomson and Planaria software LLC). The analysis of binding free energy and interactions of ligands with residues at active site was carried out by using Pymol.

Anti-Inflammatory Activity:

The newly obtained derivatives were evaluated for invitroanti-inflammatory activity by HRBC membrane stabilization method[21-25]. The synthesized compounds were made into doses of 1000µg/ml with DMSO (5.0%) solution. Diclofenac sodium was taken as the standard. The reaction mixture consisted of 2ml of hypotonic saline (0.36% sodium chloride), 1ml of 0.15M phosphate buffer (pH 7.4), and 1ml of test solution (1000µg/ml) in normal saline. For control, 1ml of isotonic saline was used instead of test solution. All the assay mixtures were incubated at 56ºC for 30 minutes and centrifuged at 3000rpm for 20 minutes. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%. The percentage inhibition is calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{Absorbance of the control- Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Results and Discussion:

The protein was docked with ten different types of ligands using Argus lab software and the docking score along with the hydrogen bond length and amino acid residue was visualized using pymolvisualiser and noted.

Table: ligands docked with COX-2

S.NO	Protein (Cox-2) "Chain-A"	Ligand	Docking Score	Bond Length	Amio Acid Residue
1.	COX-2	2-(Benzo [d] isoxazol-3-yl)-N- (4-oxo-2-phenylthiazolidin-3-yl) acetamide	-12.793 kcal/mol	16.0 10.4 17.2	PHE224 THR561 GLY552
2.	COX-2	2-(Benzo [d] isoxazol-3-yl)-N-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-yl) acetamide	-11.655 kcal/mol	16.3 15.1 7.7	SER563 ALA562 ARG321
3.	COX-2	2-(Benzo[d]isoxazol-3-yl)-N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl) acetamide.	-11.103 kcal/mol	10.8 20.4 20.5	LYS557 TRP545 ALA562
4.	COX-2	2-(Benzo[d]isoxazol-3-yl)-N-(2-(3-hydroxyphenyl)-4-oxothiazolidin-3-yl) acetamide.	-9.100 kcal/mol	15.0 10.8 18.2	GLA553 LYS557 LYS360
5.	COX-2	2-(Benzo[d]isoxazol-3-yl)-N-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl) acetamide.	-10.011 kcal/mol	7.2 24.6 20.3	PHE224 ASN104 PRO106
6.	COX-2	2-(Benzo[d]isoxazol-3-yl)-N-(2-(3-nitrophenyl)-4-oxothiazolidin-3-yl) acetamide.	-11.124 kcal/mol	24.8 23.6 16.8	RRO107 GLU107 PHE361
7.	COX-2	2-(Benzo[d]isoxazol-3-yl)-N-(2-(2-nitrophenyl)-4-oxothiazolidin-3-yl) acetamide.	-6.996 kcal/mol	15.0 9.2 14.8	LYS225 LYS224 THR105
8.	COX-2	2-(Benzo[d]isoxazol-3-yl)-N-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)acetamide	-7.271 kcal/mol	23.2 4.3 17.0	GLU228 GLU553 LYS225
9.	COX-2	2-(Benzo[d]isoxazol-3-yl)-N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)acetamide.	-8.464 kcal/mol	15.6 15.0 20.1	LYS113 ASN570 TYR225
10.	COX-2	2-(Benzo[d]isoxazol-3-yl)-N-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)acetamide.	-8.444 kcal/mol	30.3 31.5 30.8	ALA303 PRO232 ASP223

Table: Ligands docked with Thromboxane

S.No	Protein	Ligand	Docking Score	Bond Length	Amio Acid Residue
1.	Thromboxane	2-(Benzo [d] isoxazol-3-yl)- N- (4-oxo-2-phenylthiazolidin-3-yl) acetamide. (5a)	-12.57 kcal/mol	3.3 3.9 16.5	SER275 PHE205 LYS261
2.	Thromboxane	2-(Benzo [d] isoxazol-3-yl)-N-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-yl) acetamide. (5b)	-11.246 kcal/mol	10.8 13.7 15.6	SER275 LEU271 PHE217
3.	Thromboxane	2-(Benzo[d]isoxazol-3-yl)-N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl) acetamide. (5c)	-10.832 kcal/mol	12.0 16.3 5.0	SER273 PHE89 ILE43
4.	Thromboxane	2-(Benzo[d]isoxazol-3-yl)-N-(2-(3-hydroxyphenyl)-4-oxothiazolidin-3-yl) acetamide. (5d)	-12.005 kcal/mol	12.5 8.9 60.7	LEU98 ASP31 ALA243
5.	Thromboxane	2-(Benzo[d]isoxazol-3-yl)-N-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl) acetamide. (5e)	-12.439 kcal/mol	16.6 13.0 16.0	PHE217 VAL267 SER187
6.	Thromboxane	2-(Benzo[d]isoxazol-3-yl)-N-(2-(3-nitrophenyl)-4-oxothiazolidin-3-yl) acetamide. (5f)	-10.851 kcal/mol	15.1 11.8 14.2	PRO276 SER273 GLN263
7.	Thromboxane	2-(Benzo[d]isoxazol-3-yl)-N-(2-(2-nitrophenyl)-4-oxothiazolidin-3-yl) acetamide. (5g)	-11.515 kcal/mol	8.5 12.7 6.9	MET147 HIS149 LEU65
8.	Thromboxane	2-(Benzo[d]isoxazol-3-yl)-N-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)acetamide. (5h)	-9.993 kcal/mol	5.3 13.6 7.7	ASN75 SER163 GLY53
9.	Thromboxane	2-(Benzo[d]isoxazol-3-yl)-N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)acetamide. (5i)	-13.257 kcal/mol	4.0 18.1 16.8	SER275 ASN87 LYS261
10.	Thromboxane	2-(Benzo[d]isoxazol-3-yl)-N-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)acetamide. (5j)	-11.468 kcal/mol	8.7 8.5 8.9	ALA306 LEU299 ASN185

The above tabulated results exhibit good binding of the synthetic ligands with the two proteins, indicating the presence of anti-inflammatory potency. Comparison of the docking scores of the ligands 5a-5j with the selected two proteins –COX-2 and Thromboxane, showed better scores with thromboxane than with COX-2.

The ligand 2-(Benzo [d] isoxazol-3-yl)- N- (4-oxo-2-phenylthiazolidin-3-yl) acetamide (5a) gave the highest docking score of -12.793 kcal/mol when docked with COX-2 with hydrogen bond lengths of 16.0, 10.4, 17.2 with amino acid residuesPHE2247, THR561, GLY552 respectively.

The ligands2-(Benzo[d]isoxazol-3-yl)-N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)acetamide (5i) gave the highest docking score of -13.257 kcal/mol when docked with Thromboxane with hydrogen bond lengths of 4.0, 18.1, 16.8 with amino acid residues SER275, ASN87, LYS261 respectively.

These two ligands can be identified as the ones with maximum anti inflammatory activity

Ligands 5b, 5c, 5d, 5e, and 5f displayed appreciable docking scores with the protein COX-2 indicating good anti-inflammatory activity and ligands 5g, 5h, 5i, and 5j gave optimum scores when docked with the protein COX-2 indicating optimum anti-inflammatory activity.

When docked with the protein thromboxane the ligands 5a, 5b, 5c, 5d, 5e, 5f, 5g, 5h, and 5j (5i showing maximum score) revealed excellent docking scores indicating a very good anti-inflammatory activity.

Thus, it is found that the ligands 5a-5j have efficient binding with the enzymes mediating inflammation viz..COX-2 and Thromboxane.These results implicates the ligands as good anti-inflammatory agents.

Cyclooxygenase Ii Enzyme **Thromboxane**

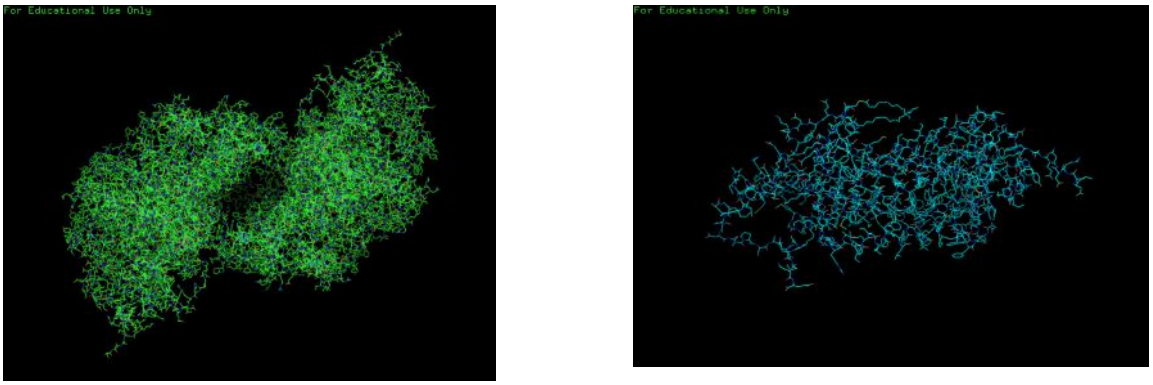
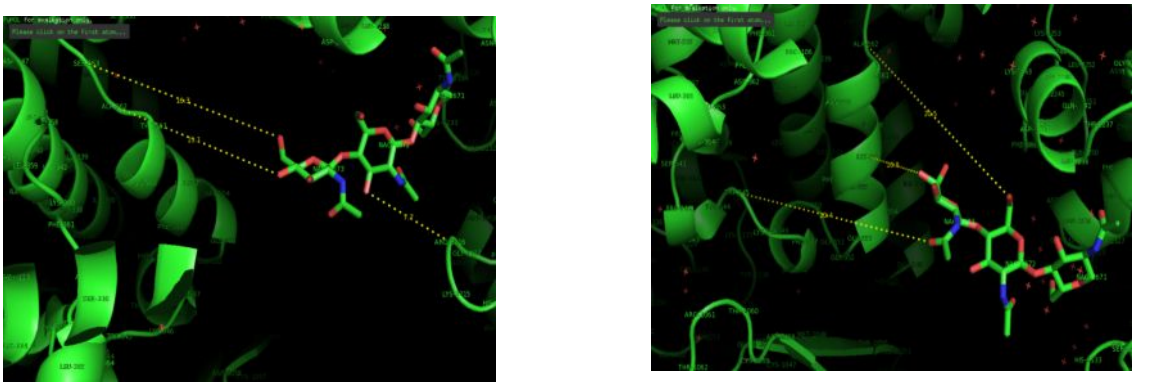
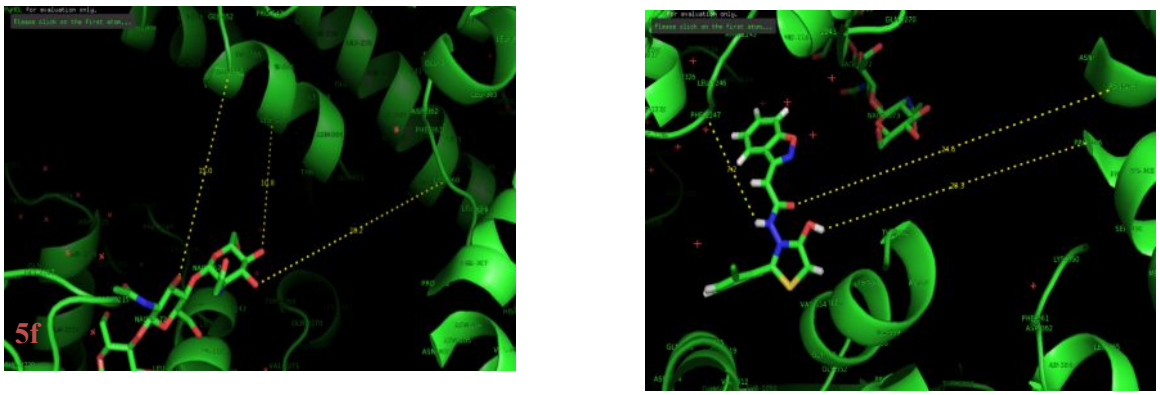


Figure 1: Crystal structure of COX-II and thromboxane from RCSB PDB.

5b **5c**



5d **5e**



5 f

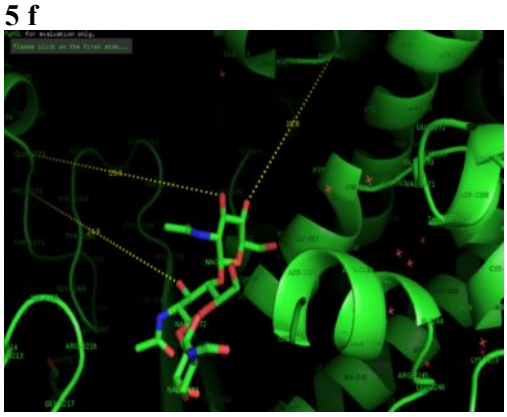
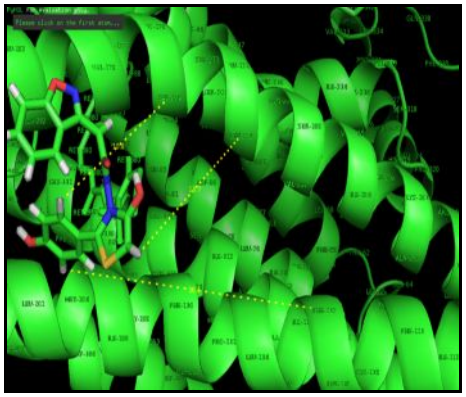
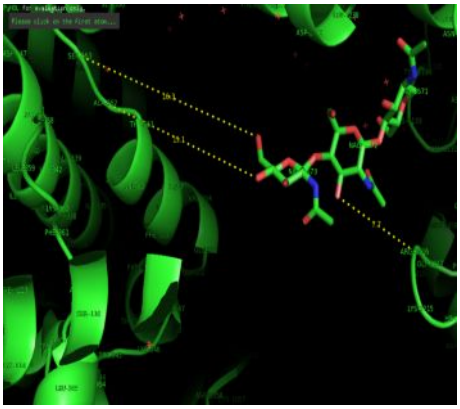


Figure 2. DCOKING OF LIGAND (5b,5c,5d,5e and 5f) with Cyclooxygenase II

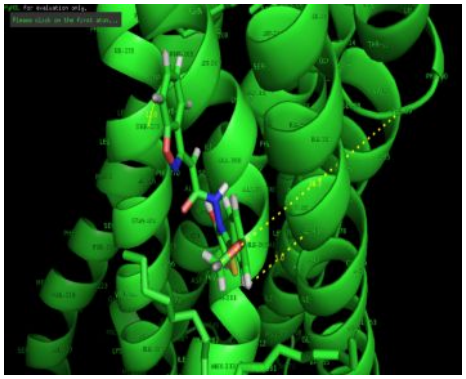
5a



5b



5c



5d

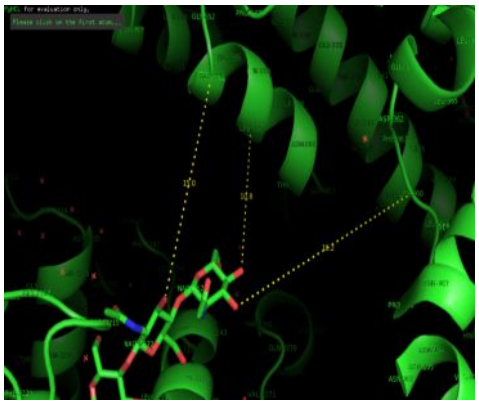


Figure 3.Docking of 5a, 5b,5c and 5 d with THROMBOXANE.

Anti –inflammatory activity:

Percentage stabilization of synthesized compounds:

S.No	Compound Code	% Stabilization
1	5a	62.84
2	5b	56.76
3	5c	56.55
4	5d	29.1
5	5e	48.62
6	5f	54.12
7	5g	55.71
8	5h	59.33
9	5i	36.26
10	5j	44.35
11	Control	---
12	Diclofenac sodium	76.80

The synthesized compounds were subjected to invitro anti-inflammatory activity using HRBC membrane stabilization method. The method involves the stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The prevention of hypo tonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of the drug. The compounds 5a, 5h,5b,5g and 5f showed better activity as compared to the standard diclofenac. Rest of the compounds also showed activity.

Conclusion:

Series of 2-(Benzo[d] isoxazole-3-yl)-N-(oxothiazolidine) synthesized derivatives were screened for anti-inflammatory activity using molecular docking and in vitro anti-inflammatory studies. Docking in AutodockVina showed compounds 5a-5j possesses good anti-inflammatory activity. Inhibiton of inflammation mediating enzyme thromboxane showed better activity when compared to COX-2. Ligands 5i and 5a showed maximum docking scores with thromboxane and COX-2 indicating maximum anti-inflammatory potency. In vitor anti-inflammatory activity by HRBC membrane stabilization method showed better activity with compounds 5a,5h,5b,5g and 5f.

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