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Original Article

DESIGN OF COX-2 INHIBITORS–AN *IN-SILICO* APPROACH

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ABSTRACT

Objective: The aim of the present work was to design the novel series of chalcone derivatives of indane-1,3-dione for its inhibition towards COX-2.

Methods: COX-2 inhibitors were designed on the binding ability of the compounds with the target. Docking analysis was performed using Acclerys discovery studio 3.5. Molecular properties, ADME parameters, Toxicity parameters were analysed using the same *in-silico* tool.

Results: Most of the designed compounds were possessing good binding affinity towards the COX-2. Other *in-silico* parameters such as ADMET and TOPKAT were within the appreciable range. Among all the designed compounds several compounds possess good CDOCKER energy and CDOCKER interaction energy with specific amino acid indicating that it could possess good binding with the target. Most of the design compounds could act as COX-2 because it forms hydrogen bonding with ARG120.

Conclusion: Compound **l** possess good binding affinity indicating that the presence of hydroxyl group in the phenyl ring possess good activity which can be further optimized for its druggability after its pharmacological activity.

Keywords: Anti-inflammatory, Docking, CDOCKER, Cyclooxygenase.

INTRODUCTION

Inflammation is a part of the defence mechanisms that involved in the inflammatory reactions associated with the release of histamine, bradykinin and prostaglandins [1, 2]. COX (Cyclooxygenase) was believed to be expressed constitutively with constant levels in individual tissues [3]. Prostaglandin synthesis was believed to increase in inflammation because of increased release of precursor [4]. COX activity increases in inflammation, and this increase can be prevented by corticosteroids [5].

X-ray crystallography of the 3-D structure of COX-1 and COX-2 has done much to show how NSAIDs work. COX-1 and COX-2 are very similar enzymes consisting of a long narrow channel with a hairpin bend [6]. Several observations have shown that NSAIDs act on COX to inhibit prostaglandin synthesis. X-ray crystallography suggested that this blocking occurs by hydrogen bonding to the polar arginine at position 120 [4].

Structure-based drug design helps in identifying the ligand with the target protein, in its complex [7]. The knowledge of binding site helps to design novel drug candidates with better potency. The goal of small-molecule drug discovery is to modulate the activity of a biological target *via* interactions with an externally administered molecule at optimal drug intervention points to afford the maximum therapeutic index [8].

This work is mainly planned to design derivatives of chalcone of indane-1,3-dione for its binding affinity towards COX-2 inhibition and also evaluate for its other *in-silico* parameters such as ADME and toxicity parameter. The chalcones were designed on the basis of binding with Arg 120, since the studies suggest [4] that blocking through hydrogen bond could inhibit COX activity.

MATERIALS AND METHODS

Molecular properties

Molecular properties were important in designing compounds [9]. It was studied using Acclerys Discovery studio 3.5 and was depicted in table 1.

Docking

Docking study was performed using Acclerys Discovery studio 3.5 versions running on windows 7 service pack 1 OS.

Protein preparation

The X-ray crystallographic structure of COX-2 (PDB ID, 1cx2) protein was obtained from the protein data bank at a resolution of 3.0Å [10]. The crystal structure of COX-2 inhibitor complex with SC-558 was obtained from protein data bank (pdb: 1CX2). The structure was tetramer. Chain A was used for docking after deleting water molecules. After importing, the chain A is subjected protein preparation wizard using CHARM force field [11].

Ligand preparation

The ligand molecules were drawn in Acclerys Discovery studio 3.5 and the energy was minimized using the same software [12]. The minimized protein and ligands were used for docking.

Docking using CDOCKER

To identify the molecular binding interaction of the designed compounds with the receptor all the compounds were docked into the active binding site of the enzyme COX-2. Docking was performed using CDOCKER for predicting the protein–ligand interactions [13]. CDOCKER energy, CDOCKER interaction energy, secondary bonding mainly hydrogen bonding and the amino acid involved in the binding were used to predict the effect of designed drug binding with the target. The docking result of the ligands was listed in table 2. The docking process involves a conformational search for compound which compliments a target binding site, with the aim of identifying the best matching pose along with the active site to perform docking. The stability of the docked ligand-protein complex is due to hydrogen bonding and Vanderwaals interactions.

ADME Parameters

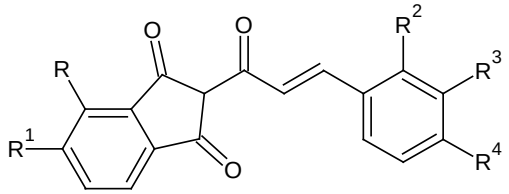
The newly designed derivatives were studied for its ADME descriptors using Discovery Studio 3.5 in which Blood Brain Barrier Penetration, Intestinal Absorption, Aqueous solubility, Hepatotoxicity, Cytochrome P450 inhibition and Plasma Protein Binding level [14] were predicted and tabulated in table 3.

Toxicity parameter

Virtual toxicity study was performed for the designed molecules using TOPKAT. This uses a training set of structure library in the database, based on the structural features in the query chemical compounds to predict the toxicity. If the query structure does not

belong to the training set, the software displays the result of the warning. Aerobic Bio-Degradability, AMES Mutagenicity, Developmental Toxicity Potential, Ocular Irritancy, Skin Irritancy, Carcinogenicity for Female Mouse, Male Mouse, Female Rat, Male Rat were calculated using TOPKAT [15]. The data of toxicity parameters were represented in table 4.

Table 1: Molecular properties of designed derivatives



Ligand code	Substituent	Mol MW	Mol surface area	No of HB donors	No of HB acceptor	AlogP
a	R^1 =CH_3	289.305	60.32	0	3	3.794
b	R^1=OH	291.278	80.55	1	4	3.066
c	R^1=F	293.269	60.32	0	3	3.513
d	R=F,R^1=F	311.259	60.32	0	3	3.719
e	R^1=NH_2	290.293	86.34	1	4	2.561
f	R^4=SH	306.335	80.22	0	4	3.747
g	R^1=SO_2NH_2	354.357	128.86	1	5	2.013
h	R^1= NH_2, R^2=CH_3	304.319	86.34	1	4	3.047
i	R^1= CH_3, R^4=CH_3	303.331	60.32	0	3	4.28
j	R^1=NH_2, R_3=SO_2NH_2	385.437	169.9	2	6	2.165
k	R^1=Cl	309.723	60.32	0	3	3.972
l	R^2=OH	291.278	80.55	1	4	3.066
m	R=NH_2, R^4=OH	306.292	106.57	2	5	2.319
n	R^4=OH	291.278	80.55	1	4	3.066
o	R^3=NH_2	290.293	86.34	1	4	2.561
p	R^4=NH_2	290.293	86.34	1	4	2.561
q	R^2=NH_2	290.293	86.34	1	4	2.561

Table 2: Docking study of the designed compounds towards COX-2

Ligand code	CDOCKER Energy	CDOCKER interaction Energy	Interactions ligand-residue	H-bond distance in Å	Interacting amino acids
a	9.0599	-17.3778	Carbonyl group of Indane-1,3-dione	2.10267	TYR355
			Carbonyl group in chalcone	2.19809	ARG513
b	9.0485	-17.3098	Carbonyl group of Indane-1,3-dione	2.48498	ARG120
			Carbonyl group of Indane-1,3-dione	2.09973	TYR355
			Carbonyl group of chalcone	2.14781	ARG513
c	18.5690	-12.8565	Carbonyl group of Indane-1,3-dione	1.91725	ARG120
			Carbonyl group of chalcone	1.85145	TYR355
d	11.6089	-18.3292	Fluoro group	2.27749	LYS83
			Fluoro group	1.95839	LYS83
			Carbonyl group of Indane-1,3-dione	2.17305	ARG120
e	9.6416	-17.9761	Carbonyl group of Indane-1,3-dione	2.06582	TYR355
			Carbonyl group of Indane-1,3-dione	2.32664	ARG513
			Carbonyl group of chalcone	2.33576	ARG513
			Carbonyl group of chalcone	2.27727	ARG513
			Amino group	2.38899	LEU352
f	11.0967	-15.8717	Carbonyl group of Indane-1,3-dione	2.10059	TYR355
			Carbonyl group of chalcone	2.21046	ARG513
g	4.9758	-20.7633	Carbonyl group of Indane-1,3-dione	2.33615	ARG120
			Carbonyl group of chalcone	2.47006	ARG513
h	17.7657	-16.0606	Carbonyl group of Indane-1,3-dione	2.33615	ARG120
			Carbonyl group of chalcone	2.47006	ARG513

i	7.35517	-18.7047	Carbonyl group of Indane-1,3-dione	2.08576	TYR355
			Carbonyl group of chalcone	2.27312	ARG513
			Carbonyl group of chalcone	2.47683	ARG513
j	14.3217	-19.1173	SO_2 group	2.19495	LYS83
			SO_2 group	1.83469	ARG120
			Carbonyl group of Indane-1,3-dione	2.40013	ARG120
			Carbonyl group of chalcone	2.09184	TYR355
			Carbonyl group of chalcone	2.15722	ARG513
k	14.0117	-15.9032	Chlorine in phenyl group	2.45276	LYS83
			Carbonyl group of Indane-1,3-dione	2.2244	ARG120
			Carbonyl group of chalcone	2.46307	ARG513
l	3.3472	-27.0175	Hydroxyl group	1.77567	TYR355
			Hydroxyl group	2.30609	ARG513
m	8.9326	-20.1671	Carbonyl group of Indane-1,3-dione	2.1153	TYR355
			Carbonyl group of Indane-1,3-dione	2.26669	ARG513
			Carbonyl group of chalcone	2.40243	ARG513
			NH_2 group in phenyl ring	2.19598	LEU352
n	7.8069	-18.8413	Carbonyl group of Indane-1,3-dione	2.12029	TYR355
			Carbonyl group of chalcone	2.21078	ARG513
			Carbonyl group of chalcone	2.48055	ARG513
o	7.9388	-18.2033	Carbonyl group of Indane-1,3-dione	2.49774	ARG120
			Carbonyl group of Indane-1,3-dione	2.128	TYR355
			Carbonyl group of chalcone	2.17392	ARG513
p	9.5658	-16.7614	Carbonyl group of Indane-1,3-dione	2.14541	TYR355
			Carbonyl group of Indane-1,3-dione	2.28169	ARG513
			Carbonyl group of chalcone	2.48836	ARG513
q	18.3698	-19.2464	-	-	-

Table 3: ADME profile of the designed derivatives

Ligand code	BBB level	Absorption level	Solubility level	Hepatotoxicity level	CYP2D6 level	PPB level
a	0	0	2	0	0	1
b	0	0	3	0	0	1
c	0	0	2	0	0	1
d	0	0	2	0	0	1
e	0	0	3	0	0	1
f	0	0	2	0	0	1
g	-	0	2	0	0	1
h	0	0	2	1	0	1
i	0	0	2	0	0	1
j	-	0	2	0	0	0
k	0	0	2	0	0	1
l	0	0	3	0	0	1
m	1	0	3	0	0	1
n	0	0	3	0	0	1
o	0	0	3	0	0	1
p	0	0	3	0	0	1
q	0	0	3	0	0	1

Table 4: Toxicity profile of the designed derivatives

Ligand code	Aerobic bio-degradability	AMES mutagenicity	Developmental toxicity potential	Ocular irritancy	Skin irritancy	Carcinogenicity			
						Female mouse	Male mouse	Female rat	Male rat
a	No	No	Yes	Mild	Yes	No	Yes	No	No
b	No	No	Yes	Mild	Yes	No	Yes	No	Yes
c	No	No	Yes	Mild	Yes	No	Yes	No	No
d	No	No	Yes	Mild	Yes	No	Yes	No	Yes
e	No	No	No	.-	Yes	No	Yes	Yes	Yes
f	No	No	Yes	Mild	Yes	No	Yes	No	Yes
g	No	No	Yes	Mild	Yes	No	Yes	No	No
h	No	No	No	Mild	Yes	No	Yes	Yes	Yes

i	No	No	Yes	Mild	No	No	Yes	No	No
j	No	No	No	Mild	Yes	No	Yes	No	Yes
k	No	No	Yes	-	Yes	No	Yes	No	No
l	No	No	Yes	Mild	Yes	No	Yes	No	Yes
m	No	No	Yes	Mild	Yes	No	Yes	Yes	Yes
n	No	No	Yes	Mild	Yes	Yes	Yes	Yes	Yes
o	No	No	No	Mild	Yes	No	Yes	Yes	Yes
p	No	No	No	Mild	No	No	Yes	Yes	Yes
q	No	No	Yes	Mild	Yes	No	Yes	Yes	Yes

RESULTS AND DISCUSSION

All the compounds possess good molecular properties. The structural studies of the new series of NSAIDs were conducted by molecular docking with COX-2 using the protocol CDOCKER in Acclerys Discovery studio 3.5. CDOCKER energy of the compound ranges from 18.3698 to 3.34729 and the CDOCKER interaction energy ranges from-27.0175 to-12.8565. Most of the designed compounds binds with TYR355, ARG120, ARG513, LYS83, LEU352.

Other than compounds A, M, L and I, amino acid ARG120 was involved in binding with the carbonyl group of the designed derivatives. ARG120 is one of the important amino acid COX-2 inhibitors mainly act by forming hydrogen bonding. This indicates the importance of carbonyl compound in COX-2inhibitor. Amino group as substituent in phenyl ring of indane-1,3-dione processes five hydrogen bonding with the receptor in which both carbonyl and amino group were involved in the binding with ARG513, LEU352, TYR355. CDOCKER score of 9.6416 and CDOCKER interaction energy off-17.9761 was observed for the above said compounds. Binding with LEU352 is one of the main interaction with the target for COX-2 inhibition.

Presence of mono and difloro substituent in phenyl ring possess good binding with the target. Mono floral substituent processes CDOCKER energy of 18.5690 and CDOCKER interaction energy of-12.8565. The hydrogen bonding was formed between ARG120 and TYR355 between carbonyl groups. At the same time presence of difloro derivative processes CDOCKER energy of 11.6089 and CDOCKER interaction energy of-18.3292. The hydrogen bonding between ARG120 and LYS83 ranges between 1.9583–2.7749 this indicates. The effective substitution in the phenyl ring with amino group at ortho, meta and para position varies the binding energy of the designed derivative with the COX-2 receptor. Good binding affinity towards the receptor with CDOCKER energy of 18.3698 and CDOCKER energy-19.2464 was observed for the compound q possessing substitution of amino group at the ortho position but at the same time it does not process any hydrogen bonding with the receptor. Substitution of amino group at the meta position of the phenyl ring processes CDOCKER energy of 7.9388 and CDOCKER interaction energy of-18.2033. It forms hydrogen bond between carbonyl group of indane-1,3-dione and chalcone.

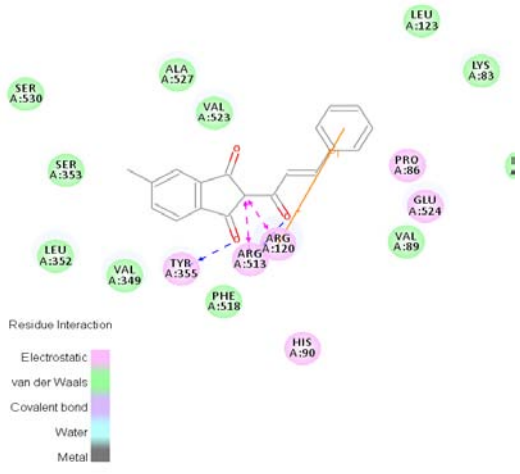


Fig. 1: Binding of compound a with 1CX2

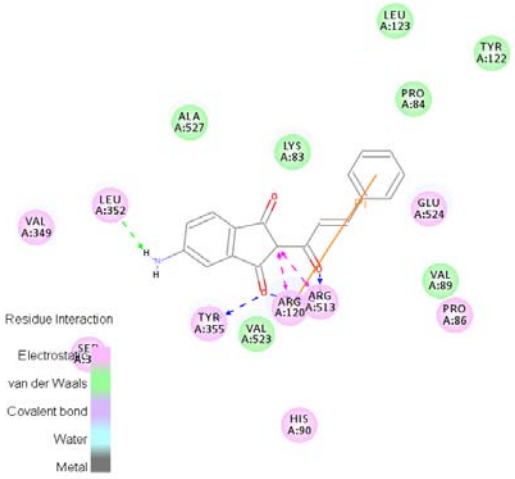


Fig. 2: Binding of compound e with 1CX2

Meanwhile substitution of an amino group at the para position in phenyl ring binds with TYR355 and ARG513 in which both the carbonyl groups were involved in binding with the target. This indicates the presence of substituent changes the binding with the specified target. Attachment of amino group and hydroxyl group individually and separately binding affinity and the interaction energy with the target differs slightly. Sulphamino group in R^1 and $R^4$with and without the presence of an amino group varied docking score and varied binding with the COX-2. Presence of SH group moderately increases binding affinity when compared to that of OH group. Binding of ligand a and e were specified in fig. 1 and 2.

Most of the compounds possess good binding with the receptor through electrostatic interaction, Vander walls force. Most of the designed derivatives possess Pi interactions specified amino acids indicating the importance of the indane-1,3-dione nucleus, Phenyl group with the target.

ADMET predication properties like blood brain barrier (BBB) penetrability, human intestinal absorption (HIA), solubility, hepatotoxicity and the ability to bind to cytochrome P450 enzymes and plasma protein binding (PPB) results were specified in table 1. All the designed molecules have the high penetration level in BBB indicating reasonable permeability, good absorption level in the human intestine. Most of the molecules possessing mono or di substituted hydroxy and amino group were predicted as low solubility. Presence of both amino and methyl group as substituent in the ring likely to cause dose-dependent liver injuries. None of designed molecules was not a likely inhibitor CYP2D6 level. Except compound **j**, other derivatives were likely to bind with the plasma protein less than other derivatives.

Toxicity profile of the designed derivatives was predicted using TOPKAT. Profiles like Aerobic biodegradability, AMES mutagenicity, Developmental toxicity potential, ocular irritancy, skin irritancy, carcinogenicity in both male and female mouse and rat model were predicted for the designed derivatives. Most of the compounds were not within the standard limit because the query structure does not belong to the training set and hence the software displays the results with warnings. Some compounds were virtually found to be more

toxic. virtual toxicity study determines did not predict the clear picture of the toxicity of the designed compounds since the query structure does not belong the training set.

In the light of above analysis, the COX-2 docked posess generated by Acclerys discovery studio produced best results. It forms hydrogen bonds, hydrophobic interaction with the important residues mainly with Arg120. Since most of the compounds were within the range of ADME and toxicity parameters it was expected to be a good drugabble target after optimization with pharmacological activity.

CONCLUSION

Our docking studies have demonstrated this new series of chalcone of indanedione derivatives for its binding affinity towards COX-2 inhibition. Among all the designed derivatives Compound **l** possess good binding affinity indicating that the presence of hydroxyl group in the phenyl ring possess good activity. Since the ADME parameters and toxicity study were within the limit of the compound indicating that the compound **l** would be an effective inhibitor of COX-2. But the durggability depends on the pharmacological studies which have to confirm it.

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CONFLICT OF INTERESTS

Declared None

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