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RESEARCH ARTICLE

MOLECULAR DOCKING STUDIES ON 2-SUBSTITUTED-6(4-METHYLPHENYL)-4,5-DIHYDROPYRIDAZIN3(2H)-ONES AND 3-SUBSTITUTED-6-(4-METHYLPHENYL)-4,5-DIHYDROPYRIDAZIN3(2H)-ONES AND PYRIDAZIN SUBSTITUTED TRIAZIN

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ABSTRACT

Estimation of pharmacotherapeutic potential, possible molecular mechanism of action, toxic/side effects and interaction with drug-metabolizing enzymes was achieved by computational studies. Computer assisted drug designing involves all computational techniques to discover, design and optimize biologically active compounds with desired structure features for putative use as drug. The Molecular Docking studies of 2-substituted-6(4-methylphenyl)-4,5-dihydropyridazin-3(2H)-ones and 3-substituted-6(4-methylphenyl)-4,5-dihydropyridazin-3(2H)-ones and pyridazine substituted triazin was carried out using Molegro Virtual Docker for determination of anti-inflammatory activity using (PDB ID: 6COX) and analgesic activity using (PDB ID: 1CX2). The novel derivatives obtained were subjected to docking against the selected proteins and the potent derivatives of pyridazinone were finally selected on the basis of Mol Dock score values. The *in-silico* docking results showed that compound XIV i.e. N-(4-(2-imino, 3,4,8,9-tetrahydro-2H-pyridazin [1,6-α][1,3,5 triazin-7-yl)phenyl)-methanesulfonamide exhibited relatively comparable binding affinity with highest Mol Dock Score value of -125.066 and formed four H-Bonds comparable to standard compound having Mol Dock Score of -86.155 on docking with (PDB ID: 6COX) and possessed highest Anti-inflammatory activity. The compound XVII i.e. 2-(4-benzylphenyl)-3,4,7,8-tetrahydropyrimdo[1,6-b]pyridazine-6-imine exhibited highest binding affinity with Mol Dock Score value of -103.947 and formed four hydrogen bonds comparable to standard compound value having Mol Dock Score -119.837 with (PDB ID: 1CX2) and possessed highest Analgesic activity. Some other compounds which showed better results are compounds XII, XVI, XIII, VIII with anti-inflammatory activity and compounds XX, XXIII, XI, VIII with analgesic activity. The SAR studies shows that the compounds XIII and compounds VIII showed good mol dock score bearing pyridazinone as central /basic moiety having sulphate group substituttion on sixth position while the compounds II and III having pyridazinone as central moiety but having hydrazine hydrate substitution on fifth position having poor docking results for anti-inflammatory and analgesic. Thus, pyridazinone-based compounds represent potential lead compounds for further development of selective COX inhibitors.

INTRODUCTION

Pyridazinone are six-member heterocyclic compounds, with two nitrogen atoms at adjacent positions. (Asif and Singh, 2010) Pyridazin-3(2H)-one derivatives have attracted the attention of medicinal chemists during the last decade due to their diverse pharmacological activities. Easy functionalization of various ring positions of pyridazinones makes them an attractive synthetic building block for designing and synthesis of new drugs. The incorporation of this versatile biologically accepted pharmacophore in established medicinally active molecules results in wide range of pharmacological effects. (Bansal and Thota, 2013) Pyridazinones constitute an interesting group of compounds, many of which possess wide spread pharmacological properties such as analgesic (Asif *et al*., 2011), anti-inflammatory (Gokce *et al*., 2009, Sahin *et al*., 2004), antidepressant (Coelho *et al*., 2003), antihypertensive (Demirayak *et al*., 2004, Anees Siddiqui *et al*., 2011). (Murty *et al*., 2012) Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. (Ratnavali *et al*., 2011) Docking is frequently used to predict the binding orientation of small drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. (David *et al*., 2007; Daniele *et al*., 2006; Himaja *et al*., 2011) It is a computational method to determine possible

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binding modes of a ligand to the active site of a receptor. Different types of interaction between receptor and ligand like Vander Waal's interaction, hydrogen bonding, electrostatic and aromatic interactions are considered to calculate the binding energy. (Silva and Taft, 2005) The calculation of binding energy between receptor and ligand molecule is more accurate by flexible docking. (Halperin *et al.,* 2002) The main objective of this study is to predict the anti-inflammatory and analgesic activity of pyridazinone derivatives with the help of modern computer aided drug designing tools i.e. Molegro Virtual Docker using (PDB ID 6COX) (Kiefer *et al.,* 2003) for anti-inflammatory and (PDB ID 1CX2) (Kurumbai and Stevens 1996) for analgesic activity. So, molecular docking has been performed to pre-access anti-inflammatory and analgesic significance of target compounds and their applications in drug discovery. (Prasanna *et al.,* 2009) Taking in to consideration the docking results, it was felt worthwhile to carry out the synthesis and biological evaluation of some novel pyridazinone derivatives.

MATERIALS AND METHODS

Computational approaches have been developed to provide practical solutions to the problem of diversity sampling. Since it is impossible to synthesize all the possible compounds and to test all the available ones so molecular modelling makes this approach easier and limits to some fixed number of compounds.

Docking Studies

To pre-asses the analgesic and anti-inflammatory activity of substituted pyridazinones and their derivatives on the structural basis, auto-mated docking studies were carried out using Molegro Virtual Docker, the scoring functions and hydrogen bonds formed with the surrounding amino acids were used to predict their binding modes, their binding affinities and orientation of these compounds. (Kumar *et al.,* 2012) On the basis of literature data, we selected sixty hypothetical compounds further we screened twenty four compounds which showed structural resemblance with 2-substituted-6(4-methylphenyl)-4,5-dihydropyridazin3(2h)-ones and 3-substituted-6-(4-methylphenyl)-4,5-dihydropyridazin3(2h)-ones and pyridazin substituted triazin. Docking studies were performed using (PDB ID: 6COX) for anti-inflammatory and (PDB ID: 1CX2) for analgesic activity using Molegro Virtual Docker. The Docking studies were performed on the Standard compounds and twenty four docked compounds and were found to possess good results for analgesic and anti-inflammatory activity.

Importing and Preparing Molecules

The molecules in the workspace were properly prepared before the docking begins. The hypothetical compounds were selected based on literature. The ligand molecules were prepared using Marvin 5. 11. 4, converted to 3D structure from the 2D using build and optimization method & finally clean in 3D. The resulting structures were saved in MDL Molfile (*. mol) format. A single, low energy, 3D structure with correct chiralities for each successfully proposed input structure were generated. Then the generated structures were imported into the workspace of docking software Molegro virtual docker 4.0.2. Molecule can be incorporated into MVD using MDL (sdf/sd/mol/mdl) file format which contains bonding information. The 3D structures of molecules to be imported were prepared. The molecules were imported by dragging or dropping a molecule structure file.

Protein Preparation and detecting cavities of protein molecules

The crystal structure (PDB code: 6COX & 1CX2) was accessed from the RCSB Protein data bank (www.pdb.org) and imported. The protein structures were prepared using the protein preparation wizard in MVD. In this step, bond orders were assigned, all hydrogens in the structure were added, and bonds to metals were deleted and the formal charge in the metal and neighboring atoms were adjusted at more than the 5A° specified distance. The energy of imported molecules was minimized using Ligand Energy inspector. The energy minimization helps in stability of molecules to be imported. Next, the protein surface was created using Protein Preparation. This step helps to inspect and change the protonation state for the residues. In order to determine the potential binding sites, a cavity prediction algorithm was performed.

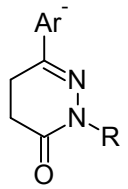
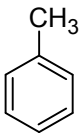
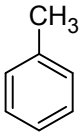
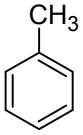
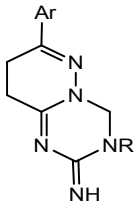
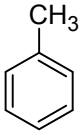
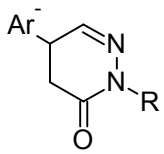
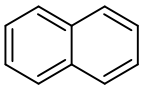
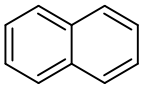
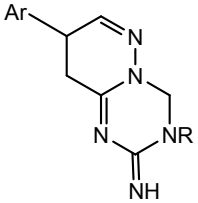
Executing a docking set up through docking wizard panel

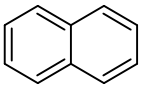
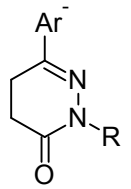
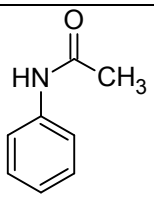
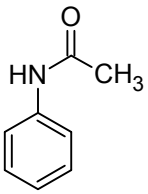
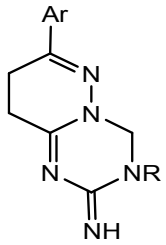
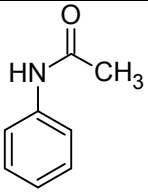
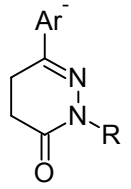
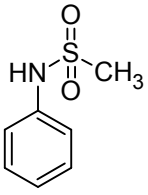
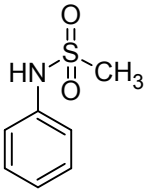
Molegro searches for favorable interactions between ligand molecules and the receptor. Next, all ligands were selected and Docking was performed through the docking wizard panel. Then in next step docking results were imported. Various possess of docked compounds were analyzed by determination of Mol Dock Score and H-Bond interaction. Compounds were ranked according to their docking scores and were visualized inside the pocket to view their fitting and closure to main residues. Molecular docking studies were revealed further insight into the nature of interactions between the compounds and the active site amino acids to rationalize the obtained biological result (Sivan and Manga, 2010).

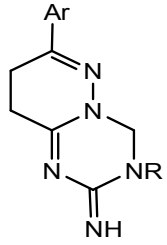
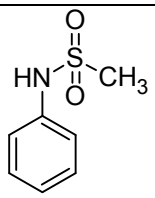
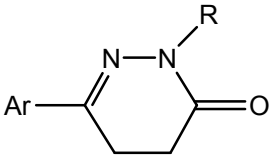
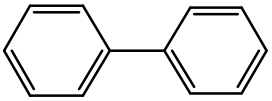
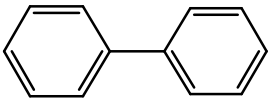
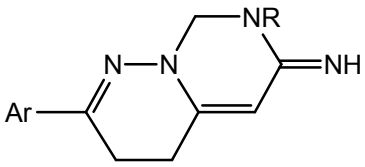
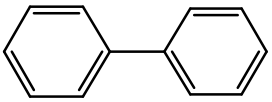
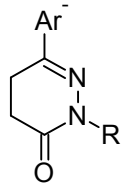
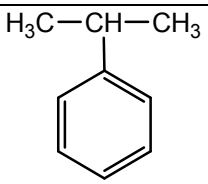
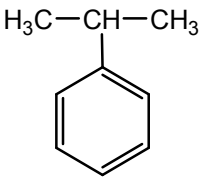
RESULTS

On the basis of literature survey, we carried out docking studies on twenty four pyridazinone derivatives and selected compound number XIV, XVII, XII, XVI, XX as potent compounds that showed good docking results. Molegro Virtual Docker allows the flexible docking of ligands into its site of action. It has the ability to use all the rotatable bonds of the ligands to give a number of conformations from which the best mode could be achieved. In the analysis of docking results, we tried to find a correlation between the structure and activity on the basis of mol dock value and we found that the hypothetical compounds selected for docking studies were able to interact with the hydrophobic pocket of the protein efficiently that were having binding affinity and also showed good results for anti-inflammatory and analgesic activity. From Table 2 it can be observed that on (PDB ID: 6COX) for anti-inflammatory activity, the Mol Dock Score of Standard Compound I was -86.155 (possessing pyridazinone as central moiety and substitutions on second and third position showing close resemblance to the docked compounds) and it formed three

Table 1. Structure of Hypothetical compounds

Compound No	Ar	R
		
II		H
III		CH_2OH
IV		C_6H_5
		
V		H
		
VI		H
VII		CH_2OH
		

VIII		H
		
IX		H
X		CH_2OH
		
XI		H
		
XII		H
XIII		CH_2OH

		
XIV		H
		
XV		H
XVI		CH_2OH
		
XVII		H
		
XVIII		H
XIX		CH_2OH

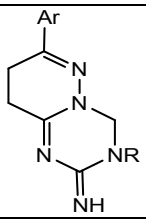
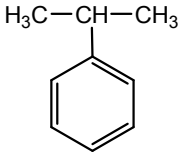
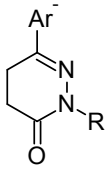
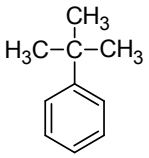
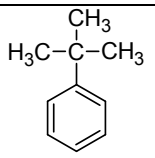
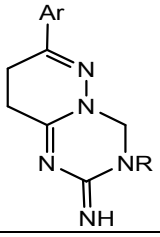
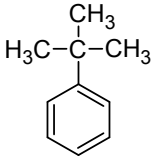
		
XX		H
		
XXI		H
XXII		CH_2OH
		
XXIII		H

Table 2. **The interactions of standard compound with amino acids residues using PDB ID: 6COX for anti-inflammatory activity**

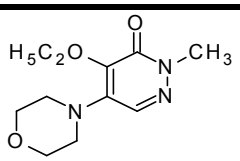
Compound No.	Structure	Mol dock score	Docking Score	H-Bond Interaction	H-Bond Distance	Interacting Residue	Interacting Atom
I	 Emorfazone (std. Compound)	-86.155	-87.0387	3	3.10 3.19 3.56	Asn 537 Asn 537 Val 228	N-N N-N N-N
	Internal Standard	-91.4689	-99.6862	8	2.60 2.40 2.07 3.11 2.78 2.73 3.11 2.97	Gln 374 Tyr 374 Asn 375 Asn 375 Asn 537 Gly 225 Gly 536 His 226	O-O O-H O-H O-O O-N O-O O-N O-O

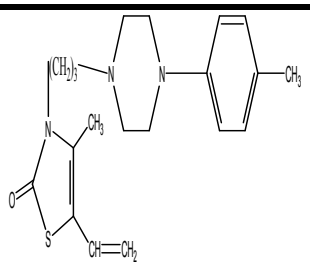
Table 3. The interactions of test compound and amino acid residues using PDB ID: 6COX for anti-inflammatory activity

Compound No. II	Mol dock score	Docking Score	H-Bond Interaction	H-Bond Distance	Interacting Residue	Interacting Atom
	-77.5642	-80.592	2	3.04	Gly 536	N of pyridazinone with O
				2.73	Val 228	O of O_2 with N
	-75.5119	-77.2933	5	3.29	Val 228	O of O_2 with N
				3.37	Asn 375	N of pyridazinone with N
				3.57	Asn 375	N of pyridazinone with O
				2.89	His 226	N of pyridazinone with O
				3.12	Gly 225	N of pyridazinone with O
III	-95.9316	-99.7047	3	3.37	Asn 537	N of pyridazinone with N
				2.60	Gly 225	O of OH with O
				2.57	Val 228	O of O_2 with N
	-95.4807	-95.294	3	2.83	Asn 375	O of OH with O
				2.96	Asn 375	O of OH with N
				3.34	Asn 375	N of pyridazinone with N
	-95.1791	-93.84998	4	3.42	Arg 376	O of OH with N
				2.97	Asn 375	O of OH with O
				3.10	Asn 375	N of pyridazinone with N
				3.47	Asn 375	N of pyridazinone with N
IV	-104.374	-102.004	1	3.60	Asn 375	O of pyridazinone with N
	-108.488	-105.58	1	3.48	Asn 375	N of pyridazinone with N
	-104.415	-102.006	1	2.60	Asn 375	N of pyridazinone with O
V	-102.49	-105.762	4	3.07	Gly 536	N of triazin with O
				2.60	Gly 533	N of triazin with O
				3.11	Val 228	N of triazin with N
				3.10	Asn 537	N of triazin with N
	-102.4	-99.0737	4	3.06	Gly 536	N of triazin with O
				2.60	Gly 533	N of triazin with O
				3.14	Val 228	N of triazin with O
				3.10	Asn 537	N of triazin with O
	-100.958	-103.869	4	3.07	Val 228	N of triazin with N
				3.10	Asn 537	N of triazin with N
				2.59	Gly 533	N of triazin with O
				2.96	Gly 536	N of triazin with O
	-100.225	-97.077	1	3.22	Asn 537	N of pyridazinone with N
VI	-99.1685	-99.8101	1	3.51	Asn 537	N of pyridazinone with N
	-93.5139	-95.0858	4	2.98	Ser 143	N of pyridazinone with O
				3.10	Phe 142	N of pyridazinone with O
				2.74	Arg 376	O of O_2 with N
				3.24	Arg 376	O of O_2 with N
VII	-106.941	-105.889	1	3.11	Gln 374	O of OH with O
	-106.541	-104.827	1	3.10	Gln 374	O of OH with O
	-104.354	-102.794	3	3.15	Arg 376	O of O_2 with N
				3.10	Arg 376	O of OH with N
				3.41	Gln 374	O of OH with O
VIII	-116.713	-116.729	4	3.22	Val 228	N of triazin with N
				3.18	Asn 537	N of triazin with N
				2.47	Gly 533	N of triazin with O
				2.73	Gly 536	N of triazin with O
	-116.43	-116.437	4	2.70	Gly 536	N of triazin with O
				2.48	Gly 533	N of triazin with O
				3.23	Asn 537	N of triazin with N
				3.27	Val 228	N of triazin with N
	-114.892	-114.74	4	3.08	Gln 374	N of triazin with O
				3.10	Arg 376	N of triazin with N
				3.49	Asn 375	N of triazin with N
				3.07	Asn 375	N of triazin with N
IX	-106.85	-113.346	4	2.81	Arg 376	O of $NHCOCH_3$ with N
				3.10	Val 228	O of O_2 with N
				2.79	Gly 536	N of pyridazinone with O
				3.33	Asn 375	N of pyridazinone with N
	-104.168	-110.105	4	3.10	Arg 376	O of $NHCOCH_3$ with N
				3.42	Asn 375	N of pyridazinone with N
				3.01	Val 228	O of O_2 with N
				3.63	Gly 536	N of pyridazinone with C
	-102.914	-103.507	3	3.38	Arg 376	O of $NHCOCH_3$ with N
				3.48	Asn 375	N of pyridazinone with N
				2.90	Val 228	O of O_2 with N
X	-112.407	-116.924	6	2.92	Arg 376	O of $NHCOCH_3$ with N
				2.74	Gly 533	O of OH with O
				3.10	Gly 536	O of OH with O
				3.09	Val 228	O of O_2 with N
				3.14	Asn 537	O of O_2 with N
				2.60	Asn 537	N of pyridazinone with N
	-109.468	-119.472	3	3.01	Arg 376	O of $NHCOCH_3$ with N
				2.63	Gly 533	O of OH with O
				2.80	Val 228	O of O_2 with N
	-108.574	-111.526	3	2.64	Gly 533	O of OH with O
				2.92	Val 228	O of O_2 with N
				2.81	Asn 537	O of O_2 with N

XI	**-120.715**	**-123.288**	**5**	**2.60**	**Ser 143**	**N of $NHCOCH_3$ with O**
				3.10	**Asn 537**	**N of triazin with N**
				3.11	**Val 228**	**N of triazin with N**
				2.59	**Gly 533**	**N of triazin with O**
				2.88	**Gly 536**	**N of triazin with O**
	-114.772	-119.211	3	2.75	Asn 537	O of $NHCOCH_3$ with N
				2.60	Arg 376	N of triazin with N
				2.64	Asn 375	N of triazin with O
	-114.605	-118.82	3	2.66	Asn 537	O of $NHCOCH_3$ with N
				2.61	Asn 375	N of triazin with O
				2.59	Arg 376	N of triazin with N
XII	-102.301	-109.2	3	2.62	Arg 376	O of $NHSO_2CH_3$ with N
				2.80	Gly 536	N of pyridazinone with O
				3.64	Val 228	O of pyridazinone with N
	-97.7544	-107.054	6	2.79	Arg 376	O of $NHSO_2CH_3$ with N
				3.28	Asn 375	N of pyridazinone with N
				3.24	Val 228	O of pyridazinone with N
				3.29	Gly225	N of pyridazinone with O
				2.84	His 226	N of pyridazinone with O
				3.49	Asn 375	N of pyridazinone with O
	-97.5094	-107.312	6	2.76	Arg 376	O of $NHSO_2CH_3$ with N
				3.28	Asn 375	N of pyridazinone with N
				3.60	Asn 375	N of pyridazinone with C
				2.87	His 228	N of pyridazinone with O
				3.25	Val 228	O of pyridazinone with N
				3.50	Asn 375	N of pyridazinone with O
XIII	-122.323	-121.663	5	2.78	Arg 376	O of $NHSO_2CH_3$ with N
				3.10	Gly 225	O of pyridazinone with O
				3.10	Val 228	O of CH_2OH with N
				3.19	Asn 537	O of CH_2OH with N
				2.92	Gly 533	O of CH_2OH with O
	-119.155	-126.172	5	2.60	Arg 376	O of $NHSO_2CH_3$ with N
				2.84	Gly 533	O of OH with O
				3.19	Asn 537	O of OH with N
				3.08	Val 228	O of OH with N
				3.26	Gly 225	O of pyridazinone with O
	-118.852	-125.025	4	2.60	Arg 376	O of $NHSO_2CH_3$ with N
				2.72	Gly 533	O of OH with O
				3.27	Asn 537	O of OH with N
				3.38	Val 228	O of OH with N
XIV	-125.066	-129.095	4	2.95	Gly 536	N of triazin with O
				3.10	Val 228	N of triazin with N
				3.08	Asn 537	N of triazin with N
				2.52	Gly 533	NH of triazin with O
	-124.853	-128.677	4	2.87	Gly 536	N of triazin with O
				3.10	Val 228	N of triazin with N
				3.10	Asn 537	N of triazin with N
				2.51	Gly 533	NH of triazin with O
	-120.22	-121.132	4	2.86	Asn 375	O of $NHSO_2CH_3$ with N
				3.29	Asn 375	N of triazin with N
				2.60	Gly 225	N of triazin with O
				3.46	His 226	NH of triazin with O
XV	-97.9218	-95.5974	0	----	----	----
	-95.1669	-95.3978	4	3.10	His 226	N of pyridazinone with O
				3.25	Asn 375	N of pyridazinone with O
				3.10	Asn 375	N of pyridazinone with N
				3.53	Gly 225	N of pyridazinone with O
	-92.9618	-93.8703	2	3.11	Val 228	O of pyridazinone with N
				3.10	Asn 537	O of pyridazinone with N
XVI	-123.974	-125.089	3	2.60	Gly 225	O of OH with O
				2.47	Val 228	O of pyridazinone with N
				3.07	Asn 537	N of pyridazinone with N
	-122.696	-122.436	2	3.35	Asn 375	O of pyridazinone with N
				2.89	Gln 374	O of OH with O
	-121.694	-120.782	2	2.92	Gln 374	O of OH with O
				3.24	Asn 375	O of pyridazinone with N
XVII	-124.537	-127.035	4	3.10	Asn 375	N of pyridazine with N
				3.02	Gly 533	N of triazin with O
				2.73	Gly 533	NH of triazin with O
				3.07	Asn 537	N of triazin with N
	-121.345	-121.53	2	2.69	Asn 375	NH of triazin with O
				3.24	Asn 375	N of triazin with O
	-121.266	-120.56	1	3.41	Tyr 373	N of triazin with O
XVIII	-87.0588	-89.745	2	2.92	Gly 536	N of pyridazinone with O
				2.87	Val 228	O of pyridazinone with N
	-86.469	-90.2217	2	2.70	Val 228	O of pyridazinone with N
				3.10	Gly 536	N of pyridazinone with O
	-85.0593	-86.3308	1	2.96	Val 228	O of pyridazinone with N
XIX	-104.91	-112.443	2	3.23	Lys 532	O of OH with O
				2.63	Val 228	O of pyridazinone with N
	-104.381	-111.736	2	3.28	Lys 532	O of OH with O
				2.62	Val 228	O of pyridazinone with N
	-103.44	-103.189	1	1.71	Gln 374	O of OH with O

XX	**-111.587**	**-102.206**	**2**	**2.69**	**Arg 376**	**N of triazin with N**
				2.93	**Asn 372**	**NH of triazin with O**
	-111.474	-114.787	2	2.67	Arg 376	N of triazin with N
				2.93	Asn 375	NH of triazin with O
	-104.267	-103.65	3	2.94	Asn 375	N of pyridazine with N
				3.55	Asn 375	N of pyridazine with N
				3.02	Gly 533	NH of triazin with O
XXI	-87.6914	-89.5102	2	3.10	Val 228	O of pyridazinone with N
				2.71	Gly 536	N of pyridazinone with O
	-87.503	-89.2002	2	3.04	Val 228	O of pyridazinone with N
				2.76	Gly 536	N of pyridazinone with O
	-87.042	-88.5106	1	3.10	Val 228	O of pyridazinone with N
XXII	-106.965	-108.829	3	2.60	Gly 225	O of OH with O
				2.57	Val 228	O of pyridazinone with N
				3.37	Asn 537	N of pyridazinone with N
	-106.936	-108.594	3	2.60	Gly 225	O of OH with O
				2.57	Val 228	O of pyridazinone with N
				3.37	Asn 537	N of pyridazinone with N
	-105.881	-110.877	4	3.54	Asn 375	N of pyridazinone with N
				2.60	Gly 533	O of OH with O
				2.90	Val 228	O of pyridazinone with N
				2.78	Asn 537	O of pyridazinone with N
XXIII	-104.198	-106.402	2	2.69	Arg 376	N of triazin with N
				3.02	Asn 375	NH of triazin with O
	-103.673	-102.003	3	3.43	Asn 375	N of pyridazine with N
				2.74	Asn 375	N of pyridazine with N
				3.10	Gly 533	NH of pyridazine with O
	-103.208	-104.145	4	3.20	His 226	NH of triazin with O
				2.86	His 226	N of triazin with O
				3.12	Gly 225	N of triazin with O
				3.42	Asn 375	N of triazin with O

Table 4. **The interactions of standard compound with amino acids residues using PDB ID: 1 CX2 for analgesic activity**

Compound No.	Structure	Mol Dock Score	Docking Score	H-Bond Interaction	H-Bond Distance	Interacting Residue	Interacting Molecule
XXIV		-119.837	-83.432	2	2.01	Asn 375	O with N
					3.10	Asn 105	O with N
		-111.949	-77.9507	2	2.51	Arg 108	N with N
					2.50	Asn 375	O with N
	Internal Standard	-95.7637	-95.4051	5	3.05	Lys 360	N with N
					2.68	Arg 109	O with N
					3.40	Pro 106	N with N
					3.07	Asn 105	O with N
					2.60	Asn 104	N with O

hydrogen bond interactions with a distance of 3.10, 3.19, 3.56 (A˚) while the internal standard had showed Mol Dock Score of -91.4689 and formed eight hydrogen bond interactions having distance of 2.60, 2.40, 2.07, 3.11, 2.78, 2.73, 3.11, 2.97 (A˚). From Table 3 it can be observed that Compound XIV i.e. N-(4-(2-imino, 3,4,8,9-tetrahydro-2H-pyridazin [1,6-α][1,3,5 triazin-7-yl)phenyl) methane sulfonamide exhibited relatively comparable binding affinity on (PDB ID: 6COX) with highest Mol Dock Score of -125.066 as compared to standard and formed four H-Bond interactions. The Compound X i.e. N-(4-(1-(hydroxymethyl)-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl)acetamide also formed maximum hydrogen bond interactions i.e. four H-Bonds with a distances of 2.92, 2.74, 3.10, 3.09, 3.14, 2.60 (A˚) and showed good Mol Dock Score followed by other compounds such as Compound XII, XVI, XIII, VIII with anti-inflammatory activity which also showed good docking results. From above observations it is concluded that the Compound XIV possesses greatest anti-inflammatory activity (Fig. I) The SAR studies illustrate that pyridazinones bearing triazine ring and methyl sulfonamide group as substituent showed good docking results.

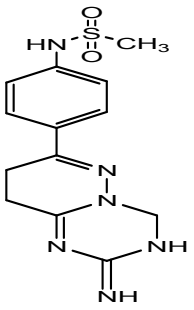


Fig. 1.

From Table 4 it can be observed that on (PDB ID: 1CX2) for determination of analgesic activity, the Mol Dock Score of Standard Compound XXIV was -119.837 while the Internal Standard had Mol Dock Score of -95.7637 and formed five hydrogen bond interactions with a distance of 3.05, 2.68, 3.40, 3.07, 2.60 (A°). From Table 5 it can be observed that the compound XVII i.e. 2-(4-benzylphenyl)-3,4,7,8-tetrahydropyrimdo [1,6-b]pyridazine-6-imine exhibited relatively comparable binding affinity with highest Mol Dock Score of -103.947 comparable to standard compound. The compound XIV which showed highest mol dock score for anti-inflammatory activity also formed maximum hydrogen bond interactions i.e. four H-Bonds with distances of 3.27, 3.51, 2.98, 2.60 (A°) respectively for analgesic activity with a mol dock score value followed by other compounds such as compound no XX, XXIII, XI, VIII with analgesic activity and also showed good docking results.

Therefore, compound XVII possesses greatest analgesic activity (Fig 2). The SAR studies illustrate that pyridazinones bearing triazine ring showed good docking results.

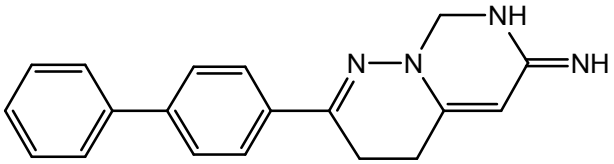


Fig. 2.

Compound No. II	Mol dock score	Docking Score	H-Bond Interaction	H-Bond Distance	Interacting Residue	Interacting Atom
	-71.0822	-64.2049	1	2.73	Cys 57	N of pyridazinone with O
	-70.734	-66.2868	1	2.62	Glu 533	N of pyridazinone with O
	-69.8039	-61.597	1	3.28	Glu 319	N of pyridazinone with O
III	-79.9933	-74.9794	2	3.27	Cys 57	O of OH with O
				3.07	Asp 58	O of OH with O
	-79.5032	-73.9525	3	3.13	Cys 59	O of OH with N
				2.79	Asp 58	O of OH with O
				3.10	Thr 60	O of OH with N
	-77.8411	-71.621	4	2.99	Glu 46	O of OH with O
				2.60	Lys 56	O of OH with O
				3.39	Met 48	O of OH with N
				3.43	Asp 58	O of OH with N
IV	-120.556	-117.9	1	2.55	Arg 101	N of pyridazinone with O
	-107.113	-103.914	1	3.10	Asn 375	N of pyridazinone with O
V	-92.6256	-91.8682	5	2.96	Glu 319	NH of triazin with O
				3.45	Gly 551	NH of triazin with O
				2.60	His 320	N of triazin with N
				3.09	Gly 551	N of triazin with O
				3.20	His 320	N of triazin with N
	-83.3703	-80.1804	1	3.49	Thr 60	N of triazin with O
	-75.5287	-71.5513	0	----	----	----
VI	-86.2407	-78.2569	2	2.66	Cys 59	O of pyridazinone with N
				3.32	Thr 60	O of pyridazinone with N
	-77.5714	-76.5668	1	3.90	Glu 553	N of pyridazinone with N
	-74.7091	-76.2435	1	2.90	Ser 548	O of pyridazinone with N
VII	-91.9791	-77.3697	0	----	----	----
	-79.528	-79.5177	1	3.39	Glu 553	N of pyridazinone with N
	-73.4104	-72.6823	1	3.39	Glu 553	N of pyridazinone with N
VIII	-94.211	-88.8542	2	3.09	Glu 553	N of triazin with O
				2.60	Glu 553	NH of triazin with O
	-93.7325	-92.6006	2	3.07	Glu 553	N of triazin with O
				2.60	Glu 553	NH of triazin with O
	-77.3242	-74.5882	0	----	----	----
IX	-90.1447	-87.4741	2	3.11	Thr 60	O of $NHCOCH_3$ with N
				2.82	Cys 59	O of $NHCOCH_3$ with N
	-85.5376	-84.1635	2	3.26	Cys 57	O of $NHCOCH_3$ with N
				3.48	Glu 553	N of pyridazinone with N
	-84.3961	-86.7127	4	3.05	Glu 553	O of $NHCOCH_3$ with N
				2.68	Cys 57	N of pyridazinone with O
				3.41	Cys 59	O of pyridazinone with N
				3.54	Thr 60	O of pyridazinone with N
X	-89.0126	-83.6959	3	2.72	Asp 58	O of OH with O
				3.50	Cys 57	O of OH with O
				3.47	Val 554	O of $NHCOCH_3$ with N
	-86.3512	-80.8398	2	2.69	Asp 58	O of OH with O
				3.51	Cys 57	O of OH with O
	-75.4363	-76.3442	4	2.99	Asn 560	O of $NHCOCH_3$ with O
				2.87	Glu 364	O of OH with O
				2.85	Arg 61	O of OH with N
				2.60	Trp 545	O of OH with O
XI	-98.2105	-90.5284	2	2.96	Gly 551	N of triazin with O
				3.10	His 320	N of triazin with N
	-89.7802	-82.9659	5	3.01	Cys 59	O of $NHCOCH_3$ with N
				3.12	Glu 319	N of triazin with O
				3.02	Glu 319	NH of triazin with O
				3.10	His 320	NH of triazin with N
				2.91	Glu 319	NH of triazin with O

	-81.5159	-80.9542	2	3.10	Asp 268	NH of triazin with N
				3.43	Gln 270	N of triazin with O
XII	-79.2947	-79.6024	2	3.52	Lys 253	O of $NHSO_2CH_3$ with N
				3.33	Gln 270	N of pyridazinone with N
	-79.1328	-79.9955	2	3.10	Lys 253	O of $NHSO_2CH_3$ with N
				3.10	Gln 270	N of pyridazinone with N
	-71.6181	-77.9888	5	3.56	Lys 342	O of $NHSO_2CH_3$ with N
				3.09	Arg 109	O of $NHSO_2CH_3$ with N
				3.13	Arg 109	O of $NHSO_2CH_3$ with N
				2.64	Trp 545	O of pyridazinone with N
				2.92	Asp 362	N of pyridazinone with O
XIII	-90.4808	-81.3801	2	2.35	Glu 319	NH of $NHSO_2CH_3$ with O
				3.55	Glu 553	O of OH with O
	-87.8606	-85.2273	2	3.01	Ser 548	O of $NHSO_2CH_3$ with N
				2.68	Gly 66	O of OH with O
	-78.5017	-83.636	4	3.10	Arg 109	O of $NHSO_2CH_3$ with N
				3.10	Arg 109	O of $NHSO_2CH_3$ with N
				3.10	Asp 362	O of OH with O
				3.28	Lys 557	O of pyridazinone with N
XIV	-83.3157	-87.4128	4	2.99	Gln 270	O of $NHSO_2CH_3$ with N
				2.56	His 242	N of triazin with O
				3.58	His 242	N of triazin with O
				2.09	Ala 562	O of $NHSO_2CH_3$ with N
	-79.2361	-81.8104	6	3.18	Lys 557	N of pyridazine with N
				3.10	Lys 557	N of triazin with N
				3.16	Glu 364	N of triazin with O
				3.00	Arg 109	O of $NHSO_2CH_3$ with N
				3.05	Arg 109	O of $NHSO_2CH_3$ with N
				3.06	Arg 109	O of $NHSO_2CH_3$ with N
	-79.2149	-80.6306	1	3.09	Ser 548	N of triazin with N
XV	-81.6135	-80.0907	0	----	----	----
	-76.914	-77.2661	2	3.10	Glu 553	N of pyridazinone with O
				3.23	Glu 553	O of pyridazinone with N
	-74.886	-72.5026	2	3.50	Gln 270	N of pyridazinone with N
				2.67	Ala 562	N of pyridazinone with O
XVI	-89.3155	-86.7509	1	3.27	Glu 553	N of pyridazinone with N
	-86.2194	-81.1544	1	2.60	Asp 58	O of OH with O
	-83.069	-82.0189	1	3.12	Glu 553	O of pyridazinone with N
XVII	-103.947	-102.231	4	3.27	Glu 319	N of triazin with O
				3.51	Gly 551	NH of triazin with O
				2.98	His 320	NH of triazin with N
				2.60	His 320	NH of triazin with N
	-93.7466	-80.2307	0	----	----	----
	-92.101	-88.6965	3	2.96	Gln 270	N of triazin with O
				3.27	Asp 268	N of triazin with O
				3.09	Asp 268	NH of triazin with O
XVIII	-75.8932	-74.3934	1	2.90	Cys 57	N of pyridazinone with O
	-73.3059	-69.9465	0	----	----	----
	-72.0755	-67.5579	0	----	----	----
XIX	-81.7644	-72.6164	2	2.91	Gly 551	O of OH with O
				3.15	His 320	O of OH with N
	-72.9963	-72.9199	1	2.96	Glu 553	O of pyridazinone with N
	-70.2318	-70.2083	3	3.08	Lys 342	N of pyridazinone with N
				2.60	Lys 342	N of pyridazinone with N
				2.68	Asn 560	O of OH with O
XX	-101.023	-98.1617	5	2.87	His 320	N of triazin with N
				3.35	Gly 551	N of triazin with O
				2.82	His 320	NH of triazin with N
				2.87	Glu 319	NH of triazin with O
				2.98	Glu 319	NH of triazin with O
	-85.4414	-83.4676	0	----	----	----
	-82.0075	-78.7008	2	3.47	Gln 270	N of triazin with N
				3.13	Asp 268	NH of triazin with O
XXI	-82.8966	-89.2031	1	3.54	Ser 548	O of pyridazinone with O
	-79.0717	-76.9254	1	3.29	Gln 270	N of pyridazine with N
	-77.6585	-77.0193	1	2.90	Glu 319	N of pyridazinone with O
XXII	-90.1231	-79.5749	1	2.81	Glu 553	O of OH with N
	-86.5472	-78.2924	2	3.14	His 320	O of OH with N
				3.09	Gly 551	O of OH with O
	-82.6211	-72.6923	4	3.09	Lys 253	O of pyridazinone with N
				3.54	Lys 243	O of OH with O
				3.00	Gln 270	O of OH with O
				3.10	Gln 270	O of OH with O
XXIII	-100.026	-100.074	5	3.13	His 320	N of triazin with N
				2.88	Gly 551	N of triazin with O
				2.68	His 320	NH of triazin with N
				3.26	Gly 551	NH of triazin with O
				2.79	Glu 319	NH of triazin with O
	-84.1591	-80.7689	0	----	----	----
	-83.0253	-79.4756	2	3.10	Asp 268	NH of triazin with O
				3.52	Gln 270	N of triazin with N

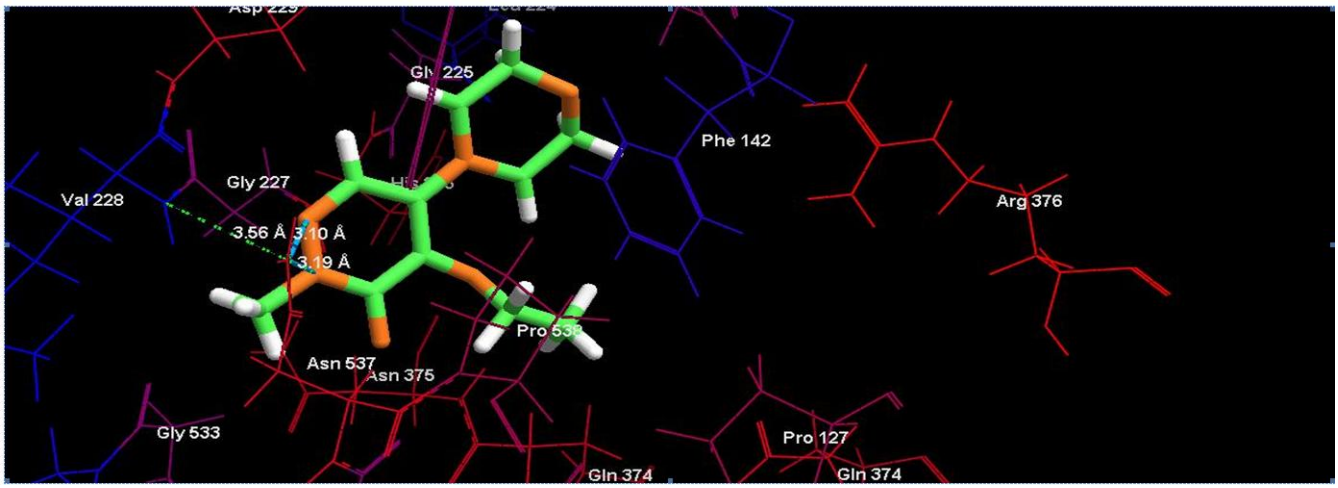


Fig A. Binding mode of Standard Compound I (for Anti-inflammatory Activity) into the binded site of pdb: 6COX. It has Mol dock score -86.155 and docking score -87.0387 and form 3 hydrogen bonds shown as blue lines, between N of N, N of N, N of N with distance of 3.10 A°, 3.19 A°, 3.56 A° respectively

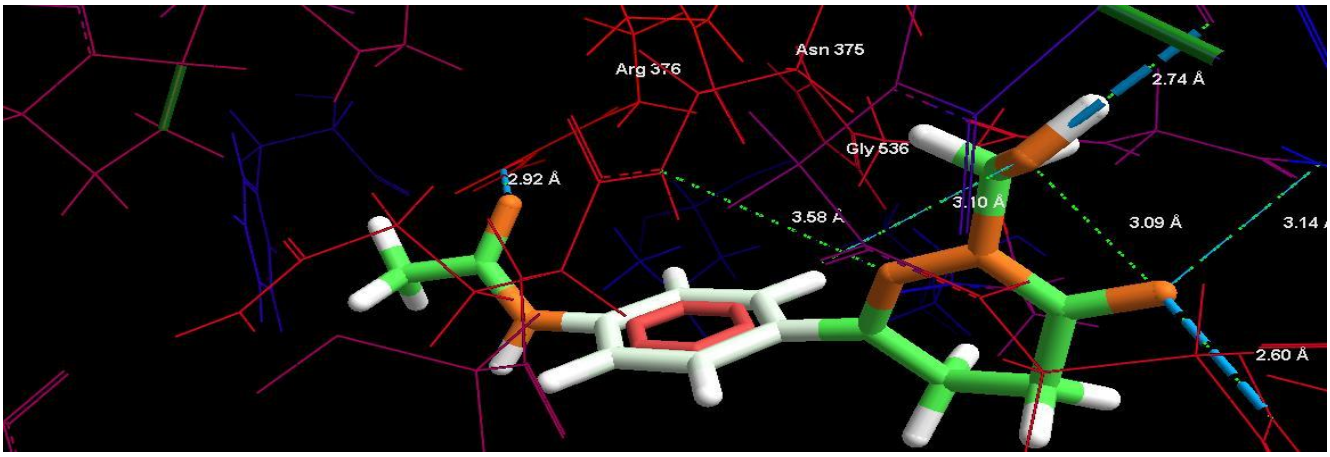


Fig B. Binding mode of Compound X(for Anti-inflammatory activity) into the binded site of pdb: 6COX. It has Mol dock score -112.407 and docking score -143.041 and form 6 hydrogen bonds shown as blue lines, between O of $NHCOCH_3$ with N, O of OH with O, O of OH with O, O of O_2 with N, O of O_2 with N, N of pyridazinone with N with distance of 2.92 A°, 2.74 A°, 3.10 A°, 3.09 A°, 3.14 A°, 2.60 A° respectively.

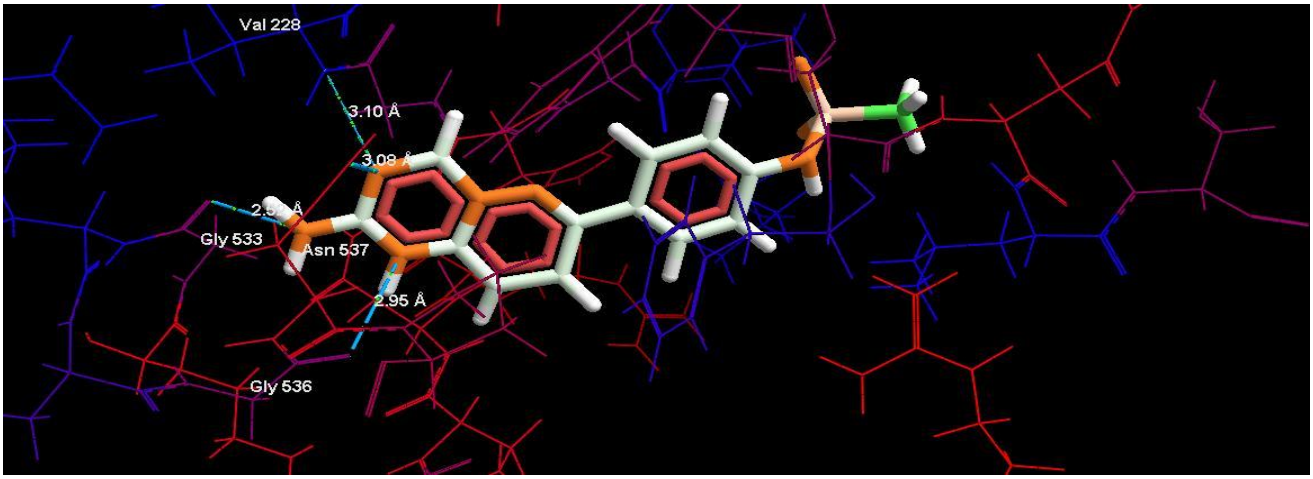


Fig C. Binding mode of Compound XIV(for Anti-inflammatory activity) into the binded site of pdb: 6COX. It has Mol dock score -125.066 and docking score -129.095 and form 4 hydrogen bonds shown as blue lines, between N of triazin with O, N of triazin with N, N of triazin with N, NH of triazin with O with distance of 2.95 A°, 3.10 A°, 3.08 A°, 2.52 A° respectively.

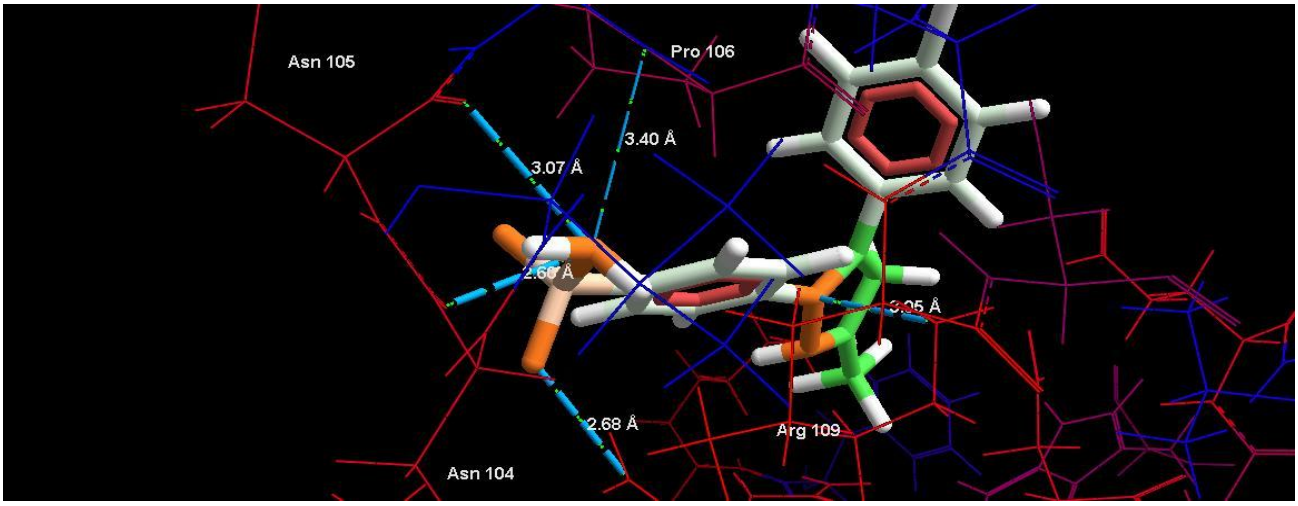


Fig D. Binding mode of Standard Compound XXIV (for Analgesic Activity) into the binded site of pdb: 1 CX2. It has Mol dock score -95.7637 and docking score -95.4051 and form 5 hydrogen bonds shown as blue lines, between N of pyridazinone with N, O of pyran with N, N of pyridazinone with N, O of pyran with N, N of piperidine with O with distance of 3.05 A°, 2.68 A°, 3.40 A°, 3.07 A°, 2.60 respectively

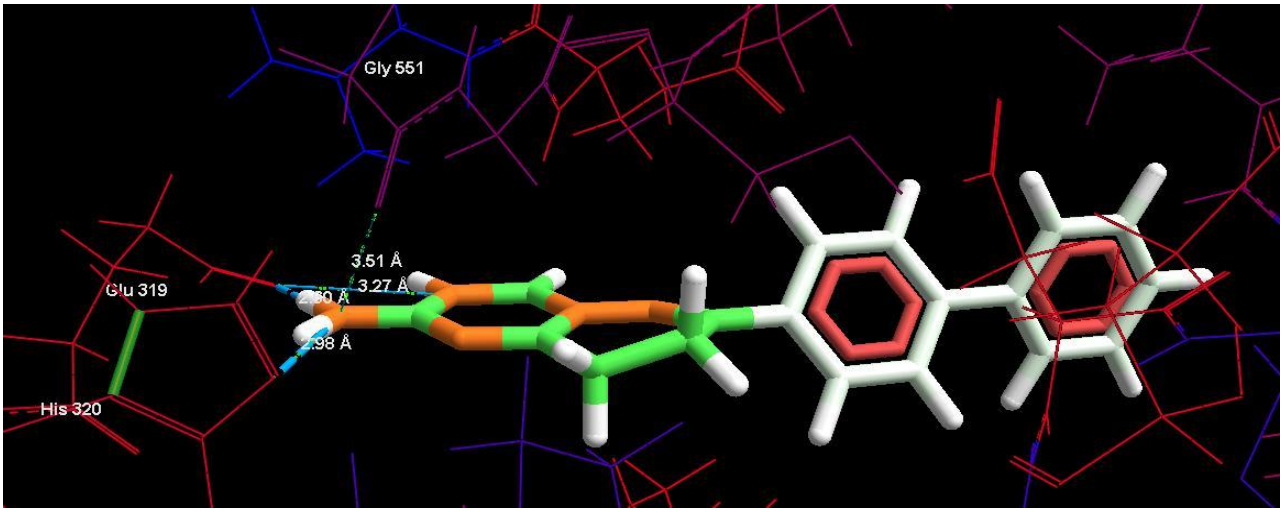


Fig E. Binding mode of Compound XVII(for Analgesic activity) into the binded site of pdb: 1CX2. It has Mol dock score -125.066 and docking score -129.095 and form 4 hydrogen bonds shown as blue lines, between N of triazin with O, NH of triazin with O, NH of triazin with N, NH of triazin with N with distance of 3.27 A°, 3.51 A°, 2.98 A°, 2.60 A° respectively

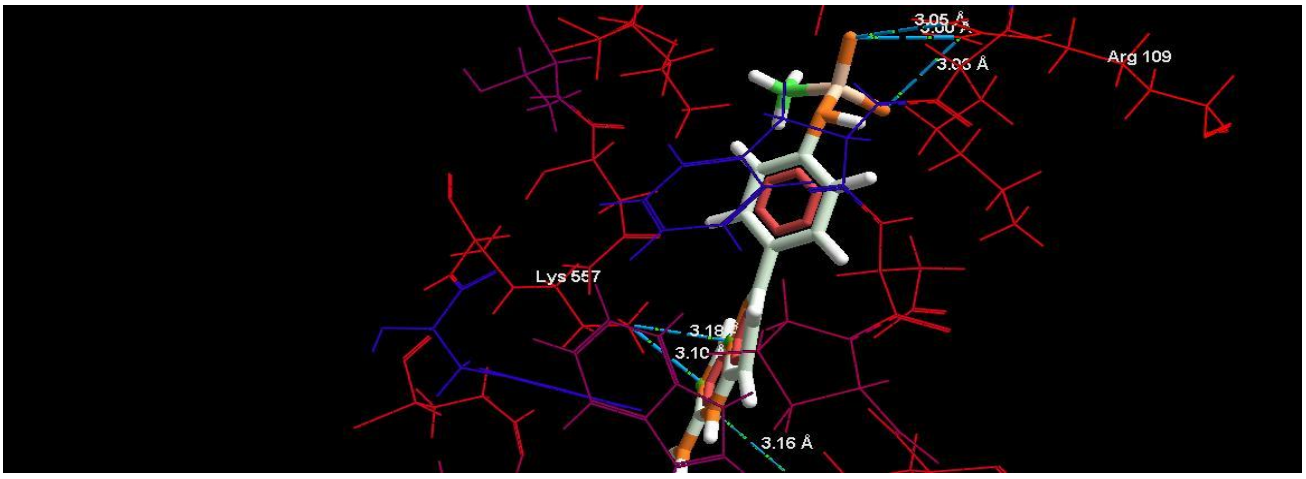


Fig F. Binding mode of Compound 1V(for Analgesic activity) into the binded site of pdb: 1CX2. It has Mol dock score -79.2361 and docking score -81.8104 and form 6 hydrogen bonds shown as blue lines, between N of pyridazinone with N, N of triazin with N, N of triazin with O, O of $NHSO_2CH_3$ with N, O of $NHSO_2CH_3$ with N, O of $NHSO_2CH_3$ with N with distance of 3.18A°, 3.10 A°, 3.16 A°, 3.00 A°, 3.05A°, 3.06 A° respectively

DISCUSSION

In the present study attempts have been made to pre-asses the analgesic and anti-inflammatory activity of substituted pyridazinones and their derivatives on the structural basis, auto-mated docking studies were carried out using Molegro Virtual Docker. On the basis of literature data, we selected sixty hypothetical compounds, further we screened twenty four compounds which showed structural resemblance with 2-substituted-6(4-methylphenyl)-4,5-dihydropyridazin3(2h)-ones and 3-substituted-6-(4-methylphenyl)-4,5-dihydropyridazin3 (2h)-ones and pyridazin substituted triazin. Docking studies were performed using (PDB ID: 6COX) for anti-inflammatory and (PDB ID: 1CX2) for analgesic activity using Molegro Virtual Docker. The Docking studies were performed on the Standard compounds and twenty three test compounds. The docked compounds showed good results for analgesic and anti-inflammatory activity. The *in-silico* Docking studies shows that compound XIV i.e. N-(4-(2-imino, 3,4,8,9-tetrahydro-2H-pyridazin [1,6-α][1,3,5 triazin-7-yl)phenyl) methanesulfonamide bearing triazine ring and methyl sulfonamide group as substituent exhibited relatively comparable binding affinity on (PDB ID: 6 COX) with highest Mol Dock Score of -125.066 as compared to standard and formed four H-Bond interactions with distance of 2.95,3.10,3.08,2.52 (Aº) with Amino Acids Gly 536,Val 228, Asn 537, Gly 533 and formed Hydrogen Bonds with atoms i.e. Nitrogen of triazin with Oxygen, Nitrogen of triazin with Nitrogen, Nitrogen of triazin with Nitrogen, Amide group of triazin with Oxygen respectively possessed greatest Anti-inflammatory acivity amongst the twenty three docked compounds.

The compound XVII i.e. 2-(4-benzylphenyl)-3,4,7,8-tetrahydropyrimdo [1,6-b]pyridazine-6-imine bearing biphenyl as basic moiety and triazine ring as substituent exhibited comparable binding affinity on (PDB ID: 1CX2) with highest Mol Dock Score of – 103.947 and with distance of 3.27, 3.51, 2.98, 2.60 with Amino Acids Glu 319, Gly 551, His 320, His 320 and formed four hydrogen bonds with atoms i.e. Nitrogen of triazin with Oxygen, Amide group of triazin with Oxygen, Amide group of triazin with Nitrogen, Amide group of triazin with Nitrogen respectively and possessed greatest Analgesic activity amongst the twenty three docked compounds. Other than these two highly potent compounds some other compounds i.e. XVII, XVI, XIII and XI having good Mol Dock Score values of -124.537,-123.974,-122.32,-120.715 and formed 4,3,5,5 Hydrogen bonds respectively were found to possess good Anti-inflammatory Activity. The SAR studies illustrate that compounds XVII and XVI showing structural resemblance and bearing pyridazinone as central moiety and triazin substitution showed good interactions with the Amino acids such as Asn 375, Gly 533 and formed hydrogen bonds with Nitrogen of triazin with Oxygen Atom. Some other compounds which showed good results for Analgesic activity were compounds IV, XX, XIV and XI having good Mol Dock Score values of -120.556,-101.023,-83.315,-98.210 and formed 1,5,4,2 Hydrogen bonds respectively. The SAR studies illustrate that compounds XI and XIV showing structural resemblance and bearing pyridazinone as central moiety and triazin substitution showed good interactions with the Amino acids such as Glu 319, His 242 and formed hydrogen bonds with Nitrogen of triazin with Oxygen atom. Various other compounds such as compounds XIII and VIII showed good mol dock score bearing pyridazinone as central /basic moiety having sulphate group substituttion on sixth position while the compounds II and III having pyridazinone as central moiety but having hydrazine hydrate substitution on fifth position having poor docking results for anti-inflammatory and analgesic. The theoretical study allowed us to predict the affinity of the new compounds as well as to infer the structural/dynamics determinants of their interaction with COX inhibitors. Thus, pyridazinone-based compounds represent potential lead compounds for further development of selective COX inhibitors. Overall the compounds XIV, XII, XVI, XIII, VIII and compounds XVII, XX, XXIII, XI, VIII selected for Docking were found to possess good anti-inflammatory and analgesic activity as compared to standard compound and can explored further.

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REFERENCES

Asif M, Singh A. 2010. Exploring Potential. Synthetic Methods and General Chemistry of Pyridazine and Pyridazinone: A Brief Introduction. *International Journal of Chem Tech Research*, 2(2):1112-1128.

Bansal R, Thota S. 2013. Pyridazin-3(2H)-ones: the versatile Pharmacophore of medicinal significance. *Med.Chem. Res*., 22(6):2539-2552.

Daniele B, Andrea B, Matteo C, Gianni P, Sergio S. 2006. Synthesis and conformational preferences of Unnatural tetrapeptides containing L-valine units. *Tetrahedron Assymetry*, 17:3273-3281.

David S, Lenka Z, Milos. 2007. High-performance liquid chromatography and nuclear magnetic resonance study of linear terapeptides and octapeptides containing N-methylated amino acid residues. *Journal of Chromatography*, 1160:128-136.

Halperin I, Ma B, Wolfson H, Nussinov R. 2002. Principles of docking: An overview of search algorithms and a guide to scoring functions. *Proteins: Structure, Function and Bioinformatics*, 47(4):409-443.

Himaja M, Vandana K, Ranjitha A, Ramana MV, Karigar AA. 2011. Synthesis, docking studies and antioxidant activity of 1,3-Benzodioxole-5-carbonyl amino acids and dipeptides. *Int. Res. J. Pharm*., 2(6):5761.

Kiefer JR, Rowlinson SW, Kozak KP. 2003. A Novel Mechanism of Cyclooxygenase -2 Inhibition Involving Interactions with Ser-530 & Tyr-385. *J. Bio. Chem.,* 278(46): 45763-45769.

Kumar A, Lohan P, Aneja DK, Gupta GK, Kaushik D, Prakash O. 2012. Design, synthesis, computational and biological evaluation of some new hydrazine derivatives of DHA and pyranopyrazoles. *European Journal of Medicinal Chemistry*, 50:81-89.

Kurumbail R.G, Stevens A.M. 1996. Structural Basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature.,* 384:644-648.

Murty M.S.R., Rao BR, Ram KR, Yadav JS, Antony J, Anto RJ. 2012. Synthesis and preliminary evaluation activity studies of novel 4-(aryl/heteroaryl-2-ylmethyl)-6-phenyl-2-[3-(4-substituted-piperazine-1-yl)propyl]pyridazin-3(2H)-one derivatives as anticancer agents. *Med. Chem. Res*., 21(10):3161-3169.

Prasanna S, Daga PR, Xie A, Doerksen RJ. 2009. Glycogen synthase kinase-3 inhibition by 3-anilino-4-phenylmaleimides: insights from 3D-QSAR and docking. *J. Comput. Aided. Mol. Des*., 23:113.

Ratnavali G, Devi NKD, Kavitha R, Sri KB, Raju JK, Sirisha B. 2011. An attempt to screen top colorectal cancer drugs by using Molegro Virtual Docker. *Annals of Biological Research*, 2(1): 114-126.

Silva CHTP, Taft CA. 2005. Molecular dynamics, database screening, density functional and docking studies of novel RAR ligands in cancer chemotherapy. *Biophysical Chem*., 117:73-77.

Sivan SK, Manga V. 2010. Molecular Docking and 3D-QSAR studies on triazolinone and pyridazinone, non-nucleoside inhibitor of HIV-1 reverse transcriptase. *Journal of Molecular Modelling,* 16:1169-1178.

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