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Molecular Docking Studies, Analgesic and Anti-inflammatory Screening of Some Novel Quinazolin-4-one Derivatives

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Molecular docking studies was performed on 20 analogous novel quinazolin-4-one derivatives as cox-2 inhibitors using glide tool of maestro 11.4 application of Schrodinger software. Anti-inflammatory and analgesic activities were further evaluated for the compounds. Based on docking studies, the binding affinity of QZN-16 was found to be -10.32 kcal/mol. In order to understand the significance of R-substituents on the quinazoline-4-one nucleus, the findings of hydrogen bonding interactions between designated ligands with binding site region of 4cox were studied. The ligands which are having high docking score were subjected to pharmacological screening. The compound QZN-16 has shown analgesic and anti-inflammatory activity at a dose level of 50 and 100 mg/kg body weight, respectively when compared with standard drug indomethacin. The newly designed quinazoline-4-one derivatives may serve as lead molecules for further development.

Keywords: Structure based drug design, Quinazolin-4-one derivatives, Molecular docking, Analgesic, Anti-inflammatory, Cox-2 inhibitor.

INTRODUCTION

Docking methodologies have been used to predict the experimental binding modes and affinities of small molecules within the binding site of particular targets like receptors and enzymes and are currently being used in virtual screening studies as a standard computational tool in drug design for lead compound optimization and to find novel biologically active compounds. The search algorithm and energy scoring functions are used as fundamental tools for generating the different poses for ligand and also its evaluation in docking studies [1]. Ligand-protein docking is performed to predict the main binding modes of a ligand with a protein with a defined three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function and give the ranking based on the binding modes [2,3]. By using docking applications, virtual screening on large libraries of analogues can be performed so that the results can be graded and structural hypotheses can be formulated on inhibition of the target by ligands, which is helpful in optimizing leads. In addition, the characterization of the binding activity plays an important role in both the logical design of drugs and the elucidation of fundamental biochemical processes [4-6].

Non-steroidal anti-inflammatory medications are among the most widely used therapeutics (NSAIDs). The pharmacological target is cyclooxygenase, which catalyzes the first and key step in arachidonic-acid metabolism. They represent a choice of treatment for different inflammatory diseases *viz.*, arthritis, rheumatism and relaxation through their anti-inflammatory, antipyretic and analgesic activities. The constitutive isozyme COX-1 plays a role in the cytoprotective mechanism of GIT and in normal functioning of the renal system [7]. In response to a pro-inflammatory stimulus, COX-2 is an inducible and short-lived enzyme that is expressed. The biosynthesis of prostaglandin requires COX-2 in inflammatory cells. Classical non-steroidal anti-inflammatory agents inhibit both isozymes to varying degrees, a characteristic that has been directly linked to the corresponding differential distribution in tissue and also represents the shared therapeutic properties and side effects of these agents [8,9]. The basis of the inhibition of COX-2

selectively is clearly demonstrated by the structural features of certain newer heterocyclic derivatives. Few COX-2 inhibitors which are selective like celecoxib are available in the market and, thus, an important objective in medicinal chemistry is the production of novel drugs operating with high efficacy using a similar overall mechanism. Based on the facts given above, we have selected some newer quinazolinone derivatives as attractive candidates to exhibit both anti-inflammatory and analgesic activities [10,11]. These derivatives have been tested biologically for anti-inflammatory and analgesic activities *in vivo*. To define the requirements of structure for inhibitory activity of these novel compounds on COX-2 [12-14], docking studies was performed.

EXPERIMENTAL

Selection of protein: While selecting protein for the docking studies, the resolution must be minimal and X-ray diffraction should be used to assess the structure in order to ensure better protein consistency and reliable docking performance. The protein structure must have a resolution of 2.0-3.0 Å and protein breaks should not be observed in the 3D structure. The protein structure should contain co-crystallized ligand which is subjected to docking simulation to study the effector and inhibitor characteristics towards ligand and it must possess complete domain along with sidechains otherwise it leads to false interpretation in the docking studies [15].

Methodology: In our molecular docking studies, software Maestro along with the Glide algorithm which was provided by Schrodinger small drug discovery suite was employed. The selected protein for the docking studies was prepared using a tool called protein preparation wizard. Ligands designed for docking on the target were prepared by using a tool called ligprep. The grid was generated for the selected binding site by using the tool receptor grid generation. Glide tool was utilized to calculate the docking score and different binding modes for the ligands, the binding modes generated by the glide was evaluated by using tools called pose organizer and ligand interaction diagram generated by Maestro.

Protein preparation: The RCSB domain of PDB was used to get the X-ray crystal structure of protein PDB id: 4COX [https://www.rcsb.org/structure/4COX] used in the docking study. In the present docking study, protein preparation wizard panel of Maestro was used to resolve common structural issues by way of pre-processing, review and modification and refinement. In the pre-processing step, the bond orders were assigned to the protein, polar hydrogens were added to the protein structure, zero-order bonds were created to the metals, if necessary, disulfide bonds are created in the protein structure and water molecules present around the co-crystalized ligand (indomethacin) beyond 5.0 Å was determined. In the review and modify step the water molecules, which are present around the co-crystallized ligand and beyond the 5.0 Å were removed in order to determine during the pre-processing step. Protonation states were generated for the co-crystallized ligand and the lowest penalty state was automatically selected. In the refinement step, the overlapping atoms have been corrected, side chains that have been flipped are labelled as flip and hydrogen bonds assigned were adjusted in the protein by using the force field OPLS in interactive optimizer tool [16,17].

Software method validation: Maestro was the program used to validate the X-ray crystal structure. 4COX, collected from the Protein data bank, was the protein used in the current docking analysis (PDB). In the PDB file format, the X-ray crystal structure of 4COX that was co-crystallized with ligand indomethacin was retrieved from the protein data bank. In the binding site region of 4COX, the co-crystallized ligand bound with protein was split and docked. The 4COX X-ray crystal structures have a resolution of 2.9 Å, suggesting that the parameters for the docking analysis are excellent for reproducing the X-ray crystal structure. Ramachandran plot is considered as an important statistical criterion for the X-ray crystal structure by considering allowed and disallowed regions.

Ligand preparation: The structure of quinazolin-4-one derivatives (Fig. 1) was converted into a 3D model by using a graphical user interface provided by Schrodinger and finally subjected to energy minimization using the tool ligprep. A series of steps are involved in the ligand preparation process including corrections, generate variations, eliminate unwanted moieties and optimize the structures. Hydrogen atoms are added which is consistent with a particular force field before the 3D structures can be minimized by applying the program htreat of the maestro. Before the generation of ionization states, charged groups must be neutralized, after which the ionizer generates various ionization states, tautomerize generates various tautomer's ligfilter filters the structures possessing molecular weight greater than 1000 or the specific functional groups, which may be present or absent in the structure. The chirality of the atoms are varied to generate possible structures using stereoizer, low-energy ring conformations generated by ring_conf, followed by short conformational search to adopt proper chirality's in 3D structure to optimize the geometries. The OPLS force field was used for the preparation of ligands [18].

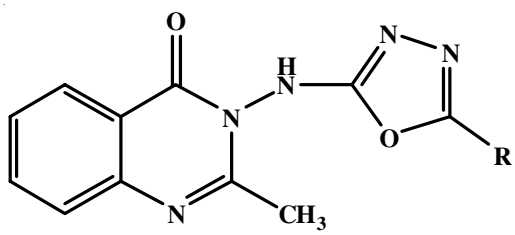

Fig. 1. General structure of quinazolin-4-one derivatives

Receptor grid generation: The grid was generated around the active binding site of protein where the co-crystallized ligand was present using the tool called receptor grid generation panel. The binding site was defined in the grid box, which surrounds the bound co-crystallized ligand atom within 15 Å dimensions. According to the given dimensions, the receptor grid generation panel runs calculation for the target binding site and generated a grid file. This grid file was uploaded into the glide to perform the docking simulation.

Ligand docking: In this present study, we have used a docking algorithm called Glide. Glide works on the Emodel1 scoring function, which gives ranking based on the coulomb-

vdW energy of protein-ligand complexes of a set of ligands with a small contribution from Glide Score. The compounds possessing strong binding affinity relative to those possessing little to no binding affinity can be separated by using GlideScore2, which is an empirical scoring function. It gives a ranking between the docked ligands based on the active and inactive compounds [19,20]. Docking simulation was performed to dock the designed quinazolin-4-one derivatives QZN1–QZN-20 against cyclooxygenase-2 (4COX) using glide to study the interaction between quinazoline-4-one derivatives and cyclooxygenase-2 enzyme. The ligands, which were prepared and the grid file which was generated before docking was uploaded into glide. In this present docking study, the virtual screening was performed using the extra precision mode to study the physico-chemical descriptors. We used glide because it gives fast and higher docking accuracy results than other docking software (Glide: 82%, Surflex: 75%, FlexX:58%) [21-23].

Anti-inflammatory activity

Experimental animals: Albino rats of Wistar were collected from an animal house attached to the Department of Pharmacology from strains of either sex between 150 and 250 g. Throughout the study, the animals were fed a regular diet of rats and water adlibitum. In the laboratory state, they were acclimatized for two weeks before the experimentation. The housing is maintained with the following conditions: 12:12 h light and dark cycle regulated lighting at 25 ºC and around 50% relative humidity.

IAEC approval: Pharmacological evaluation of quinazoline-4-one derivatives for various screening methods has been approved (approval No: 16/IAEC/CLPT/2018-19) by the Institutional Animal Ethics Committee (IAEC) of Chalapathi Institute of Pharmaceutical Sciences, Guntur, India (Reg.No.: 1048/PO/Re/S/07/CPCSEA).

Animals: Adult Wistar albino rats of either sex with a weight of 180 to 220 g were divided into five separate groups each of four rats. The control group was administered with normal water at a dosage of 10 mL/kg for 0.5% gum acacia, the regular group was administered with indomethacin 100 mg/kg and the test groups were administered with quinazoline-4-one derivatives at a dose of 100 mg/kg body weight.

Acute anti-inflammatory activity: The hind paw edema test induced by Carrageenan was performed in rats using the method of Winter *et al.* [24]. The acute anti-inflammatory activity in Wistar albino rats was assessed using the carrageenan induced paw edema technique. By injecting 0.1 mL of 1% carrageenan solution into the sub-plantar surface of the hind paw of the rat, acute inflammation was produced. The group specific drugs were administered 1 h before the carrageenan injection. A Plethismometer (PLM-01 PLUS Orchid Scientifics) was used to measure the paw volume up to the tibiotarsal articulation at baseline, 30, 60, 90 and 120 min after injection of carrageenan. Anti-inflammatory behaviour was expressed by measuring the percentage decrease in paw edema (V-paw volume).

Analgesic activity

Animals: Using Swiss male albino mice weighing 20 to 25 g, pharmacological studies were carried out. In the animal house attached to the Pharmacology Department, mice were raised. The animals were grouped randomly in polyacrylic cages and held under normal conditions of animal housing (25 ± 2 ºC) and relative humidity (40-70%) with dark-light cycles (12/12 h). Water *ad libitum* and regular laboratory chow were been fed to the mice. For 1 day prior to experimentation, the mice were acclimatized to laboratory conditions. Throughout the entire day of the experiment, animals had no access to food.

Adult Swiss albino mice of either sex weighing between 20 and 25 g were divided into five groups each consisting of four animals. The control group was administered with normal water at a dosage of 10 mL/kg in 0.5% gum acacia and the standard group was administered with pentazocine 6 mg/kg in 0.5% gum acacia suspension. Quinazoline-4-one derivatives at a dosage of 50 mg/kg were administered as a suspension in 0.5% gum acacia to the test groups.

Eddy's hot plate method: The analgesic efficacy of quinazoline-4-one derivatives was tested using the Eddy & Leimbach hot plate technique [25]. A temperature of 55 ± 0.2 ºC was maintained. As a sign of discomfort, animals licked their limbs and jumped. These mice were treated with suspension as follows: in 0.5% of gum acacia the control group were administered with regular water and 50 mg/kg of quinazoline-4-one derivatives was administered to the test groups. Pentazocine (6 mg/kg) standard was administered to the regular group *via* oral route. Mice were put on the hot plate 1 h after dosing group specific drugs and the time was monitored by a stopwatch before either licking or jumping occurred. The latency period was reported after oral administration of group-specific drugs, before and after 1, 2, 3 and 4 h. The 12 s cut-off time was used for the hot plate test [26].

RESULTS AND DISCUSSION

Docking studies: Extra precision docking approach (XP mode) has been used as a tool in the present docking studies. This method was employed to study the physico-chemical properties of designed ligands. The binding of small molecules into the enzyme complex was studied by utilizing glide, which gives a reliable prediction and also in identifying the active ligands. The hydrogen bond interactions of the ligand with macromolecule were represented in the form of a ligand interaction diagram. Evaluation based on the various R-substituents, which contribute to hydrogen bond interactions with the enzyme was considered. The functional groups responsible were analyzed on the basis of the ligand's interaction with the enzyme's binding site and the result obtained was interpreted. The molecule QZN-16 was found to possess minimum energy of interaction with a docking score of -10.36 kcal/mol in comparison with standard indomethacin with a docking score of -12.09 due to the additional availability of amino acid residue Arg-120. The binding site interactions with the ligands are represented in Fig. 2 and results are tabulated in Table-1.

The activities of quinazoline-4-one derivatives were studied using standard drug. Even though the derivatives QZN-16, QZN-08, QZN-20 differ in chemistry, they showed a positive result for anti-inflammatory and analgesic activities (Table-2). The difference between the values of paw edema for the anti-

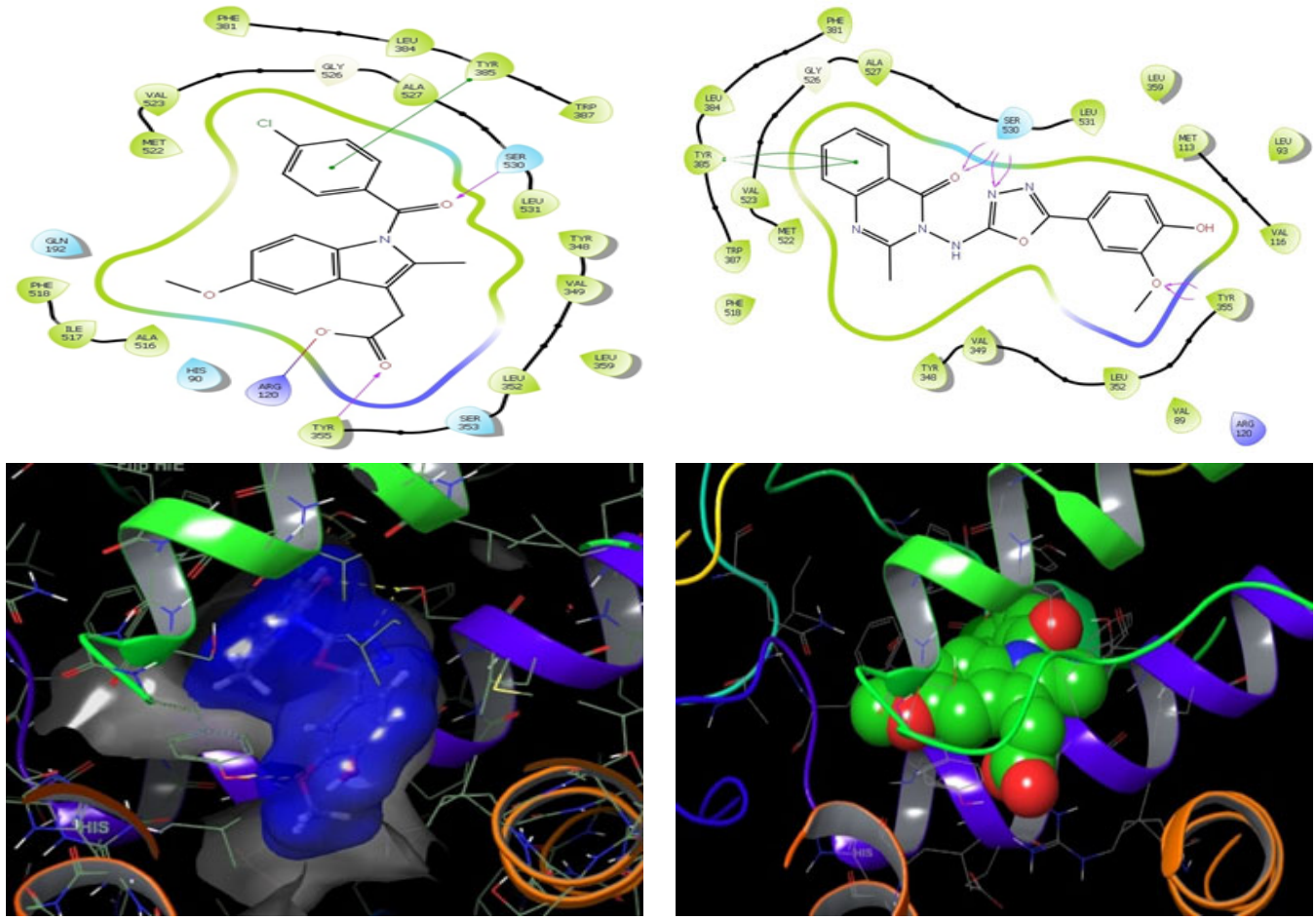

Fig. 2. 2D and 3D structures of interaction of QZN-16 and indomethacin with active site of 4COX

TABLE-1
DOCKING RESULTS OF NEWER
QUINAZOLIN-4-ONE DERIVATIVES

R group substituent's	Binding affinity	H-bond interacting residues
-C_6H_5	-9.16	TYR-355, ARG-120
2-Cl C_6H_4	-9.21	TYR-385, TRP-387, SER-530, TYR-355
3-Cl C_6H_4	-9.11	TYR-385, SER-530
4-Cl C_6H_4	-8.44	TYR-385, SER-530, TYR-355
2,4-Cl C_6H_3	-9.04	ARG-120, TYR-355
2-F C_6H_4	-9.45	TRP-387, TYR-385, SER-530
4-F C_6H_4	-9.66	TYR-385, TYR-355
2-$NO_2C_6H_4$	-9.47	SER-530, TYR-385, ARG-120
4-$NO_2C_6H_4$	-8.45	TYR-385, TYR-355, SER-530, ARG-120
3-$NO_2C_6H_4$	-8.72	TYR-385
2-OHC_6H_4	-9.29	ARG-120, TYR-385, SER-530,
4-OHC_6H_4	-9.17	TYR-385, TYR-355
2-$OMeC_6H_4$	-9.34	TYR-385, ARG-120, SER-530
4-$OMeC_6H_4$	-8.89	SER-530, TYR-355, TYR-385
2,4,6 $OMeC_6H_3$	-6.01	TYR-355, ARG-120
4-OH,3-$OMeC_6H_4$	-10.36	TYR-385, TYR-355, SER-530
$(CH_3)_2N$-C_6H_4	-7.66	ARG-120, TYR-355
2-Pyridyl	-9.33	TYR-385, SER-350
4-Pyridyl	-8.3	TYR-385, SER-530
2-Furanyl	-9.17	TYR-385, SER-530
Indomethacin	-12.09	TYR-385, TYR-355, SER-530, ARG-120

inflammatory activity of all the three derivatives was found to be similar to the standard drug. Also, much variation was not observed between all the three derivatives as the changes with time interval was found to be 2% at 30 min, 4% at 60 min, 6% at 90 min and 3% at 120 min. The observed experimental values of the test were compared with the standard and found to be very similar as there is a slight variation between the values and were found to be 7% at 30 min, 5% at 60 min, 5% at 90 min and 9% at 120 min. In case of analgesic activity, the time to withstand pain by the animal was also increased and the values are nearer for three derivatives when compared with standard. The observed experimental values of the standard to withstand pain was found to be 2.28 s at 3 h as threshold and 1.63 s for QZN-16, 0.66 s for QZN-8 and 0.72 s for QZN-20 as depicted in Table-3. On an average, the test compounds possess the ability to withstand pain for 1.00 s and 56.15% similar to that of standard. In case of independent compounds, QZN-16 is 71.49% similar to that of standard, QZN-08 is 28.94% similar to that of standard, QZN-20 is 31.57% similar to that of standard.

Conclusion

By using the Schrodinger small drug discovery suite using glide algorithm, novel quinazoline-4-one derivatives were subjected to docking studies. Cyclooxygenase-2 (PDB ID-4COX) from the protein data bank was the enzyme selected

TABLE-2
ANTI-INFLAMMATORY SCREENING OF NEWER DERIVATIVES

Treatment groups	Reduction in paw volume per time (min) and percentage difference								
	Basal	30 min	Change (%)	60 min	Change (%)	90 min	Change (%)	120 min	Change (%)
Control	0.86	0.84	2	0.86	0	0.83	3	0.81	5
Standard	0.93	0.58	35	0.54	39	0.49	44	0.45	48
QZN-16	0.74	0.45	29	0.39	35	0.32	42	0.33	41
QZN-08	0.81	0.54	27	0.51	30	0.46	35	0.44	37
QZN-20	0.78	0.50	28	0.43	35	0.39	39	0.41	37

TABLE-3
SCREENING OF NEWER DERIVATIVES
FOR ANALGESIC ACTIVITY

Treatment groups	Time (s) when paw licking and jumping was observed				
	Basal	1 h	2 h	3 h	4 h
Control	5.71	5.54	5.42	5.45	5.18
Standard	5.12	5.43	6.30	7.40	6.04
QZN-16	6.02	6.41	7.18	7.65	7.07
QZN-08	5.86	6.04	6.31	6.52	6.11
QZN-20	5.78	5.91	6.12	6.50	6.03

for the study. The protein under study was subjected to the 3-step refinement process for energy minimization of protein. The ligands understudy was drawn and converted into 3D structures using a tool called 2D sketcher and subjected to energy minimization. The receptor grid generation was done to define the binding site for the ligands. The grid file generated was uploaded into the glide to perform ligand docking. After the completion of docking, the score was generated for the ligand based on the interaction with the active site of the enzyme. The ligands with high docking scores were selected and subjected to binding site interaction and the results were interpreted and compared with the standard drug. The docking score of QZN-16 was found to be -10.32 kcal/mol. Compound QZN-16 showed analgesic and anti-inflammatory activity nearer to the standard drug at dose 50 mg/kg and 100 mg/kg body weight, respectively.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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