WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 4, 1734-1742 Research Article ISSN 2278 – 4357

DOCKING STUDIES OF 4 - (3*H*)-QUINAZOLINONE DERIVATIVES AS COX-2 INHIBITOR

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Article Received on
15 Feb. 2019,
Revised on 08 March 2019,
Accepted on 29 March 2019
DOI: 10.20959/wjpps20194-13568

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ABSTRACT

A series of novel *Some 4(3H)*-quinazolinone derivatives containing primary aromatic amines were synthesized, characterized and subsequently evaluated for anti inflammatory property. Docking studies with these compounds against cyclooxygenase-2 receptor (PDB 1D: 1PXX) indicated that they exhibit specific interactions with key residues located in the site of the COX-2 structure, which colloborates the hypothesis that these molecules are potential ligands of COX-2. Molecular modeling studies were used to assess the fit of these compounds within the active site of human DHFR. The structural analyses indicate that the coordinate bond interactions, the hydrogen bond interactions, the Vander Waals interactions as well as the hydrophobic interactions between ligand and receptor are responsible simultaneously for the preference of inhibition and potency. In this study, fast flexible docking simulations were performed on quinazolinone derivatives as human COX-2 inhibitors. The results indicated that the quinazolinone ring of the inhibitors forms hydrophobic contacts with Tyr384,Ser529, Arg119 and stacking interaction is conserved in complex with the inhibitor and cofactor .The analysis of the docking results, which takes into account the hydrophilic and hydrophobic interactions between the ligands and the target, identified 3h,3e and 3f (comparable with standard diclofenac sodium) and the best docking score, indicating effective binding of the compound **3h,3e** and **3f** at the active site.

KEYWORDS: Anti-inflammatory, Molecular docking, COX-2, *4(3H)*-Quinazolinone.

INTRODUCTION

The main objective of medicinal chemistry is to synthesize the compounds that show promising activity as therapeutic agents with lower toxicity. *4(3H)-quinazolinone* have been reported to show abroad spectrum of biological activity.[1,2] The substituted *4(3H)*-quinazolinone[7-11] have been shown to exhibit antitumor, antihistaminic, anti-inflammatory, herbicidal, antiallergic, antihelmintic, COX-2inhibitory,antifungal,antibacterial, antitubercular, anticonvulsant, diarrhea redominant irritable bowel syndrome, hypoglycemic, HIV-1 reverse transcriptase inhibitor & insecticidal activities. *Insilico* modeling of different derivatives will be carried out by using software such as Chemsketch and Molinspiration. Compounds having drug likeness and molecular descriptors, resembling those of standard molecules, and which obeys the Lipinski Rule of Five will be selected for wet lab synthesis.[3-6]

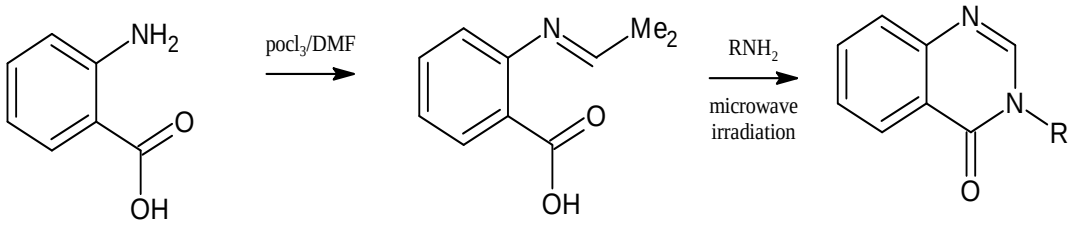
The amount of resolved X-ray structures of protein-ligand complexes have exploded during the last decade. This has initiated much improvement of docking methods by an advanced knowledge about the key interactions in the complexes, nevertheless, it still remains a challenge even to reproduce known experimental results by ligand docking. A number of docking methods for predicting binding modes of small molecules have been developed, methods which are also thought to help to quantify energetics of different molecular interactions. Ligand docking is mainly used by the pharma industry for identifying possible compounds for development in the drug discovery process, usually in the very early hit identification phase, but also at later stages of lead optimisation. The quality of different docking methods has been thoroughly investigated, however, the relationship between methods, scoring functions and target proteins on one hand, and docking performance on the other hand still seems poorly understood. Scoring functions are especially important since minimisation algorithms relay on these functions. Therefore, an accurate scoring function is absolutely crucial to obtain correct results, i.e. correct binding modes but also correct ranking of docked ligands. The accuracy of scoring functions is target dependent, which implies that it is important to study the scope and limitations of these functions. In this report, The present study is a continuation of our previous efforts aiming to create novel synthetic lead compound and its in vitro testing for future development as COX-2 inhibitor.

4.3.1. General procedure for the synthesis of 4-(3*H*)- Quinazolinones

To an ice-cold solution[2] of $POCl_3$ (5.6 ml) in DMF (10 ml) was added anthranilic acid 2gm (0.01458 mol)and stirred for 5-10 minutes until the disappearance of anthranilic acid as indicated by TLC. The reaction mixture was treated with the respective primary aromatic amine (0.01458 mol) and supported on to anhydrous sodium sulphate (five times the weight of anthranilic acid) and exposed to micro wave (BPL company) irradiation (600 Watts) for 1.5- 2 minutes with 30 sec pulse. The reaction mixture was quenched with water (50 ml) and extracted with ethyl acetate (2 x50ml). The organic layer was dried over anhydrous sodium sulphate, concentrated and purified by silica gel column chromatography (60-120 mesh) using hexane/Ethyl acetate (7.5:2.5) as eluent to yield the pure product.

By following the above procedure, compound **3a-3j** were synthesized .**Table 1.1**

General scheme of 4-(3*H*)-Quinazolinone derivatives



Anthranilic acid Intermediate ion 3-Substituted Quinazolinones

Sl.No	Compound code	R	Substituents (X)		
			o	m	p
1	3a	Phenyl	H	H	H
2	3b	Phenyl	H	H	Cl
3	3c	Phenyl	Br	H	H
4	3d	Phenyl	CH_3	H	CH_3
5	3e	Phenyl	OCH_3	H	H
6	3f	Phenyl	CH_3	H	H
7	3g	Phenyl	Cl	H	H
8	3h	Phenyl	H	NO_2	H
9	3i	Phenyl	NO_2	H	H
10	3j	Phenyl	H	CH_3	CH_3

MATERIALS AND METHODS1

Docking studies were performed for of 4-(3*H*)Quinazolinone with target proteins by Glide 5.5 module of Schrodinger suite.

Molecular docking studies[15-19]

Comparative docking of set of ligands with specific proteins involves methodology with Easy user interface and their respective scoring function provided by Molegro Virtual Docker. Molecular docking studies were performed to target COX-2. The compound which have shown potent anti inflammatory and analgesic activity were subjected to molecular docking to target COX-2 (Pdb-1COX2).

Steps in methodology

1) Importing a protein file and ligand file and preparation of ligands.

2) Protein preparation and detecting cavities of protein molecules.

3) Executing a docking set up through docking wizard panel.

4) Poses of protein-ligand complex obtained after docking process with their specific mol dock scores displayed in output file.

Computational Methods with Glide Version5.5

All computational studies were carried outusing Glide version 5.5, installed in a single machine running on Intel Core TM 2 Duo processor with 1GB RAM and 160GB hard disk with Red Hat Linux Enterprise version 5.0 as the operating system.

Ligand Preparation

The structure of the compound, 4 -(3*H*)-Quinazolinone (C14H13N2O) was drawn by using ChemSketch (ACDLABS 12.0) and converted to 3D structure with the help of 3D optimization tool. By using the LigPrep (2.3)B – 71 module (LigPrep, Version 2.3,2009), the drawn ligand was geometry optimized by using the Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field with the Steepest Descent followed by truncated Newton Conjugate gradient protocol. Partial atomic charges were computed using the OPLS-2005 force field. The LigPrep is a utility in Schrodinger software suite that combines tools for generating 3Dstructures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers and steric isomers and geometry minimization of ligands. Finally, 32 poses had been prepared with different tautomeric and steric features for docking studies.

Validation of the Docking Protocol in Glide

The most suitable method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose predicted by the scoring function resembles an experimental binding mode as determined by X-ray crystallography. In the present study, the

docking of proteins with their already presented ligand was performed to test the reliability and reproducibility of the docking protocol for our study. The root mean square deviations (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of the ligand by Glide (3 A) was analyzed. This indicates the reliability of the docking method in reproducing the experimentally observed binding mode for target proteins.

Docking and Scoring Function[19-24]

The ligands were docked with the active site using the 'Extra precision' Glide algorithm. Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. Final scoring of docked ligand is carried out on the energy-minimized poses Glide Score scoring function. Glide Score is based on ChemScore, but includes a stericclash term and adds buried polar terms devised by Schrödinger to penalize electrostatic mismatches.

GScore = 0.065*vdW + 0.130*Coul + Lipo + Hbond + Metal + BuryP + RotB + Site

Where,

VdW : - Van der Waal energy

Coul : - Coulomb energy

Lipo : - Lipophilic contact term

HBond : - Hydrogen-bonding term

Metal : - Metal-binding term

BuryP : - Penalty for buriedpolar groups

RotB : - Penalty for freezing rotatable bonds

Site : - Polar interactions at the active

Site; and the coefficients of vdW and Coul are: - a = 0.065, b = 0.1

Docking Studies

The docking studies were done for all the prepared proteins separately. Docking studies on compounds prepared through Lig Prep were carried out in the active site of the protein. Receptor Vander Waals scaling for the non polar atoms was set to 0.9 which makes the protein site "roomier" by moving back the surface of non Polar Regions of the protein and ligand. This kind of adjustments emulate to some extent the effect of breathing motion to the protein site, it is a kind of giving breathing to the receptor, this approach softens the active site region of the receptor making it flexible (Taverna and Goldstein, 2002).The prepared protein and the ligand were employed to build energy grids using the default value of protein

atom scaling (1.0A) within a cubic box, centered around the centroid of the X-ray ligand pose. After Grid generation, the ligand was docked with the protein by using Glide 5.5 module (Glide, Version 5.5, 2009) in extra precision mode (XP) which uses MCSA (Monte Carlo Based Simulated Algorithm) based minimization. The best docked pose (with lowest Glide Score value) obtained from Glide(Hamilton-Miller,1995; Friesner et al., 2004; Friesner et al.,2006; Halgren et al., 2004) was analyzed.The binding energy was calculated by Liaison module (Liaison, Version 5.5,2009).

RESULTS AND DISCUSSION

The docking simulation technique was performed using Glide module (Schrodinger suite) with 4 - (3*H*)- Quinazolinone derived compounds 3-mono substituted quinazolinone and it was docked into each of COX-2 targets. For protein were selected after evaluating number of geometries from protein data bank (PDB) for docking studies. For validating the software, the proteins were redocked with the already bound ligand. In that 32 poses, the best 10 poses (1 to 10) were selected according to the Glide XP score and lowest energy docked conformation and subjected to the energy minimization using Liasion module. **Table 1.2** summarizes the result of the docking study presented as Glide score and Glide energies.

Docking Score and Energy Score of Antiinflammatory

S.NO	Glide Energy (Kcal / Mol)	Glide Score	D-H….A	Distance Between Donor And Acceptor
3a	-28.8	-4.1	N-H…O (Tyr 384)	2.83
3b	-29.8	-4.5	N-H…O (Tyr 384)	2.91
3c	-27.8	-4.5	N-H…O (ser 529)	2.7
3d	-27.1	-4.9	N-H…O (Tyr384)	2.9
3e	-30.6	-4.9	N-H…O (ser 529)	2.8
3f	-30.9	-4.9	N-H…O (ser529)	2.8
3g	-27.4	-4.8	N-H…O (ser 529)	2.6
3h	-33.9	-4.9	N-H…O (ser 529)	2.8
3i	-28.8	-4.1	N-H…O (Arg 119)	3.0
3j	-26.5	-4.2	Hydrophobic	-
STD	-28.7	-5.2	Hydrophobic	-

CONCLUSION

The preliminary *insilico* screening of various analogues was performed to assess the drug like properties using Molinspiration software. Drug likeness properties and bio activity of the proposed analogues were studied and all the compounds obeyed Lipinski rule of five were selected for further studies. Docking score of derivatives find out using Schrodinger software and depending on good docking scores compound were selected for wet lab synthesis and further pharmacological screening. The prepared compounds were characterized using FTIR, 1HNMR and MASS spectroscopy.

ACKNOWLEDGEMENT

The authors wish to express their deep gratitude to D.Vel Murugan, Dean of crystallography, Madras university, Chennai and Mr.Kiran, secretary, RR Group of institution, Bangalore for their help in carrying out the Research work .

REFERENCE

1. Ugi I, Domling A and Werner B, J Heterocycl Chem., 2000; 37: 647.
2. Shirakawa S and Kobayashi S, Org Lett., 2006; 8: 4939-4942.
3. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deli Rev., 2001; 46(1-3): 3–26.
4. Jeffrey R. Deschamps The role of crystallography in drug design The AAPS Journal; E813-E819.
5. James R. Connolly. Introduction to X-Ray Powder Diffraction, Spring, 2012.
6. Morris G M, Huey R, Lindstrom W, Sanner M F, Belew R K, Goodsell D S and Olson A J AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility J. Comput. Chem., 2009; 30: 2785.
7. Zarghi A, Rao P N P and Knaus E E, Designand synthesis of new rofecoxib analogs as selective cyclooxygenase-2 (COX-2) inhibitors: Replacement of the methanesulfonyl pharmacophore by a N-acetylsulfonamido bioisostere J. Pharm. Pharm. Sci., 2007; 10: 159.
8. Pinto D J P, Batt D G, PittsWJ, Petraitis J J, OrwatMJ, Wang S, Jetter J W, Sherk S R, Houghton G C, Copeland R A, Covington M B, Trzaskos J M and Magolda R L.
9. Terphenyl cyclooxygenase-2 (COX-2) inhibitors: Optimization of the central ring and o-biphenyl analogs Bioorgan. Med. Chem. Lett., 1999; **9:** 919.

10. Pinto D J P, Copeland R A, Covington M B, Pitts W J, Batt D G, Orwat M J, Lam G N, Joshi A, Chan Y-C, Wang S, Trzaskos J M, Magolda R L and Kornhauser D M Chemistry and pharmacokinetics of diarylthiophenes and terphenyls as selective COX-2 inhibitors. *Bioorgan. Med. Chem. Lett.*, 1996; **6**: 290.
11. Wierzbicki M, Sauveur F, Bonnet J and Tordjman C 1998 Thiophene compounds U.S. Patent 5705525.
12. Mizushima Y and Kobayashi M Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins J. Pharm. Pharmacol, 1968; 20**:** 169.
13. Rajadurai R, Padmanabhan R and Ananthan S Synthesis and biological evaluation of diamide derivatives of (S)-BINOL and biphenyl as potential antiinflammatory/anti-arthritic agents Med. Chem. Res., 2013; 48: 76.
14. Sanner M F Python: A programming language for software integration and development J. Mol. Graph. Model, 1999; 17: 57.
15. Morris G M, Huey R, Lindstrom W, Sanner M F, Belew R K, Goodsell D S and Olson A J AutoDock4 and Auto Dock Tools 4: Automated docking with selective receptor flexibility J. Comput. Chem., 2009; 30: 27-85.
16. Kiefer J R, Rowlinson S W, Prusakiewicz J J, Pawlitz J L, Kozak K R, Kalgutkar A S, Stallings W C, Marnett L J and Kurumbail R G Crystal structure of Diclofenac bound to the cyclooxygenase active site of COX-2, 2003.
17. Lil lMA and Danielson ML Computer-aided drug design platform using PYMOL J. Comput. Aided Mol. Des., 2010; 25: 13.
18. Tordjman C, Sauveur F, Droual M, Briss S, Andre N, Bellot I, Deschamps C and Wierzbicki M Synthesis of the butanamide derivative, 2003; 53: 774.
19. Wang Z, Yang Q, Bai Z, Sun J, Jiang X, Song H, Wu Y and Zhang W Synthesis and biological evaluation of 2,3-diarylthiophene analogues of combretastatin Med Chem Comm, 2015; 6: 971.
20. Paolo C and Flavio V New derivatives of 1,2,3,4-tetrahydronaphthalene, process for their preparation and associated pharmaceutical compositions, 1984; Patent 2123410A
21. Zarghi A, Rao P N P and Knaus E E Design and synthesis of new rofecoxib analogs as selective cyclooxygenase-2 (COX-2) inhibitors: Replacement of the methanesulfonyl pharmacophore by a N-acetylsulfonamido bioisostere J. Pharm. Pharm. Sci., 2007; 10: 159.

22. Pinto D J P, Batt D G, PittsWJ, Petraitis J J, OrwatMJ, Wang S, Jetter J W, Sherk S R, Houghton G C, Copeland R A, Covington M B, Trzaskos J M and Magolda R L Terphenyl cyclooxygenase-2 (COX-2) inhibitors: Optimization of the central ring and o-biphenyl analogs Bioorgan. Med. Chem. Lett., 1999; 9: 919.
23. Pinto D J P, Copeland R A, Covington M B, Pitts W J, Batt D G, Orwat M J, Lam G N, Joshi A, Chan Y-C, Wang S, Trzaskos J M, Magolda R L and Kornhauser D M Chemistry and pharmacokinetics of diarylthiophenes and terphenyls as selective COX-2 inhibitors *Bioorgan. Med. Chem Lett.*, 1996*;* **6**: 2907.
24. Kiefer J R, Rowlinson S W, Prusakiewicz J J, Pawlitz J L, Kozak K R, Kalgutkar A S, Stallings W C, Marnett L J and Kurumbail R G Crystal structure of Diclofenac bound to the cyclooxygenase active site of COX-2 2003; doi: 10.2210/pdb1pxx/pdb