Research Article

Docking Studies of 4 - (3H)-Quinazolinone Derivatives as DHFR Inhibitor

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ABSTRACT
In order to produce potent new leads for anticancer drugs, a new series of quinazoline analogs was designed to resemble methotrexate (MTX, 1) structure features and fitted with functional groups believed to enhance inhibition of mammalian DHFR activity. 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 quinazoline antifolates derivatives as human DHFR inhibitors. The results indicated that the quinazoline ring of the inhibitors forms hydrophobic contacts with Ala 9, Leu252, Thr196 and Lys 352 and stacking interaction is conserved in complex with the inhibitor and cofactor.

Keywords: Quinazolin-4- (3H) one, DHFR and Molecular docking, anticancer.

INTRODUCTION

Dihydrofolate reductase (DHFR) is an enzyme of pivotal importance in biochemistry and medicinal chemistry.DHFR catalyzes the reduction of folate or 7, 8-dihydrofolateto tetrahydrofolate and intimately couples with thymidylate synthase (TS). Inhibition of DHFR or TS activity in the absence of salvage leads to 'thyminelessdeath.' [1–3] Compounds that inhibit DHFR exhibit an important role in clinical medicine as exemplified by the use of methotrexate in neoplastic diseases,[4,5] inflammatory bowel diseases,6 and rheumatoid arthritis,[7] as well as in psoriasis,[8,9] and in asthma.[10] A new generation of potent lipophilic DHFR inhibitors such as trimetrexate and piritrexim.

Cancer is continuing to be a major health problem in developing as well as undeveloped countries. Surpassing heart diseases, it is taking the position number one killer due to various worldwide factors. Although major advances have been made in the chemotherapeutic management of some patients, the continued commitment to the laborious task of discovering new anticancer agents remains critically important. In the course of identifying various chemical substances which may serve as leads for designing novel antitumor agents, we are particularly interested in the present work with quinazoline derivatives which have been identified as a new class of cancer chemotherapeutic agents with significant therapeutic efficacy against solid tumors. It is well known that quinazoline derivatives are potent inhibitors of Epidermal growth factor receptor (EGFR) . Combining the inherent DHFR inhibition activity of the quinazolines and those functional groups in one structure was expected to produce more active compounds. Most of those functional groups are also known to increase lipid solubility. 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 DHFR inhibitor.

Chemistry

[11]Anthranilic acid was treated with Vilsmeier reagent at 0 ^{o}C and the reaction mixture was stirred until TLC monitoring indicated the disappearance of anthranilic acid. Subsequently aniline was added to the reaction mixture and supported on sodium suphate and subjected to microwave irradiation. The reaction was quenched with water and extracted with ethyl acetate, dried over sodium sulphate, concentrated and column purified. IR absorption at 1668 and 1591 cm-1
Corresponding to carbonyl and imine functionality respectively indicated the formation of the product. Further a singlet at 8.11 ppm for quinazolinone proton confirmed the formation of the product. Following the same procedure substituted anilines were treated with anthranilic acid and Vilsmeier reagent under microwave conditions. The products were characterized by IR, NMR and Mass spectra. (**Scheme1)** and the results are summarized in **Table 1.**

MATERIALS AND METHODS

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

Computational Methods with Glide Version 5.5

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

Ligand Preparation

The structure of the compound, 4 -(3H)-Quinazolinone ($C_{14}H_{13}N_2O$) 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 3D structures 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 Studies

[12-13]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 (1.0 A) 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).

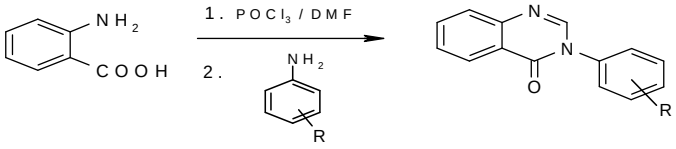
RESULTS AND DISCUSSION

The docking simulation technique was performed using Glide module (Schrodinger suite) with 4 - (3H)-Quinazolinone derived compound 3-mono substituted quinazolinone and it was docked into each of DHFR 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 2 summarizes the result of the docking study presented as Glide score and Glide energies.

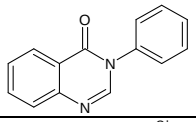
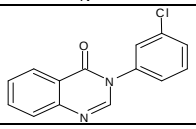
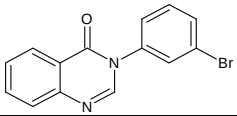
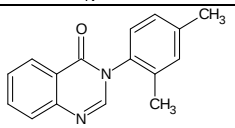
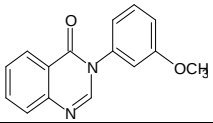
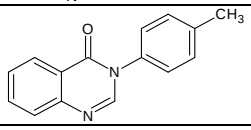
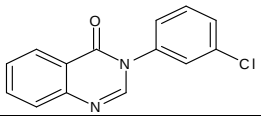
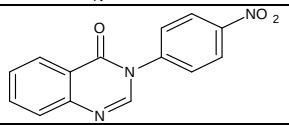
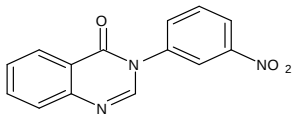
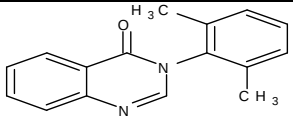
Table 2: Docking result of the ligand, 3- mono substituted quinazolinones with 32 Poses

S.NO	GLIDE ENERGY (Kcal / Mol)	GLIDE SCORE	D-H….A	DISTANCE BETWEEN DONOR AND ACCEPTOR
3a	-33.6	-7.3	N-H….O (Ala9)	3.2
3b	-37.2	-7.0	N-H…O(Ala 9)	2.96
3c	-41.9	-7.1	N-H…O(Ala 9)	3.3
3d	-39.0	-7.7	N-H…O(Ala 9)	3.2
3e	-31.0	-6.6	Hydrophobic	2.8
3f	-35.7	-8.1	N-H…O(Ala 9)	3.2
3g	-39.1	-7.1	N-H…O (Gly117)	2.9
3h	-39.8	-7.5	O-H….O (Thr196)	3.2
3i	-43.5	-7.1	N-H…O (Gly117)	3.3
3j	-37.8	-7.5	N-H…O(Ala 9)	3.4
STD	-50.6	-6.9	N-H….O (Thr56)	3.1

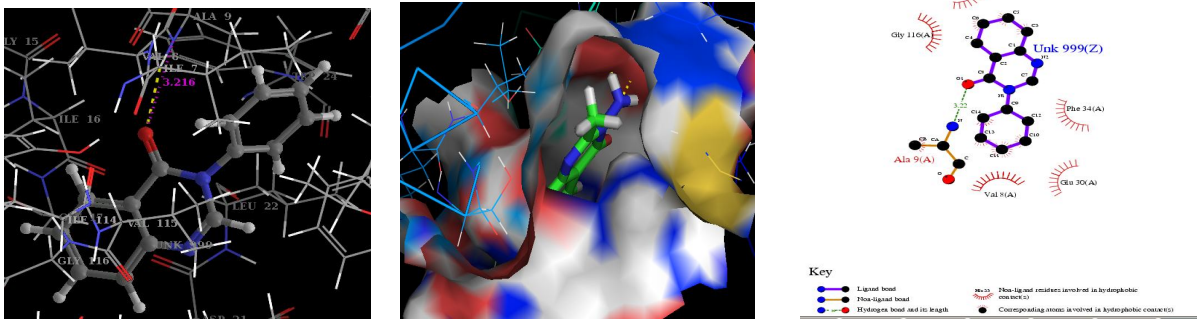
Table 1 and Scheme 1



1 **2 a - j** **3 a - j**

S.No	quinazolinone	Reaction time (Min)	% yield	M.P (ºC)
3a		1.5	83	132
3b		1.7	81	178
3c		1.5	88	200
3d		1.7	84	Liquid
3e		2	85	240
3f		2	83	152
3g		1.9	84	194
3h		2	84	200
3i		2	85	198
3j		2	84	liquid

Docking of Compound 3a with the DHFR Receptor



CONCLUSION

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the Present work we have docked the ligand, 3-mono substituted quinazolinone ($C_{14}H_{13}ON_2$) with the proteins that are used as the target for DHFR. The analysis of the docking result allowed us to know the efficiency of the natural bioactive compound to control the cancer. The docking study revealed the binding orientation of the natural ligand 3-monosubstituted Quinazolinone in the target protein's active site which resulted in inhibition of enzyme activity. The binding energy of the ligand-protein interactions also confirmed that the ligand tightly fit to the macromolecule, protein. From the results obtained, it will be essential to understand the important structural features required to enhance the inhibitory activities and further it will help to produce augmented inhibitory compound

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