Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Suppl 1, 2012 ISSN - 0974-2441

Research Article

DOCKING STUDIES OF NOVEL COUMARIN DERIVATIVES AS ARYLAMINE N-ACETYLTRANSFERACE 2 INHIBITORS

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Received: 18 October 2011, Revised and Accepted: 09 December 2011

ABSTRACT

Arylamine N-acetyltransferase 2 catalyze the metabolism of various aromatic amine drugs and carcinogens. It catalyzes the N- or O-acetylation of various arylamine and heterocyclic amine substrates and is able to bioactivate several known carcinogens. A series of coumarin derivatives were computationally designed and optimized with the AutoDock 4.0.1 to investigate the interactions between the target compounds and the amino acid residues of the NAT2. In this study, the docking studies were done using auto dock between coumarin derivatives and NAT 2 receptor. Among all the designed compounds 3, 6- dibutyl-7-hydroxy-4-oxo-2-chlrobenzyl -4H chromene -8- carbaldehyde (compound 5) shows more binding energy values (-9.08). These values suggested that the designed coumarin derivatives are excellent promoters of NAT2.

Key words: Colorectal cancer, N-acetyltransferase, Coumarin, Docking, Auto Dock.

INTRODUCTION

Cancer chemotherapy has been one of the major medical advances in the last few decades. However, the drugs used for this therapy have a narrow therapeutic index, and often the responses produced are only just palliative as well as unpredictable. In contrast, targeted therapy that has been introduced in recent years is directed against cancer-specific molecules and signaling pathways and thus has more limited nonspecific toxicities [1]. Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year [2].

Colorectal cancer is the fourth most common type of cancer and the second most common cause of cancer death. Fewer than 5% of colon cancers arise in the presence of a clear hereditary cancer condition; however, current estimates suggest that an additional 15–25% of colorectal cancers arise on the basis of unknown inherited factor (3). Colorectal cancer is a complex disease resulting from somatic genetic and epigenetic alterations, including locus-specific CpG island methylation and global DNA or LINE-1 hypomethylation (4) .Colorectal cancer occurs when some of the cells that line the colon or the rectum become abnormal and grow out of control. Being overweight or obese is an established risk factor for colorectal cancer, more so for men than for women. Approximately 10%–20% of colorectal tumors display microsatellite instability (MSI), defined as the expansion or contraction of small repeated sequences in the DNA of tumor tissue relative to nearby normal tissue (5).

Arylamine N-acetyltransferase 2 (NAT 2) metabolizes arylamines and hydrazines moieties found in many therapeutic drugs, chemicals and carcinogens. The gene encoding NAT2 is polymorphic, thus resulting in rapid or slow acetylator phenotypes. The acetylator status may, therefore, predispose drug-induced toxicities and cancer risks, such as bladder, colon and lung cancer. Indeed, some studies demonstrate a positive association between NAT2 rapid acetylator phenotype and colon cancer (6).

Two *N*-acetyltransferase isozymes, NAT1 and NAT2, are polymorphic and catalyze both *N*-acetylation (usually deactivation) and *O*-acetylation (usually activation) of aromatic and heterocyclic amine carcinogens. Epidemiological studies suggest that the NAT1 and NAT2 acetylation polymorphisms modify risk of developing urinary bladder, colorectal, breast, head and neck, lung and possibly prostate cancers. Associations between slow NAT2 acetylator genotypes and urinary bladder cancer and between rapid NAT2 acetylator genotypes and colorectal cancer are the most consistently reported. The individual risks associated with NAT1 and/or NAT2 acetylator genotypes are small, but they increase when considered in conjunction with other susceptibility genes and/or aromatic and heterocyclic amine carcinogen exposures. Because of the relatively high frequency of some NAT1 and NAT2 in the population, the attributable cancer risk may be high (7).

Dietary heterocyclic aromatic amines (HAAs) are members of a family of chemicals that comprise highly mutagenic compounds related to colon cancer. The polymorphic N-acetyltransferase 2 enzyme plays a key role in the transformation of HAAs to ultimate carcinogens. NAT2 enzyme activity is expressed in a genotype-dependent manner in colon epithelium. Therefore local activation of HAAs in colon, and hence increased risk to develop colon cancer, is likely to be related to high NAT2 enzyme activity. (8).

Coumarin (2*H*-pyran-2-one) and its derivatives are widely distributed in nature and exhibit a broad pharmacological profile (9). A number of natural and synthetic coumarin (2-oxo-2H-chromen) derivatives have been reported to exert antimicrobial (10, 11), analgesic- anti-inflammatory (12) and anticancer (13) activity.

Taking these points in to account several coumarin ligands having different substitutions were designed computationally. In this study, we designed some coumarin derivatives as targeted for colorectal cancer based on molecular docking between designed new inhibitors and NAT 2 using Auto dock.

MATERIALS AND METHODS

Computer –Aided Drug Design (CADD) is a specialized discipline that uses computational methods to simulate drug – receptor interactions. CADD methods are heavily dependent on bioinformatics tools, applications and databases (14). Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an enzyme receptor fit together and dock to each other well. The molecules binding to a receptor, inhibit its function, and thus act as drug. The collection of drug and receptor complex was identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations.

Auto Dock is an automatic docking tool. It is designed to predict how small molecules, such as substrates, bind to a receptor of known 3D structures. A graphical user interface called Auto Dock Tools or ADT was utilized to generate grids, calculate the dock score and evaluate the conformers (15). A total of 15 entries of NAT2 were selected from RCSB protein data bank, based on the presence of ligand, X-ray diffraction and 2.0-2.5 Aº resolution. Out of the entries, 2PFR was taken for docking analysis.

A comparative protein-ligand dock analysis was performed using 2PFR extracted from Protein Data Bank (PDB) (16) to evaluate the algorithm and scoring function efficiency between Auto Dock 4.0.1 and experimental activities. Figure 1shows structure of Arylamine N-acetyltransferase 2 protein (PDB ID: 2PFR). All these computationally designed molecules as well as the bound ligand of the protein 2PFR were docked by using the software Auto Dock and

the score values are predicted. The protein ligand interactions were also studied in web server. Based on the score values against the activity the molecules were represented as active, moderately active and inactive. All molecules were drawn using integrated Chem Draw tool). The possible binding sites of NAT 2 were searched using Q-site finder. Figure 2 shows binding sites of 2PFR from Q-Site finder. These include pockets located on protein surfaces and voids buried in the interior of proteins. Automated docking was used to locate the appropriate binding orientations and conformations of various inhibitors into the 2PFR binding pocket. To perform the task, the powerful genetic algorithm method implemented in the program Auto Dock 4.0.1 was employed.

All water molecules were removed from the original Protein Data Bank file. Polar hydrogen atoms and Kollman charges 18 were added. Grid maps were generated by Auto Grid program. Each grid was centred at the crystal structure of the corresponding 2PFR bound ligand. The grid dimensions were 80 Aº X 84 Aº X 88 Aº with points separated by 0.375 Aº.

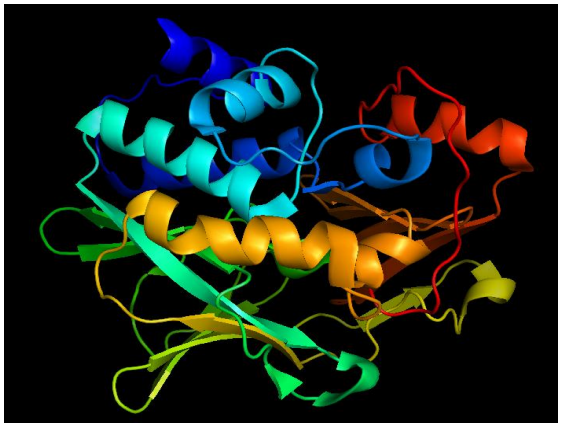
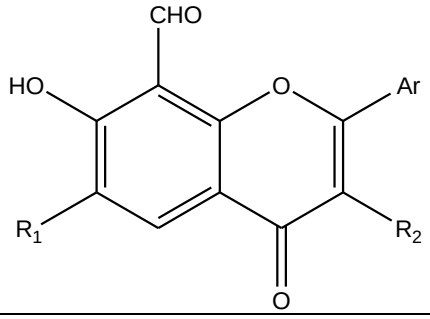


Fig 1: Structure of Arylamine N-acetyltransferase 2 protein (PDB ID: 2PFR)

Table 1: Docked scores of newly designed compounds



Compound	R_1	R_2	Ar	Binding energy (K Cal/mol)	No of H bonds	Interacting residues
1	$-C_4H_9$	$-C_4H_9$	C_6H_5	-8.57	1	LYS 272
2	$-C_4H_9$	$-C_4H_9$	$C_6H_4(p)NO_2$	-7.47	1	LYS 272
3	$-C_4H_9$	$-C_4H_9$	$C_6H_4(p)Cl$	-8.66	0	-
4	$-C_4H_9$	$-C_4H_9$	$C_6H_3(3,4)Di\text{-}Cl$	-8.85	2	GLY 11, LYS 272
5	$-C_4H_9$	$-C_4H_9$	$C_6H_4(o)Cl$	-9.08	2	GLY 11, LYS 272
6	$-C_4H_9$	$-C_4H_9$	$C_6H_4(m)Br$	-8.48	1	LYS 272
7	$-C_4H_9$	$-C_4H_9$	$C_6H_4(p)OCH_3$	-8.45	0	-
8	$-C_4H_9$	$-C_4H_9$	$C_6H_5CH_2$	-8.52	0	-
9	$-C_4H_9$	$-C_3H_7$	$C_6H_4(p)Cl$	-9.14	0	-
10	$-C_4H_9$	$-C_3H_7$	$C_6H_3(3,4)Di\text{-}Cl$	-8.97	1	LYS 272
				-8.62	1	LYS 272
Capecitabine						

RESULTS AND DISCUSSION

Docking results between Arylamine N-acetyltransferase 2 receptor and designed coumarin derivatives are reported in Table 1. Computational strategies for structure based drug discovery offer a valuble alternative to the costly and time consuming process of random screening. Auto Dock is employed to study the docking molecules within active site region of 2PFR. At the end of each run, docked orientations are saved and the resultant molecules are checked for geometry and number of hydrogen bonds. The newly designed molecules were docked against the protein 2PFR. The standard chemotherapeutic agent capecitabine on docking with 2PFR produce energy values of -8.62. When the designed coumarin derivatives were docked against the same receptor the energy values are greater than the standards for some derivatives. Compound 5 shows energy values of -9.08. It was observed that the compound 5 containing chloro group at 3rd and 4th position of benzene is showing better binding nature, which resulted in a decrease in the energy value.This particular compound showed a decreased in energy values which means it was more compatible with the receptor than the standard and other designed coumarin derivatives. Figure 3 shows the interaction mode of compound 10 with 2PFR receptor site.

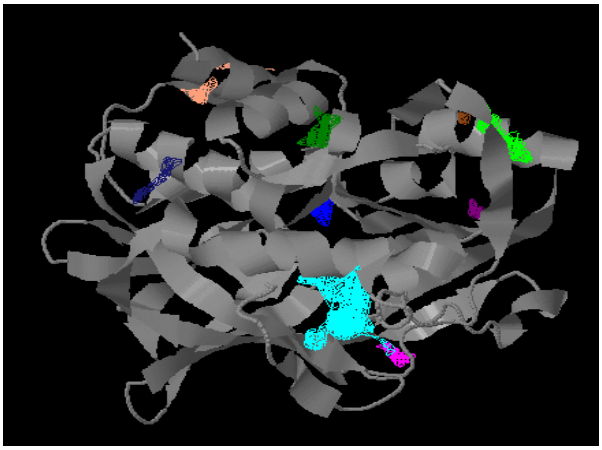


Fig 2: Binding sites of 2PFR from Q-Site finder

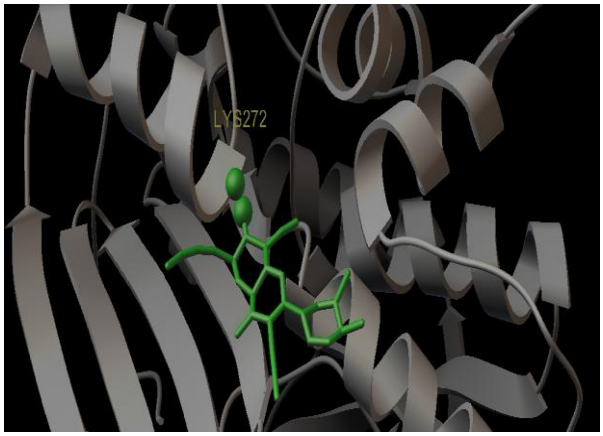


Fig 3: Binding mode of Compound 10 in the active site of 2PFR along with interacting aminoacid

CONCLUSION

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. The present study concludes that 3, 6- dibutyl-7-hydroxy-4-Oxo-2-chlro benzyl -4H chromene -8- carbaldehyde (compound 5) is found to be most active against Arylamine N-acetyltransferase 2. Coupling of these compounds with other known antineoplastic natural products for the finding of more promising anticolorectal cancer compounds are being analysed.

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