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Novel fluorescent antifolates that target folate receptors α and β: Molecular dynamics and density functional theory study

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ABSTRACT

Nine novel fluorescent antifolates, 1–9, were designed and docked with FRα and FRβ. The binding energies of the bound complexes were determined by molecular docking and MM-PBSA studies. The structural properties of the complexes FR-FOL, FR-7, FR-8 and FR-9 were analyzed in detail via molecular docking and molecular dynamics studies. We further calculated the root mean square displacement and root mean square fluctuation of the bound complexes using molecular dynamics simulations. Since compounds 7, 8 and 9 are promising candidate in distinguishing FRα from FRβ, the hydrogen bond properties of complexes FRα-7, FRα-8 and FRα-9 were studied by a dispersion complemented density functional tight-binding method. The purpose of this study is to provide a rationale for the design of novel fluorescent antifolates targeted with FRα and FRβ.

1. Introduction

Folate receptors (FRs) [1,2] are glycosylphosphatidylinositol-linked proteins. They are inaccessible in normal tissues and expressed on plasma membranes which are most commonly presented on the apical surface of polarized epithelial cells. FRs have four isoforms: FRα, FRβ, FRγ and FRδ. FRα is expressed at high levels in epithelial-derived cancers, such as ovarian, endometrial cancers and non-small cell lung cancers; FRβ is expressed in hematologic malignancies such as acute myeloid leukemias [3], tumor associated macrophages, and sites of inflammation [4]. Therefore, FRs have attracted a great deal of attention in the field of drug delivery [5–18]. For example, Tang et al. [19] optimized the density of magnet-guided iron oxide nanoparticles combined with folic acid as a drug delivery system. Chen et al. [20] reported a template of human FRα bound with folic acid and found that the pteroate moiety of folic acid is docked deep inside the negatively charged pocket of FR. Studies focusing on FRs have been strongly propelled by the identification of 3D structures of FRs [20,21].

Classic antifolates, such as methotrexate (MTX), pralatrexate (PDX), pemetrexed (PMX), and others, serve key roles in the therapy of cancer. In spite of demonstrated clinical efficacy, none of these compounds are selectively targeted to the tumor and inflammation diseased tissue; as they are substrates for both FRα and FRβ, they cause toxic effects toward normal tissues [22–24]. Since folic acid and classical antifolates cannot be distinguished by FRα and FRβ, novel specific antifolates targeted with FRα and FRβ must be designed. For example, Gangjee et al. [25–27] studied a series of novel 6-substituted straight side chain pyrrolo [2,3-d]pyrimidines antifolates and determined that a side chain benzoyl group is not essential for tumor-selective drug uptake by FRα. They also demonstrated the importance of the α- and γ-carboxylic acid groups, the length of the amino acid, and the conformation of the side chain for transporter binding and biological activity of 6-substituted pyrrolo [2,3-d] pyrimidine thienoyl antifolates.

Fluorescent dyes, which can be used as probes for detection and treatment tools in biological systems, have attracted a great deal of attention [28–33]. For example, Nygren et al. [34] studied the interactions between the fluorescent dye thiazole orange (TO) and DNA, and found that the TO had enhanced fluorescence when bound to DNA. There have been numerous studies on optical imaging-based FR expression studies. For example, Qiao et al. [35] reported a nano conjugate, RhB-PVDMA-FA, with fluorescent properties and indicated that they have good biocompatibility and intracellular dispersion.

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Molecular simulations at the atomic level, could reveal details unable to be observed experimentally, which are useful in the design of targeted drug delivery system. They have been widely used by many researchers. In this report, we designed nine novel fluorescent antifolate (NFA) molecules and studied their interactions with FRs to improve the design process for future generations of targeted drug delivery systems. To understand the binding mechanisms of FR-NFA complexes, we generated the 3D model of FR-NFA using molecular docking simulations followed by molecular dynamics (MD) and density functional tight-binding method (DFTB) analyses. This research will facilitate further design of novel fluorescent antifolate targeted with FRs.

2. Models and methods

2.1. Model preparation

Targeted antifolates with fluorescent dye replacements of the phenyl ring of folic acid by imidazole (1), oxazole (2), thiazole (3), indole (4), benzofuran (5) and benzothiophene (6), and the pyrazine by imidazole (7), oxazole (8) and thiazole (9), were tested for selective cellular uptake by folate receptor α (FRα) and β (FRβ). The single bond of the -CH_2-NH- bridge of folic acid was substituted by the double bond to increase the fluorescence of the antifolate. Fig. 1 demonstrates the structures of the nine NFAs. The crystal structures of FRα and FRβ were obtained from the Protein Data Bank (PDB). The PDB IDs are 4LRH [20] and 4KMZ [21] for FRα and FRβ, respectively.

2.2. Molecular docking of NFA with FR

The molecular docking studies of the nine novel compounds with FRs were carried out using the AutoDock 4.2 Release 4.2.6 [36]. The solvent molecules were removed from the folate receptor to obtain the docking grid, and the ligands were set to be flexible. We used the Lamarckian genetic algorithm to find the favorable bonding conformations of the ligand at the negatively charged pocket of the folate receptor. The best docking poses which have best binding energies were considered for building FR-NFA complex and further studied.

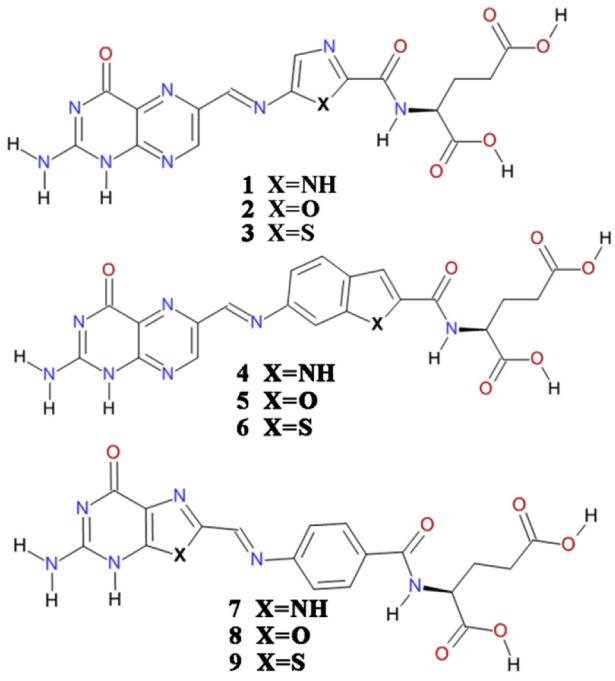

Fig. 1. Structures of novel fluorescent antifolates 1 to 9.

2.3. MD simulation of the complex

The MD simulation was carried out using GROMACS software version 5.1.4 [37]. We selected the best docking posed complexes by molecular docking studies for all the FR-FOL and FR-NFA complexes to further MD simulation. In order to be more precise, the FR-NFA complex was embedded in water box with the size of $7.49 \times 7.49 \times 7.49\ nm^3$ and $7.67 \times 7.67 \times 7.67\ nm^3$ for FRα and FRβ complexes, respectively. The box size was used to ensure that the distance between the box faces and the molecular surface is at least 1.0 nm, and about 12570 and 13390 water molecules were used for FRα and FRβ complexes, respectively. In this study, the AMBER99SB-ILDN [38] force field was used for proteins, and TIP3P for water. For NFAs, the general Amber force field (GAFF) was used [39]. The partial charges of NFAs were calculated through the restrained electrostatic potential (RESP) fitting procedure using HF/6-31G(d) method. The AMBER format files of ligands were converted to the GROMACS format by the antechamber program of AmberTools. A steepest descent algorithm with a tolerance of $100\ kJ\ mol^{-1}\ nm^{-1}$ was used to minimize the system, and the step size of 2 fs (fs) was used for the MD runs. The FR-NFA complexes were equilibrated for 0.1 ns (ns) and 0.2 ns (ns) under the constant volume (NVT) and constant pressure (NPT) conditions, respectively. The pressure and temperature for all the systems were set to be 1 bar and 300 K, respectively, with semmisotropic coupling to a Parrinello-Rahman barostat with a time constant of 2ps. The neighbor list was updated with a grid search using the switching algorithm, with a van-der-Waals cutoff of 1.0 nm and short-range neighbor list and electrostatic cutoff of 1.0 nm. After the two equilibration phases, final production MD simulation of 50 ns was carried out for the FR-NFA system. The binding free energies were obtained by the MM/PBSA calculation. Further, the hydrogen bond, Root mean square displacement (RMSD) and Root Mean Square Fluctuation (RMSF) were analyzed.

2.4. DFTB simulation of the complex

Because of the ring structures in the compound, weak interactions play significant roles in stabilizing the structures of compounds bound with the FRs. Weak interaction remains a challenging issue for the force field studies. In this research, we further studied the geometry structure of the bound complex by an empirical London dispersion energy term complemented self-consistent charge density functional tight-binding method (acronym SCC-DFTB-D) [40–45].

The London dispersion energy term is defined as

$$E_d = -\sum_{\alpha\beta} f\left(R_{\alpha\beta}\right) C_6^{\alpha\beta}\left(R_{\alpha\beta}\right)^{-6}$$

where α and β refer to the atoms on which the wavefunction and potential are centered, $R_{\alpha\beta}$ is the distance between the atoms, and $f(R)$ is a damping function.

The coefficient C_6 is calculated by

$$C_6^a = 0.75\sqrt{N_a p_a^3}$$

where N_a is the Slater-Kirkwood effective number of electrons and p_a is the polarizability of atom α.

A comprehensive description of the method can be found in the