A molecular docking study of N-ferrocenylmethylnitroanilines as potential anticancer drugs

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ABSTRACT In the present study, the interaction of the protein structure of Escherichia coli DNA Gyrase-A (EcGyr-A) extracted from protein data bank (PDB Code: 1AB4) with ligands N-ferrocenylmethyl-2-nitroaniline (2FMNA), N-ferrocenylmethyl-3-nitroaniline (3FMNA) and N-ferrocenylmethyl-4-nitroaniline (4FMNA) were investigated by performing docking studies using the Molegro Virtual Docker (MVD) software. The results obtained showed that the best poses which is derived from MolDock score for Escherichia coli DNA Gyrase-A were respectively equal to -92.0111, -96.0866 and -95.6808 with reranking score equal to -40.9575, -73.4476 and -73.6423. Calculations revealed that 3FMNA react strongly with EcGyr-A followed by 4-FMNA and 2-FMNA.

1. INTRODUCTION

Cancer is a genetic disease caused by changes to genes that control the way the cells function, especially how they grow and divide. It can be inherited from parents or arise during a person's lifetime as a result of errors that occur as cells divide or because of damage to DNA caused by certain environmental exposures. Cancer is the second leading cause of death worldwide, with 8.2 million deaths in 2012, exceeded only by heart disease. An estimated 14.1 million new cancer cases occurred in 2012 [1].

Escherichia coli DNA Gyrase-A is a type II DNA topoisomerase from bacteria that introduces supercoils into DNA. It catalyses the breakage of a DNA duplex (the G segment), the passage of another segment (the T segment) through the break, and then the reunification of the break, this activity involves the opening and dosing of a series of molecular 'gates' which is coupled to ATP hydrolysis [2].

Ferrocene derivatives has drawn interest due to their stable chemical structure and large variety of biological properties [3-14], ferrocenes incorporated heterocyclic ring systems have a huge potential to become successful drug candidates which was proven in the recent past few years [15-16]. Ferrocenylmethylnitroanilines exhibit diverse biological activities such as antioxidant activities [17].

Molecular Docking is the computational modelling of the structure of complexes formed by two or more interacting molecules. It is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule [18]. It can be applied to study the biological interactions between two macromolecules (protein/protein or DNA/protein) or between ligand and the receptors of macromolecules. In general docking only produces plausible candidate structures [19, 20]. Docking can be derived into three types: (a) rigid body docking, where both the receptor and the ligand are treated as rigid, (b) flexible ligand docking, where the receptor is held rigid, but the ligand is treated as flexible; and (c) flexible docking, where both receptor and ligand are considered as flexible [20]. In this study the rigid receptor/flexible ligand model was used.

2. MATERIAL AND METHODS

The molecular docking study of N-ferrocenylmethylnitroanilines with well established structure of EcGyr-A was done using MolDock docking engine of Molegro Virtual Docker, version 5.5.0

(MVD) software from CLC Bio (<http://www.clcbio.com/products/molegro>, Aarhus, Denmark) [21]. Calculations were carried out using an Intel ® Core™ i3 3217U CPU, 1.80 GHz dual processing machine.

3. RESULTS AND DISCUSSION

In our previous research, we have described the synthetic routes and full characterisation of compounds 2a, 2b and 2c as illustrated in Scheme 1 [22, 23].

Scheme 1. Synthesis of N-ferrocenylmethylnitroanilines 2a: X = 2-NO₂, 2b: X = 3-NO₂, 2c: X = 4-NO₂

3.1. Docking study

Docking of N-ferrocenylmethylnitroanilines with Escherichia coli DNA Gyrase-A proceeds in three steps; the first is receptor and ligand preparation; second is retrieval, preparation and validation of 3D X-ray crystal structure of EcGyr-A and third is identification of interactions. All docking simulation were carried out using the grid-based Mol Dock score (GRID) function with a grid resolution of 0.30 Å°. The Mol Dock optimization search algorithm with a maximum of 10 runs was used through the calculations, with all other parameters kept as defaults. All the poses were examined manually, and the best pose was selected on the basis of the Mol Dock score and re-rank score.

3.2. Preparation of Receptor

The protein structure of Escherichia coli DNA Gyrase-A (EcGyr-A) (PDB Code: 1AB4) was imported in MVD. The model on figure 1 was used as the best 3D backbone visualization structure for DNA Gyrase-A.

Fig.1. Imported structure of protein of Escherichia coli DNA Gyrase-A (EcGyr-A) chain as viewed in visual molecular dynamics

3.3.Optimisation and preparation of ligands

Initial calculations were optimized using HyperChem 8.03 software [24]. The geometries of N-ferrocenylmethylnitroanilines were first fully optimized by molecular mechanics, with MM+ force-field ($\text{rms} = 0001 \text{ Kcal/\AA}$). Further, geometries were fully re-optimized by using RM1 method, a parallel study has been made using Gaussian 09 program package, at various computational levels, HF/6-31++G(d,p), MP2/6-31++G(d,p) and B3LYP/6-31++G(d,p). Finally the ligand structure saved in MOL format and was imported in MVD. Figures 4, 7 and 10 show optimised structures of studied ligands.

3.4.Finding ligand binding sites

To obtain better potential binding sites in the protein of Escherichia coli DNA Gyrase-A, maximum of five cavities were detected using parameters such as molecular surface (expanded van der Waals), maximum number of cavities ($n = 5$), minimum cavity volume (10), the probe size (1.20), the maximum amount of ray checks ($n = 16$), minimum number of ray hits ($n = 12$), and grid resolution (0.80). Figure 2 shows the five detected cavities for prepared protein structure, cavities are collared in green.

Fig.2. Detected five cavities in protein of Escherichia coli DNA Gyrase-A 3D structure

Among the five detected cavities, cavity number 1 of volume equal to $114.176 \text{ \AA}^3$ was selected for further studies. The chosen cavity was further refined using side chain minimization by selection of add visible option set at a maximum of 10,000 steps per residue and at a maximum of 10,000 global steps. The initial energy was set at 11911.9, whereas the energy after side chain minimization was set at 9268.35. The side chain flexibility was set by selecting the ‘‘add visible’’ option. The same was selected during docking, and the remaining parameters were kept as fixed variables. Figure 3 shows the predicted pending site along with the chosen cavity.

Fig.3. Predict pending site

N-ferrocenylmethyl-2-nitroaniline

The best pose revealed the docking score -87.44 and form three interactions as shown by green dotted lines in figure 5, due to two hydrogen bonds between oxygen of the nitro group with the residues Lys 270, and Gly 300 of distances 2.82 Å and 3.21 Å respectively. Second nitrogen of the nitro group of N-ferrocenylmethyl-2-nitroaniline also showed strong hydrogen bonding with protein residue Lys 270 of distance 2.21 Å.

Fig.4. 3D conformation of ligand 2FMNA (ORTEP View 03, V1.08)

Fig.5. Interaction between active site residues of EcGyr-A and ligand 2FMNA

Binding parameters of ligand 2FMNA with active site residues of EcGyr-A are shown in table 1, based on energy values of the bond residue-ligand it can be concluded that the ligand 2FMNA is strongly attached to the residue Lys 270.

Table 1. Interaction of 2FMNA to EcGyr-A

Residue	Interaction	Distance (Å)	Energy (kcal/mol)
Lys 270	N-H	2.82	-1.75550
Gly 300	H-O	3.21	-1.20533
Lys 270	O-H	2.21	-1.20588

N-ferrocenylmethyl-3-nitroaniline

In a same manner as for ligand 2FMNA, the best pose revealed the docking score -96.08 and form two interactions as shown by green dotted lines (Figure 8), due to two hydrogen bonds between H of NH group with Gly 300, and the nitrogen group of the nitro atom with Lys 270 of distances 2.73 Å and 2.60 Å respectively. One more weak hydrogen bonding was also detected between the nitrogen of the nitro group and the residue Lys 270 of distance 3.12 Å.

Fig.7. 3D conformation of ligand **3FMNA** (ORTEP View 03, V1.08) **Fig.8.** Interaction between active site residues of EcGyr-A and ligand **3FMNA**

The ligand 3FMNA binds very strongly with the active site residue Lys 270 of EcGyr-A as it can be seen from energy values in table 2,

Table 2. Interaction of 3FMNA to EcGyr-A

Residue	Interaction	Distance (Å)	Energy
Lys270	N-H	2.60	-2.49101
Gly300	H-N	2.73	-2.50000
Lys270	N-H	3.12	-2.38193

N-ferrocenylmethyl-4-nitroaniline

The best pose revealed the docking score -95.6808 and form two interactions as shown by green dotted lines (Figure 11), due to two hydrogen bonds between hydrogen of amino group of the ligand 4FMNA and the oxygen of the protein residue Gly 300, the second hydrogen bond is between the nitrogen of the nitro group with Lys 270. The distance of the two bonds is 3.16 and 2.86 Å respectively.

Fig.10. 3D conformation of ligand 4FMNA (ORTEP View 03, V1.08)

Fig.11. Interaction between active site residues of EcGyr-A and ligand 4FMNA

Interaction parameters of ligand 4FMNA and active site residues of EcGyr-A are summarised in table 3.

Table 3. Interaction of 4FMNA to EcGyr-A

Residue	Interaction	Distance (Å°)	Energy
Lys270	N-H	2.86	-1.60469
Gly300	O-H	3.16	-2.10684

4. CONCLUSION

A series of three N-ferrocenylmethylnitroanilines have been docked successfully, the docking results of N-ferrocenylmethylnitroanilines ligands with Escherichia coli DNA Gyrase-A indicated the existence of strong interactions between ligands and DNA Gyrase-A. Among the studied ligands, 3FMNA exhibited the most favourable binding to the receptor EcGyr-A followed by 4FMNA and 2FMNA.

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6. REFERENCE

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