Molecular docking studies on thirteen fluoroquinolines with human topoisomerase II a and b

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Abstract DNA relaxation is an important step in DNA replication. DNA topoisomerases play a major role in DNA relaxation. Hence these enzymes are important targets for cancer drugs. DNA topoisomerase inhibitors bind to the transient enzyme–DNA complex and inhibit DNA replication. Various inhibitors of topoisomerase I and II are prescribed as drugs. Topoisomerase II is considered an important target for the development of anticancer drugs. In this study we have demonstrated molecular docking of thirteen fluoroquinolines with human DNA topoisomerase II alpha (a) and beta (b). Fluoroquinolines are broad spectrum antibacterial antibiotics and it is highly effective against various bacterial infections. Some of the fluoroquinolines like moxifloxacin exert antifungal as well as anti-cancer activity. It forms complexes with topoisomerase II a and are responsible for stoppage DNA replication. Molecular docking studies showed that fluoroquinolines has shown formation of hydrogen bond and good binding affinity with human Topo2a and Topo2b. Hence FQs may inhibit the activity of enzyme topoisomerase by binding at its active site. Ofloxacin, sparfloxacin, ciprofloxacin and moxifloxacin are predicted to be the most potent inhibitors among the thirteen FQs docked. GLN773, ASN770, LYS723 and TRP931 amino acid residues of Topo2a are involved in binding with FQs while ASP479, SER480, ARG820, ARG503, LYS456 and GLN778 amino acid residues of Topo2b are involved in binding with FQs. Our in silico study suggests that fluoroquinolines could be repositioned as DNA topoisomerase II inhibitors hence can be used as anticancer drugs. In vitro and in vivo experiments need to be done to confirm their efficacy.

Keywords Human DNA topoisomerase II · Fluoroquinolines · Anticancer · Molecular docking · In silico · Drug repositioning

Introduction

DNA topological states depend upon the activity of topoisomerase I and II (Wang et al. 2002). Their activity is important for cellular activities like replication, recombination, transcription and DNA repair (Bertozzi et al. 2011, 2014; Marinello et al. 2013). Topoisomerase II is present in two isoforms Top2a and Topo2b in mammals (Chung et al. 1989; Drake et al. 1987). Top2a is actively involved in replicating cells while Top2b is constitutively expressed in specific cellular processes like neural development (Yang et al. 2000; Tsutsui et al. 2001). Top2a introduce double-strand nick into DNA during DNA replication. Top2 enzymes are important targets for several antitumour drugs (Nitiss 2009). Anti-cancer drugs inhibit the activity of DNA Topo2 enzyme which may responsible for cell death (Pommier 2013; Azarova et al. 2007). Although these drugs are having some side effects but still they are prescribed as anti-cancer drugs (Nitiss 2009). These drugs may act on Topo2b in normal cells in human body (Azarova et al. 2007; Bromberg et al. 2003; Zhang et al. 2012). Therefore screening of Topo2a inhibitors is an important task for drug developers. The exploration of differences in structure and function between the two Topo2 isoforms is...
Fig. 1 Binding of ciprofloxacin with human topoisomerase II a

Fig. 2 Binding of enoxacin with human topoisomerase II a

Fig. 3 Binding of finafloxacin with human topoisomerase II a

Fig. 4 Binding of flumequine with human topoisomerase II a
important, and the identification Topo2a inhibitors from available and already approved drugs may be a beneficial strategy in the search for new cancer drugs with improved clinical safety. The combination of structure- and ligand-based computational methods has been successfully applied in many drug design projects (Drwal et al. 2013). Pharmacophores, representing the spatial arrangement of chemical features necessary for binding of a small molecule to its biological target, can be developed in different ways, depending on the available information.
The broad spectrum antibacterial antibiotics, fluoroquinolines are highly effective against various bacterial infections. Quinolone-induced inactivation of Topo II at high concentrations is proposed to involve direct binding of quinolones to DNA (Bromberg et al. 2003). Moxifloxacin inhibited purified human topoisomerase II and acted synergistically with VP-16 at the purified enzyme level and enhanced its anti-proliferative effects in THP-1 and Jurkat cells (Fabian et al. 2006). In earlier studies we have reported that Moxifloxacin can inhibit growth and virulence factors of Candida albicans. Molecular docking of Candida DNA topoisomerase II with FQs was showed...
good binding affinity (Jadhav et al. 2017). In this study we are reporting molecular interactions of thirteen fluoroquinolines with human DNA topoisomerase II α and β by using molecular docking studies.

### Materials and methods

#### Protein structure preparation

Human DNA topoisomerase II alpha (PDB ID: 4fm9) and beta (PDB ID: 3QX3) were retrieved from PDB (http://www.rcsb.org/pdb/). Autodock Tools version 1.5.6rc2 was used to generate the docking input files. Using AutoDock Tools (ADT) 1.5.6rc2 nonpolar hydrogens were merged, Gasteiger charges were assigned saved in PDBQT file format. Grid and docking parameter files were prepared using ADT and molecular docking studies were performed with AutoDock 1.5.6rc2 considering all the bonds of ligands as rotatable and macromolecules as rigid (Morris et al. 1998). Grid box size of $60 \times 60 \times 60$ Å with 0.375 Å spacing centered at the site of DNA cleavage of topo–DNA complexes was selected. Hetero-atoms and drug molecules were removed, Gasteiger charges were assigned.

#### Table 1 Molecular interactions of fluoroquinolines with human DNA topoisomerase II (4fm9)

| Ligand    | Run no. | Interacting residue in human Topo II α | Interacting atoms (amino acid……ligand) | H bonds formed | Binding energy | Electrostatic energy |
|-----------|---------|----------------------------------------|----------------------------------------|----------------|----------------|---------------------|
| Ciprofloxacin | 51      | GLN773                                 | HE22…..O3                             | 1              | −6.54          | −0.07               |
| Enoxacin  | 37      | GLN773                                 | HE22…..O4                             | 1              | −6.06          | 0.05                |
| Finafloxacin | 43     | ASN770                                 | OD1…..O4                             | 1              | −6.41          | −0.02               |
| Flumequine | 4       | GLN726                                 | HE21…..O2                             | 3              | −6.15          | −0.55               |
| Gatifloxacin | 58     | GLN773                                 | HE22…..O4                             | 1              | −6.86          | −0.04               |
| Levofloxacin | 46     | TRP931                                 | HE1…..O3                             | 2              | −8.06          | −0.38               |
| Lomefloxacin | 1      | GLN773                                 | HE22…..O2                             | 1              | −8.64          | −0.0                |
| Moxifloxacin | 12     | GLN773                                 | HE22…..O2                             | 1              | −7.7           | −0.09               |
| Norfloxacin | 22      | GLN773                                 | HE22…..O2                             | 2              | −6.6           | −0.01               |
| Ofloxacin  | 48      | GLN773                                 | HE22…..O1                             | 2              | −8.05          | −0.36               |
| Prulifloxacin | 22     | TRP931                                 | HE1…..O3                             | 1              | −8.0           | 0.3                 |
| Sarafloxacin | 38     | GLN773                                 | HE22…..O2                             | 1              | −7.23          | 0.01                |
| Sparafloxacin | 49    | GLN773                                 | HE22…..O1                             | 2              | −8.17          | −0.05               |
| Etoposide  | 56      | SER800                                 | O…..H26                              | 3              | −7.9           | −0.15               |
|           |         | GLY796                                 | O…..H20                              |                |                |                     |
|           |         | GLN773                                 | HE22…..O8                             |                |                |                     |

Fig. 13 Binding of sparafloxacin with human topoisomerase II α
Fig. 14 Binding of ciprofloxacin with human topoisomerase II b

Fig. 15 Binding of enoxacin with human topoisomerase II b

Fig. 16 Binding of finafloxacin with human topoisomerase II b

Fig. 17 Binding of flumeoxacin with human topoisomerase II b
assigned and both the macromolecules were saved in PDBQT file format using Autodock tool (Kumar and Bora 2014).

**Ligand structure preparation**

The structures Fluoroquinolines were retrieved from Pubchem (http://pubchem.ncbi.nlm.nih.gov/) in SDF format.
Fig. 22 Binding of norfloxacin with human topoisomerase II b

Fig. 23 Binding of prulifloxacin with human topoisomerase II b

Fig. 24 Binding of ofloxacin with human topoisomerase II b

Fig. 25 Binding of sarafloxacin with human topoisomerase II b
Structure followed by 2D cleaning, 3D optimization and viewing is done by using MarwinView and saved in Mol2 file format. Mol2 files were then converted to PDBQT file format by in Autodock Tools version 1.5.6rc2 (Kumar and Bora 2014).

**Molecular docking**

Molecular docking study of Fluoroquinolines against Human Topoisomerase II was done using AutoDock1.5.6rc2 suite as molecular-docking tool (Kumar and Bora 2014; Morris et al. 1998). Empirical-free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy evaluations, a mutation rate of 0.02 and a crossover rate of 0.80 were used. AutoDock1.5.6rc2 tools generated sixty possible binding conformations, i.e. sixty runs for each docking was done using genetic algorithm (GALS) searches. Autogrid was used to obtain pre calculated grid maps (Kumar and Bora 2014).

![Fig. 26 Binding of sparafloxacin with human topoisomerase II b](image)

**Table 2 Molecular interactions of fluoroquinolines with human DNA topoisomerase II b (3QX3)**

| Ligand      | Run no. | Interacting residue in human Topo II b | Interacting atoms (amino acid........ligand) | H bonds formed | Binding energy | Electrostatic energy |
|-------------|---------|---------------------------------------|---------------------------------------------|----------------|----------------|---------------------|
| Ciprofloxacin | 54      | ASP479                                | HN......O1                                  | 1              | -9.62          | 0.11                |
| Enoxacin    | 32      | ASP479                                | HN......O1                                  | 2              | -9.96          | 0.1                 |
|             |         | SER480                                | HN......O2                                  |                |                |                     |
| Finafloxacin| 44      | ARG820                                | HE......O4                                  | 1              | -8.92          | -0.15               |
| Flumequine  | 41      | ARG820                                | HH12......O3                                | 2              | -8.79          | -0.76               |
|             |         | ARG820                                | HH22......O3                                |                |                |                     |
| Gatifloxacin| 15      | ARG503                                | HH12......O3                                | 1              | -10.73         | -0.3                |
| Levofloxacin| 59      | ASP479                                | HN......O2                                  | 2              | -11.78         | -0.18               |
|             |         | SER480                                | HN......O3                                  |                |                |                     |
| Lomefloxacin| 54      | ASP479                                | HN......O1                                  | 1              | -10.35         | -0.1                |
|             |         | SER80                                 | HN......O2                                  | 1              |                |                     |
| Moxifloxacin| 17      | LYS456                                | HZ1......O2                                 | 1              | -9.95          | -0.24               |
| Norfloxacin | 29      | ASP479                                | HN......O1                                  | 2              | -10.43         | -0.16               |
|             |         | SER480                                | HN......O2                                  |                |                |                     |
| Ofloxacin   | 16      | ASP479                                | HN......O2                                  | 2              | -11.8          | 0.18                |
|             |         | SER480                                | HN......O3                                  |                |                |                     |
| Prulifloxacin| 47     | SER480                                | HN......O6                                  | 1              | -10.77         | 0.07                |
| Sarafloxacin| 56      | ASP479                                | HN......O2                                  | 1              | -10.78         | 0.01                |
| Sparafloxacin| 39     | GLN778                                | HE22......O3                                | 2              | -11.43         | -0.83               |
|             |         | ARG820                                | HH12......O2                                |                |                |                     |
| Etoposide   | 37      | DG10                                  | H19......O2                                 | 1              | -9.38          | -0.51               |
|             |         | DA12                                  | H12......O11                                |                |                |                     |
|             |         | DG10                                  | H10......O5                                 |                |                |                     |
|             |         | DC11                                  | H11......O5,02                              |                |                |                     |
Molecular docking analysis

After completion of docking most suitable conformation was chosen to prepare Ligand–protein complex preparation using AutoDock 1.5.6rc2. Docked structures saved in the PDBQT format were analyzed for the amino acids involved in the ligand binding sites of the enzyme along with the type of interactions like hydrogen bonding etc. involved in the docking. This was performed using the Discovery Studio 4.1 Visualizer (http://accelrys.com/products/discovery-studio). 13 Docked structures showing the amino acid residues involved in the docking as well as the interactions between the residues and ligand were image captured using image save option in Discovery Studio 4.1 Visualizer (Kumar and Bora 2014).

Results

Fluoroquinolines docked with human DNA topoisomerase II alpha

Molecular docking of thirteen fluoroquinolines with human topoisomerase II a (4fm9) showed that each FQs formed hydrogen bond with the active site of topoisomerase II (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13). Ciprofloxacin and enoxacin were found to bind to human topoisomerase II at GLN773 with binding energies $-6.54$ and $-6.06$ kcal/mol. Similarly gatifloxacin, lomefloxacin, moxifloxacin, norfloxacin and sarafloxacin formed hydrogen bonds with GLN773 with binding energies $-6.86$, $-6.84$, $-7.7$, $-6.6$, $-7.23$ kcal/mol respectively (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13).

Ciprofloxacin exhibited binding at the active site of human topoisomerase II b at ASP479 amino acid residue with $-9.62$ and $-10.78$ kcal/mol binding affinities. Finafloxacin and flumequine formed hydrogen bond with ARG820 amino acid residue with binding energies $-8.92$ and $-8.79$ kcal/mol respectively (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13).

Gatifloxacin and moxifloxacin formed H bond with ARG503 and LYS 456 amino acid residue and binding energies were $-10.73$ and $-9.95$ kcal/mol respectively. Prulifloxacin was formed H and with SER480 residue and binding energy was $-10.77$ kcal/mol while sparafloxacin bound at GLN778 and ARG820 of Topo2b with $-11.43$ kcal/mol binding score (Table 1).

Fluoroquinolines showed efficient binding with human DNA topoisomerase II beta

Molecular docking of thirteen fluoroquinolines with human topoisomerase II b (3Q3X) revealed that there was significant docking between ligand and active site of topoisomerase II b protein (Figs. 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26). Enoxacin and levofloxacin were found to bind with human topoisomerase II b at ASP479 and SER480 residues with binding energy $-9.96$ and $-11.78$ kcal/mol. Similarly norfloxacin, ofloxacin, lomefloxacin formed hydrogen bonds with ASP479 and SER480 with binding energies $-10.43$, $-11.8$ and $-10.35$ kcal/mol respectively (Figs. 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26).

Ciprofloxacin and sarafloxacin exhibited binding at the active site of human Topoisomerase II b at ASP479 amino acid residue with $-9.62$ and $-10.78$ kcal/mol binding affinities. Finafloxacin and flumequine were observed to bind with ARG820 residue and binding energy was $-8.92$ and $-8.79$ kcal/mol (Figs. 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26).

Gatifloxacin and moxifloxacin docked with ARG503 and LYS 456 amino acid residue and binding free energies were $-10.73$ and $-9.95$ kcal/mol respectively. Prulifloxacin was formed H and at SER480 residue and $-10.77$ kcal/mol binding energy while sparafloxacin binds at GLN778 and ARG820 of Topo2b with $-11.43$ kcal/mol binding score (Table 2).
Discussion

Human topo2 is the target of several anticancer agents (Nitiss 2009) which includes doxorubicin, amascrine, mitoxantrone and etoposide (Shen et al. 1989). Our molecular docking studies revealed that fluoroquinoline antibiotics are showing molecular interactions with human topoisomerase II (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26).

FQs are the broad spectrum antibiotics and inhibit the activity of topoisomerase II (Shen et al. 1989; Aldred et al. 2014). Fluoroquinolones and several classes of anti-cancer agents affect type II a topoisomerase activity and function (Aldred et al. 2014; Chen et al. 2013; Chan et al. 2013; Robinson et al. 1991).

Docking of Etoposide showed interactions with GLN778, SER800 and GLY796 residues in Top2a and DG10, DG11, DG12 in Topo2b complex (Figs. 27, 28). In this study we found that FQs majorly formed hydrogen bond with GLN773, ASN770, LYS723 and TRP931 in human DNA Topo2a and ASP479, SER480, ARG820, ARG503, LYS456 and GLN778 in binding pocket of human DNA Topo2b. Out of thirteen FQs sparafloxacin has shown lowest binding energy −8.17 kcal/mol with Topo2a. Ofloxacin showed binding energy of −11.8 kcal/mol with Topo2b. Anticancer activity of norfloxacin, ofloxacin, prulifloxacin, sarafloxacin, flumequine and flumequine are not reported. These FQs are showing good binding score in molecular docking studies with Topo2 enzyme and there is need for in vitro and in vivo studies of these FQs. There is a possibility for repositioning of these antibiotics as anticancer agents.

Conclusion

In this study we have explored topoisomerase II as an effective target for FQs by in silico studies. All thirteen FQs have shown significant binding affinity with both human topoisomerase II a and b. FQs formed significant molecular interactions with topoisomerase II at active sites. FQs may form a complex with DNA and topoisomerase II, and cause inhibition of the activity of Topoisomerase leading to the inhibition of growth. As FQs are showing promising anticancer activity against various cancer cell lines, in vitro and in vivo experiments are necessary to put forward therapeutic repositioning of FQs as anticancer agents.

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