Design, synthesis, *in-silico* study and anticancer potential of novel *n*-4-piperazinyl-ciprofloxacin-aniline hybrids

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Abstract: Synthesis and characterization of novel structural hybrids of ciprofloxacin linked with a variety of anilides have been described in this paper. Antitumor activity of these derivatives was assessed against liver cell line (Huh-7) using MTT assay. Among the synthesized derivatives, compound 6a inhibited the growth of tumor cells by displaying 68.36% cell viability at 100 µg/mL concentration which was then *in-silico* modelled to delineate the potential mechanistic insights for its antiproliferative activity. The PASS prediction indicated the TopII as potential anticancer target of compound 6a. The induced fit docking revealed that compound 6a inhibits the TopII with superior binding affinity and forms stronger contacts with active site's key residues responsible for DNA-TopII intercalation and catalytic inhibition consistent with its cytotoxic potential. Therefore, compound 6a can be considered as a potential lead for further optimization in the development of ciprofloxacin-derived anticancer drugs.

Keywords: Fluoroquinolones, cytotoxic activity, human liver cell line, anti-cancer, induced fit docking.

INTRODUCTION

Drug design and discovery have always been a fascinating, challenging as well as exciting field for medicinal chemists. In this regard, great emphasis is placed on antimicrobial chemotherapy as it has not only helped to control infectious diseases but also improved the fate of mankind. However, rapid onset of bacterial resistance to existing antibiotics is a serious health hazard. Multiple factors are responsible for changes in microbial genomes leading to development of resistant genotypes. Increased and prolonged usage of antimicrobials in substandard dosage is especially important in this regard. Flare up of these multidrug resistant strains stimulated researchers to design and synthesize new antimicrobial agents for the treatment of MDR strains (Sheppard and Long 2016; Ibrahim *et al.,* 2014).

Fluoroquinolones (FQs) were introduced in late 1970s, since then they grew in popularity among researchers and remain a most challenging modality. Initially structural modifications improved the potency, spectrum and their effectiveness (Buglak *et al*., 2019; Akhtar *et al.,* 2016). Fluoroquinolones are clinically under trial as second line therapy for tuberculosis due to their favorable pharmacokinetic profile, good adsorption and proficient penetration into host macrophages (Wang *et al.,* 2018). In addition some of fluoroquinolones also exhibit antiproliferative activities against tumor cells (Kassab and Gedawy 2018; Hernández-López *et al*., 2019). For example, ciprofloxacin (CP) a well-accepted broad spectrum potent antibiotic creating minimum side effects has shown antiproliferative activity against different tumors (Sedghizadeh *et al*., 2017; Shavit *et al*., 2017).

Since the emergence of norfloxacin and ciprofloxacin, functional groups at C-7 position is the main target for FQ antibacterial researchers. At this position chemical interference can assess the effectiveness of quinolone derivatives. This site is also important to determine the mechanism of action, cell permeability as well as pharmacokinetic properties of the drug when exposed to enzymes. With addition of piperazine substituent at C-7 position, a no of clinically effective FQs emerged leading to shortened course of illness. The piperazine moiety of FQs exhibit sufficient structural flexibility to generate novel molecules (Esfahani *et al*., 2019; Sharma *et al.,* 2015; Shamsa *et al.,* 2011; Shafiee *et al.,* 2009; Emami *et al.,* 2013; Zahoor *et al.,* 2017). Hence, participating the ongoing striving research program for discovery of new potent antimicrobial agents, the prime focus of this research was to prepare a variety of ciprofloxacin derivatives having significant cytotoxicity. Therefore, we combined ciprofloxacin molecule with 2-bromo-*N*-acetamide derivatives to synthesize *N*-4 piperazinyl ciprofloxacin-aniline hybrids. Cytotoxicity of the designed derivatives was evaluated against liver cell line (Huh-7) using MTT assay.

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MATERIALS AND METHODS

General experimental part

Chemicals, bromoacetyl bromide, pyridine, dichloromethane, silica gel and anilines were purchased from Alfa-Aesar (Germany), Merck, Scharlau and Daejung through local suppliers. To get pure compounds silica gel was used in column chromatography technique. Melting points of the final derivatives were checked by using Gallenkamp equipment. To obtain ^{1}H-NMR and FT-IR spectra Bruker spectrometer at 400 MHz and Bruker fourier-transform spectrometer were used, respectively.

Common preparation method for ciprofloxacin-aniline derivatives 6a-j

Stirred the mixture of ciprofloxacin ester 2 (1.45 mmoles) and pyridine (9 mmoles) in 20 mL dichloromethane (DCM) for 15 min. After the addition of anilide 5a-j (2.17 mmoles), it was stirred at r.t. till the completion of reaction (72-96 h). Then n-hexane was added in the mixture and resulting precipitates were collected and recrystallized with methanol to get pure compounds 6a-j.

Cytotoxic activity

The synthesized derivatives 6a-j were assessed against human liver tumor cell line (Huh7). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was utilized to check viability of the cells. To culture human Huh7 cells, temperature was maintained at 37°C. Dulbecco's Modified Eagle's Medium with ten percent fatal bovine serum, 100 units/mL penicillin and 100 µg/mL streptomycin was used. Cells were treated with DMSO dissolved ciprofloxacin derivatives (having 0.05% DMSO concentration) for 48 h. Cells which were treated with DMSO used as control in all the experiments. After that 500 µg/mL MTT reagent was added and incubated the cells for 4 h. Farmazan crystals was dissolved in 150 µL dimethyl sulfoxide and checked the absorbance at 570 nm. Results are described as % of cell viability.

In-Silico Modeling

The experimental compounds were further *in-silico* modeled to provide the insights into their potential mechanism of action. The PASS prediction tool was utilized to explore the biological spectrum of experimental compounds which employs the Bayesian approach based algorithm to predict the therapeutic targets with 95% accuracy (Parasuraman 2011). The anticancer targets with Pa >50% were selected as potential therapeutic targets, and inhibitory potential of experimental compounds was investigated by the induced fit docking in Molecular Operating Environment (MOE) 2015.10. The chemical structures of compounds were sketched with ChemDraw Ultra, and energy minimized using CHARMm forcefield with MMFF94x partial charge in Accelry's Discovery Studio Visualizer 17.2. The 3D conformer of ciprofloxacin (PubChem CID: 2764) was retrieved from PubChem Database and optimized in Accelry's Discovery Studio Visualizer 17.2. The X-ray crystallographic 3D structure of Topoisomerase II (PDB ID: 4G0U, 2.7 Å resolution) was retrieved from RSCB Protein Data Bank (https://www.rcsb.org/). These structures were prepared and optimized by the QuickPrep function of MOE 2015.10. The binding site was identified by the Site Finder application of MOE. The docking application of MOE was used to dock the ligands onto binding pocket through Triangle Matcher placement method with subsequent induced fit refinement. The docked pose with lowest conformational energy (i.e. S) was utilized for further conformational analysis and complexation of ligand within the binding pocket of receptor using Accelry's Discovery Studio Visualizer 17.2.

RESULTS

Condensation of ciprofloxacin 1 with methanol in the presence of sulfuric acid produced the corresponding ciprofloxacin ester 2 in 70% yield (Scheme 1) (Shaharyar *et al.,* 2007). Anilides 5a-j were prepared by the reaction of anilines with bromoacetyl bromide according to the known procedure (Cormier *et al.,* 2012). *N*-Alkylation of ciprofloxacin ester 2 with anilides 5a-j in the presence of pyridine and dichloromethane led to the formation of *N*-4 piperazinyl ciprofloxacin derivatives 6a-j as shown in scheme 2.

DISCUSSION

Spectral studies of compound 6d

Structure of the molecule compound 6d was confirmed by infrared, proton and carbon NMR spectroscopy (fig. 1). Functional groups in the molecule were analyzed by IR spectroscopy. Absorption bands in IR spectrum were observed at 1086 for C-F bond, 1347, for C-N bond, 1581 for carbon double-bond with carbon, 1726 for carbonyl group of amide, 1619 for carbonyl group of ester and 1257 (C-O) cm^{-1}. ^{1}H-NMR of 6d gave singlet at 3.93 ppm for protons of methoxy group (C-9). The protons of aryl group in quinolone ring showed 2 doublets at 8.04 and 7.28 ppm. Four protons of cyclopropyl ring showed multiplet at 1.14-1.38 ppm and one proton gave multiplet at 3.43-3.47 ppm. The hydrogens of piperazine ring appeared as triplet at 2.78-3.32 ppm. Hydrogens of CH_2 (linker) connected to piperazine ring gave chemical shift at 3.31 ppm. Multiplet signal at 3.65-3.72 ppm appeared for morpholine ring. ^{13}C NMR spectra of compound 6d showed peaks at 110.27-152.16 ppm for fluoroquinolone ring, while cyclopropyl ring attached to quinolone ring appeared at 8.11-34.46 ppm. Carbons of piperazine and morpholine rings displayed chemical shifts at 49.84-51.98 ppm and 46.14-66.97 ppm respectively. 166.44 ppm (chemical shift) was assigned for carbonyl carbons, and

Table 1: Spectral data of compounds 6a-j

Compound*	M.P. (^oC)	Yield (%)	FT-IR (KBr, cm^{-1})ν_{max} / ^{1}H-NMR (400 MHz, DMSO-d_6/ $CDCl_3$)/ MS (EI) (*m/z*)
6a	204	67	1019 (C-F), 1313 (C-N), 1429 (C=C), 1716 (C=O), 1256 (C-O), 1626 (C=O)/ 3.74 (s, 3H, $COOCH_3$), 8.45 (s, 1H, H_2 quinolone), 3.64-3.68 (m, 1H, cyclopropyl), 1.09-1.27 (m, 4H, cyclopropyl), 7.77 (d, 1H, J = 8 Hz, H_5 quinolone), 7.05-7.09 (m, 1H, H_8 quinolone), 2.77 (t, 4H, J = 4 Hz, piperazine), 2.48 (t, 4H, J = 4 Hz, piperazine), 3.25 (s, 2H, CH_2N), 9.78 (s, 1H, NH), 7.31-7.66 (m, 5H, Ar-H)/ 479.3 [M^+]
6b	233	68	1014 (C-F), 1313 (C-N), 1490 (C=C), 1618 (C=O), 1257 (C-O), 1723 (C=O)/ 3.94 (s, 3H, $COOCH_3$), 8.58 (s, 1H, H_2 quinolone), 3.44-3.48 (m, 1H, cyclopropyl), 1.16-1.37 (m, 4H, cyclopropyl), 8.09 (d, 1H, J = 8 Hz, H_5 quinolone), 7.30 (d, 1H, J = 8 Hz, H_8 quinolone), 3.38 (t, 4H, J = 4 Hz, piperazine), 2.92 (t, 4H, J = 4 Hz, piperazine), 3.31 (s, 2H, CH_2N), 9.09 (s, 1H, NH), 2.29 (s, 3H, methyl), 2.32 (s, 3H, methyl), 7.03 (s, 1H, Ar-H), 7.07 (d, 1H, J = 8 Hz, Ar-H), 7.95 (d, 1H, J = 8 Hz, Ar-H)/ 507.3 [M^+]
6c	270	72	1016 (C-F), 1313 (C-N), 1596 (C=C), 1730 (C=O), 1256 (C-O), 1677 (C=O)/ 3.92 (s, 3H, $COOCH_3$), 8.58 (s, 1H, H_2 quinolone), 3.45-3.49 (m, 1H, cyclopropyl), 1.17-1.38 (m, 4H, cyclopropyl), 8.40 (dd, 1H, J = 4 Hz, 8 Hz, H_5 quinolone), 7.31 (d, 1H, J = 4 Hz, H_8 quinolone), 3.40 (t, 4H, J = 4 Hz, piperazine), 2.89 (t, 4H, J = 4 Hz, piperazine), 3.29 (s, 2H, CH_2N), 9.71 (s, 1H, NH), 3.94 (S, 3H, OCH_3), 6.91-7.11 (m, 3H, Ar-H). 8.08 (d, 1H, J = 12 Hz, Ar-H)/ 509.4 [M^+]
6d	228	70	1086 (C-F), 1347 (C-N), 1581 (C=C), 1726 (C=O), 1257 (C-O), 1619 (C=O)/ 3.93 (s, 3H, $COOCH_3$), 8.56 (s, 1H, H_2 quinolone), 3.43-3.47 (m, 1H, cyclopropyl), 1.14-1.36 (m, 4H, cyclopropyl), 8.04 (d, 1H, J = 12 Hz, H_5 quinolone), 7.27 (d, 1H, J = 4 Hz, H_8 quinolone), 2.78 (t, 4H, J = 4 Hz, piperazine), 3..32 (t, 4H, J = 4 Hz, piperazine), 3.31 (s, 2H, CH_2N), 3.65-3.72 (m, 8H, morpholine) / 473.3 [M^+]
6e	195	68	1015 (C-F), 1311 (C-N), 1594 (C=C), 1731 (C=O), 1255 (C-O), 1674 (C=O)/ 3.75 (s, 3H, $COOCH_3$), 8.48 (s, 1H, H_2 quinolone), 3.67-3.70 (m, 1H, cyclopropyl), 1.12-1.35 (m, 4H, cyclopropyl), 7.82 (d, 1H, J = 12 Hz, H_5 quinolone), 4.32 (s, 1H, H_8 quinolone), 3.38 (t, 4H, J = 4 Hz, piperazine), 2.92 (t, 4H, J = 4 Hz, piperazine), 3.31 (s, 2H, CH_2N), 11.09 (s, 1H, NH), 7.44-7.70 (m, 4H, Ar-H)/ 514.3 [M^+]
6f	225	71	1015 (C-F), 1311 (C-N), 1595 (C=C), 1674 (C=O), 1255 (C-O), 1731 (C=O)/ 3.75 (s, 3H, $COOCH_3$), 8.48 (s, 1H, H_2 quinolone), 3.67-3.71 (m, 1H, cyclopropyl), 1.11-1.31 (m, 4H, cyclopropyl), 7.84 (s, 1H, H_5 quinolone), 4.33 (s, 1H, H_8 quinolone), 3.38 (t, 4H, J = 4 Hz, piperazine), 2.92 (t, 4H, J = 4 Hz, piperazine), 3.31 (s, 2H, CH_2N), 10.66 (s, 1H, NH), 7.82 (s, 1H, Ar-H), 7.40-7.53 (m, 2H, Ar-H), 7.21 (d, 1H, J = 8 Hz, Ar-H)/ 514.4 [M^+]
6g	272	75	1086 (C-F), 1347 (C-N), 1581 (C=C), 1726 (C=O), 1257 (C-O), 1619 (C=O)/ 3.77 (s, 3H, $COOCH_3$), 8.69 (s, 1H, H_2 quinolone), 4.12 (m, 1H, cyclopropyl), 1.08-1.33 (m, 4H, cyclopropyl), 8.40 (d, 1H, J = 4 Hz, H_5 quinolone), 7.31 (d, 1H, J = 4 Hz, H_8 quinolone), 3.29 (s, 2H, CH_2N), 7.01-7.96 (m, 4H, Ar-H), 2.48-3.44 (m, 8H, piperazine), 7.23 (s, 1H, NH)/ 497.3 [M^+]
6h	220	77	1086 (C-F), 1347 (C-N), 1581 (C=C), 1726 (C=O), 1257 (C-O), 1619 (C=O)/ 3.77 (s, 3H, $COOCH_3$), 8.69 (s, 1H, H_2 quinolone), 4.12 (m, 1H, cyclopropyl), 1.11-1.31 (m, 4H, cyclopropyl), 7.84 (s, 1H, H_5 quinolone), 4.33 (s, 1H, H_8 quinolone), 2.48-3.44 (m, 8H, piperazine), 7.22-7.60 (m, 4H, Ar-H), 3.34 (s, 2H, CH_2N), 7.23 (s, 1H, -NH)/ 497.3 [M^+]
6i	239	69	1019 (C-F), 1313 (C-N), 1429 (C=C), 1716 (C=O), 1256 (C-O), 1626 (C=O)/ 3.77 (s, 3H, $COOCH_3$), 8.69 (s, 1H, H_2 quinolone), 3.67-3.71 (m, 1H, cyclopropyl), 1.11-1.31 (m, 4H, cyclopropyl), 7.84 (s, 1H, H_5 quinolone), 4.33 (s, 1H, H_8 quinolone), 3.38 (t, 4H, J = 4 Hz, piperazine), 2.92 (t, 4H, J = 4 Hz, piperazine), 7.43-7.87 (m, 3H, Ar-H), 3.34 (s, 2H, CH_2N), 7.23 (s, 1H, -NH)/ 548.3 [M^+]
6j	274	70	1015 (C-F), 1311 (C-N), 1594 (C=C), 1731 (C=O), 1255 (C-O), 1674 (C=O)/ 3.77 (s, 3H, $COOCH_3$), 8.69 (s, 1H, H_2 quinolone) 3.44-3.48 (m, 1H, cyclopropyl), 1.16-1.37 (m, 4H, cyclopropyl), 8.09 (d, 1H, J = 8 Hz, H_5 quinolone), 7.30 (d, 1H, J = 8 Hz, H_8 quinolone), 3.38 (t, 4H, J = 4 Hz, piperazine), 2.92 (t, 4H, J = 4 Hz, piperazine), 3.31 (s, 2H, CH_2N), 9.09 (s, 1H, NH), 7.43-7.87 (m, 3H, Ar-H)/ 514.3 [M^+]

*All the synthesized compounds in this work are white, off white, grey, light yellow and brown solids.

Table 2: Cytotoxic potential of synthetic derivatives 6a-j against liver cancer cells (Huh-7).

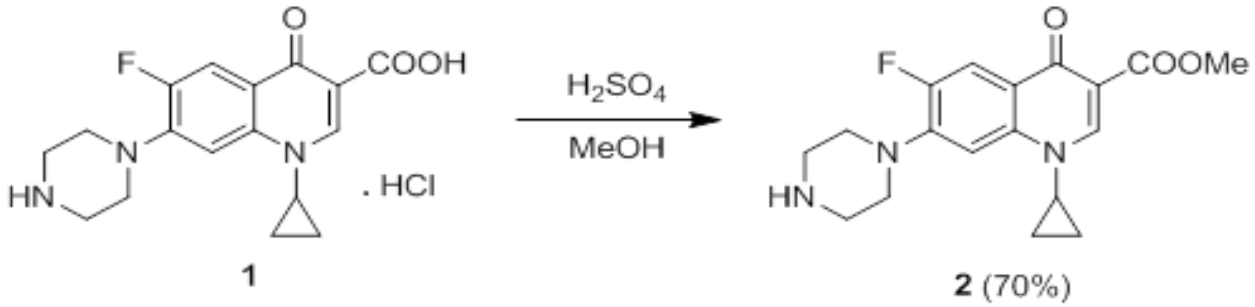
Compounds	Huh7 % Cell viability ± SD
6a	68.36 ± 4.97
6b	88.50 ± 2.97
6c	115.60 ± 1.91
6d	132.13 ± 2.84
6e	401.65 ± 24.14
6f	194.96 ± 11.91
6g	220.79 ± 9.05
6h	141.98 ± 4.21
6i	144.27 ± 3.97
6j	81.59 ± 8.07

Table 3: Cytotoxic potential of compound **6a** against liver cancer cells (Huh-7) at different concentrations

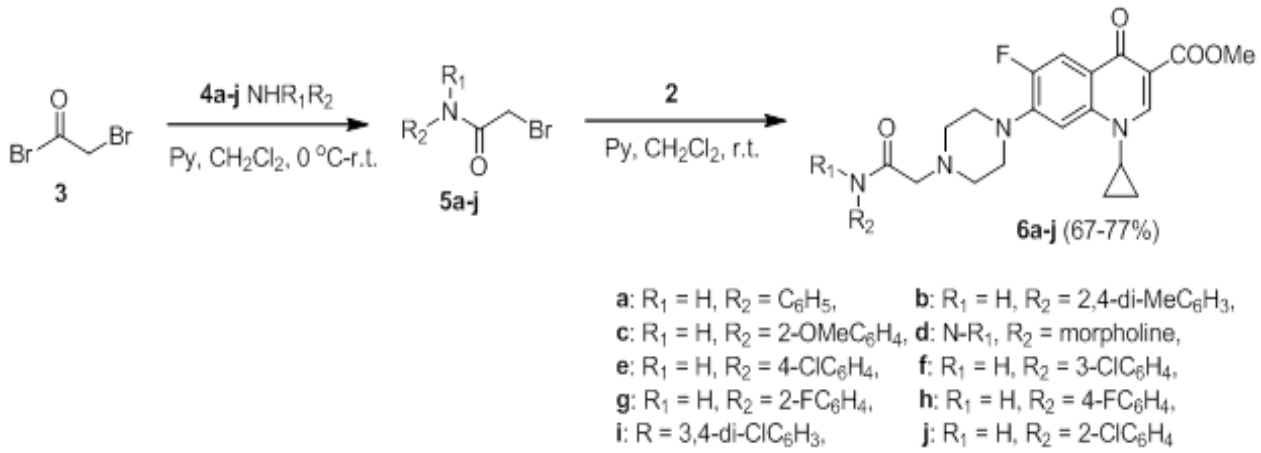
Sr. No.	Concentrations	Huh7 % Cell viability ± SD
1	500	48.31 ± 1.46
2	250	50.28 ± 1.02
3	125	53.34 ± 0.82
4	62.5	57.63 ± 3.18
5	31.25	60.15 ± 3.73
6	15.6	65.21 ± 4.30
7	8	67.00 ± 3.27
8	4	71.94 ± 3.52
9	2	79.66 ± 1.79
10	1	81.19 ± 1.93

Table 4: Analysis of parameters in induced fit docking simulation

Target	Ligand	Binding score (S) Kcal/mol	Interacting residues	Type of interaction
Topoisomerase II	Ciprofloxacin	-5.3	TYR821, SER480, SER818, ARG820, ARG503, GLU477, GLY504, LYS505, GLN778, GLY776, GLY478, ASP479	H-bonding, π – Cation, π-Anion, π-π Stacked, Alkyl, Attractive Charge, Salt Bridge, Van der Waals
	Compound 6a	-6.9	TYR821, GLY776, GLN778, ASP479, ARG820, HIS775, ARG503, ASP561, ASP557, ASP559, HIS774, ARG729, GLY504, GLU447, ILE565, LEU502, GLY478, ALA481, SER480, SER818	H-Bonding, Halogen bonding, π-Cation, π – Anion, π – Alkyl, Van der Waals



Scheme 1: Synthesis of ciprofloxacin ester 2.



Scheme 2: Synthesis of designed compounds 6a-

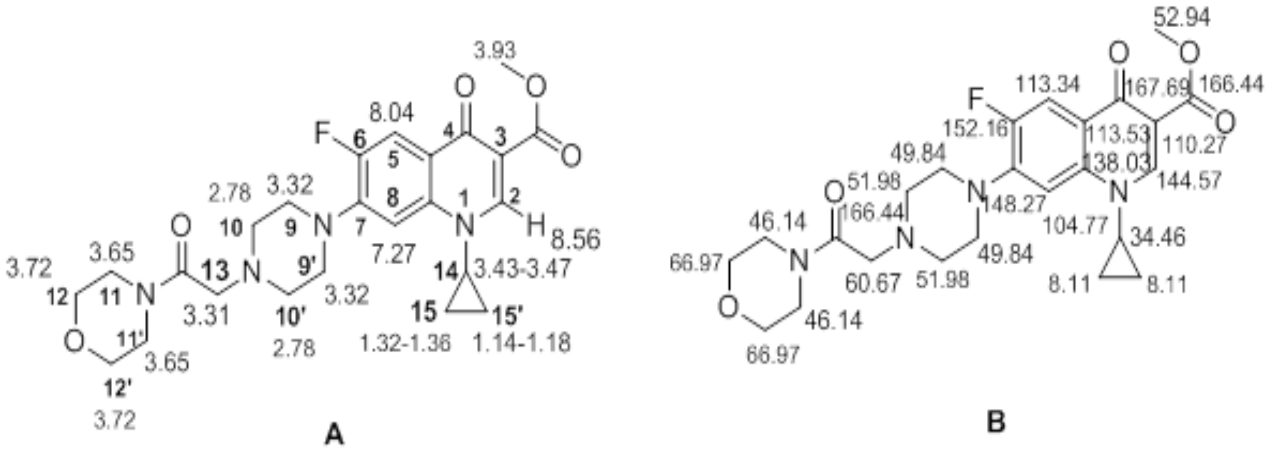


Fig.1: ^{1}H (A) and ^{13}C NMR (B) Chemical shifts of compound 6d

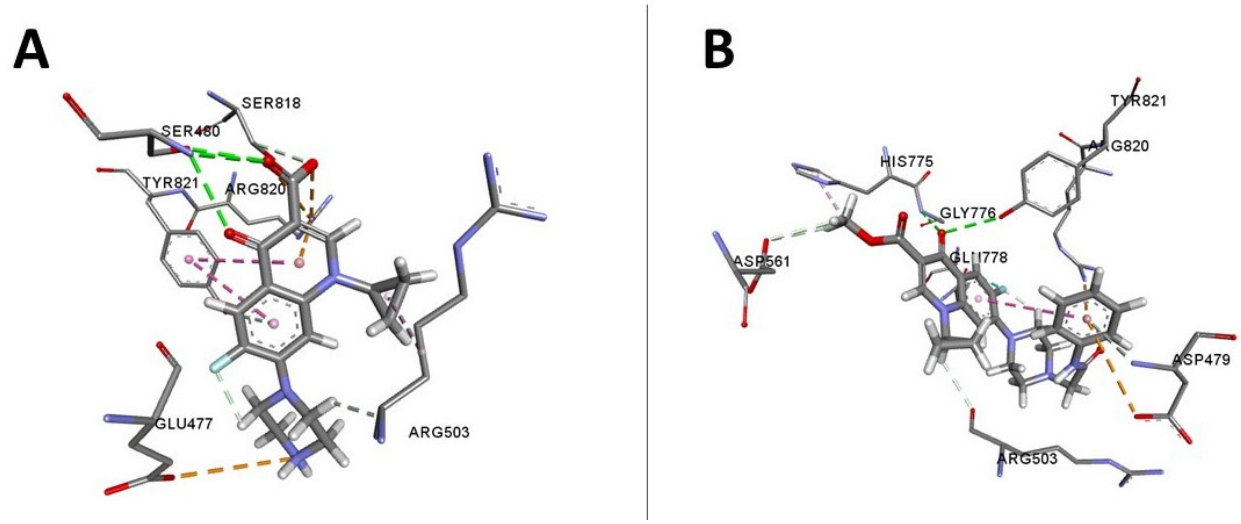


Fig. 2: Conformational analysis of ciprofloxacin and compound 6a at the binding position of TopII. Simulated best binding pose of ciprofloxacin (A) and compound 6a (B) interaction in three dimensional (3D) space of binding pocket.

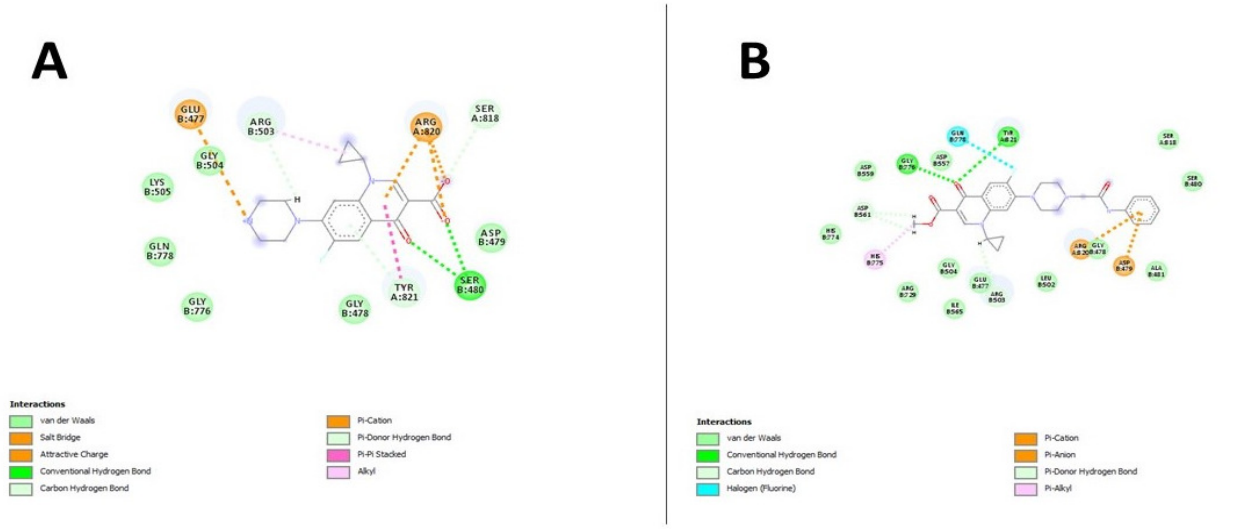


Fig. 3: Ciprofloxacin and compound 6a interactions with residues of topoisomerase II active site; Ciprofloxacin (A) and Compound 6a (B) in two dimensional (2D) space to visualize their interactions with residues shown as balls colored by type of interactions.

CH_2 (linker) peak appeared at 60.67 ppm. Other synthetic derivatives were also characterized by similar approach.

Cytotoxic activity

The role of substitution patterns and their nature at aniline ring was investigated in order to get detailed information about cytotoxic activity of the synthesized derivatives. The results for the antiproliferative activity are summarized in table 2 which revealed that the presence of different substituents on aniline could not enhance the cytotoxic activity of the synthesized ciprofloxacin derivatives. Among all the derivatives the viability of the cells treated with 100µg/mL of compound 6a was 68.36% which was significantly lower than the other synthesized derivatives. After applying different concentrations of compound 6a, 48.31% cell viability was obtained at 500µg/mL as depicted in table 3.

In-silico modeling

The compound 6a was further *in-silico* modeled to investigate its improved anticancer property as compare to ciprofloxacin. The PASS prediction indicated the topoisomerase II (Pa~0,5) as potential anticancer therapeutic target for compound 6a. Topoisomerase II (TopII) actively alters the DNA topology to resolve the DNA geometric entanglement or supercoiling by formation of DNA adducts and subsequent resealing process – that, if fails, may lead to cell death (Schoeffler and Berger 2008; Vos *et al.,* 2011). It has been emerged as a primary target of vital anticancer drugs such as etoposide, doxorubicin, and daunorubicin. The chemotherapeutic drugs have exploited this enzyme to induce the cytotoxic-effect either by accumulation of intercalated DNA-TopII complexes, or via competitive inhibition of TopII catalysis (Nitiss 2009). Ciprofloxacin has also been found to inhibit the eukaryotic topoisomerase II that may be responsible for its cytotoxic activity in certain type of malignancies (Lynch *et al.,* 2003; Kloskowski *et al.,* 2012). Interestingly, the compound 6a was found to dock on topoisomerase II binding site with -6.9 Kcal/mol conformational energy as a function of its binding affinity for topoisomerase II. The lower conformational energy of compound 6a revealed its greater binding affinity as compare to ciprofloxacin which was found to bind topoisomerase II with -5.3 Kcal/mol of conformational energy (table 4).

The conformational analysis revealed that compound 6a conformed into the spatial configuration that allowed it to interact more actively with diversity of residues at the binding site of topoisomerase II. The configuration of compound 6a preserved the interactions with majority of conserved residues for ciprofloxacin activity (fig. 2). However, the functional diversity and conformational alignment of compound 6a allowed it to establish the contact with other vital residues of topoisomerase II binding site.

The complex of compound 6a with topoisomerase II was mainly stabilized by the H-bonding with TYR821, GLY776, ASP561, ARG503 and ionic interactions with ASP479 and ARG820. In addition, it was found to form one π- alkyl and one halogen bond with HIS775 and GLN778, respectively (fig. 3). Notably, compound 6a interacted with the majority of residues, conserved for etoposide activity that may explain its transition toward

anticancer property (Wu *et al.,* 2011). The ciprofloxacin complex was stabilized by the H-bonding with SER480, SER818, ARG503 and TYR821. It also shared the strong ionic contact with ARG820, GLU477 and hydrophobic interaction with TYR821 and ARG503. Interestingly, the compound 6a preserved the interactions with conserved residues (i.e. TYR821, ARG821, ARG503, ARG820, GLU477 and SER480) of ciprofloxacin interaction at the binding site of topoisomerase II. However, it was found to interact these residues with distinct nature of bonding. Intriguingly, the comparable binding of compound 6a may be justified by H-bond with key residue ARG503 and Van der Waals interactions with LEU502 and GLY504 at the TopII binding site for DNA minor groove. However, its superior affinity towards topoisomerase II can be corroborated by its stronger H-bond with GLY776 and Halogen interaction with GLN778, respectively, at the binding site for DNA minor and major binding groove, thereby suggesting cytotoxic effect by the formation of DNA-TOPII intercalated complexes. Moreover, the formation of H-bond with major catalytic residue, i.e. TYR821, may further support the compound 6a's potential to inhibit the catalytic activity of TopII. Therefore, these insights may reasonably justify the endurance of compound 6a complexation with TopII and improved anticancer activity consistent with experimental results.

CONCLUSION

Fluoroquinolone antimicrobials are well known potent inhibitors of bacterial DNA gyrase and topoisomerase IV which they achieve by efficient inhibition of DNA replication and transcription. Some quinolone derivatives exhibit inhibitory potential against eukaryotic topoisomerase II as well as cytotoxic potential against some cancerous cells. In present study, preparation and antitumor evaluation of *N*-4 piperazinyl ciprofloxacin-aniline hybrids have been described. Among all the derivatives, compound 6a displayed moderate activity against human liver tumor cell line (Huh7) which could possibly be further enhanced keeping the results (obtained in this study) in view. Consistently, the computational studies indicated 6a with better TopII inhibitory potential and suggest further development in this regard.

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REFERENCES

Akhtar R, Yousaf M, Naqvi SAR, Irfan M, Zahoor, AF, Hussain AI and Chatha SAS (2016). Synthesis of ciprofloxacin-based compounds: A review. *Synth. Commun.,* **46**(23): 1849-1879.

Buglak AA, Shanin IA, Eremin SA, Lei H-T, Li X, Zherdev AV and Dzantiev BB (2019). Ciprofloxacin and clinafloxacin antibodies for an immunoassay of quinolones: Quantitative structure-activity analysis of cross reactivities. *Int. J. Mol. Sci.,* **20**(265): 265-278.

Cormier R, Burda WN, Harrington L, Edlinger J, Kodigepalli KM, Thomas J, Kapolka R, Roma G, Anderson BE, Turos E and Shaw LN (2012). Studies on the antimicrobial properties of *N*-acylated ciprofloxacins. *Bioorg. Med. Chem. Lett.,* **22**(20): 6513-6520.

Emami S, Ghafouri E, Faramarzi MA, Samadi N, Irannejad H and Foroumadi A (2013). Mannich bases of 7-piperazinylquinolones and kojic acid derivatives: Synthesis, *in vitro* antibacterial activity and in silico study. *Eur. J. Med. Chem.,* **68**: 185-191.

Esfahani EN, Mohammadi-Khanaposhtani M, Rezaei Z, Valizadeh Y, Rajabnia R, Hassankalhori M, Bandarian F, Faramarzi MA, Samadi N, Amini MR, Mahdavi M and Larijani B (2019). New ciprofloxacin-dithiocarbamate-benzyl hybrids: Design, synthesis, antibacterial evaluation, and molecular modeling studies. *Res. Chem. Intermediat.,* **45**(2): 223-236.

Hernández-López H, Sánchez-Miranda G, Araujo-Huitrado JG, Granados- López AJ, López JA, Leyva-Ramos S and Chaćon-Garćia L (2019). Synthesis of hybrid fluoroquinolone-boron complexes and their evaluation in cervical cancer cell lines. *J. Chem-NY.,* **2019**: 1-6.

Ibrahim MA, Panda SS, Birs AS, Serrano JC, Gonzalez CF, Alamry KA and Katritzky AR (2014). Synthesis and antibacterial evaluation of amino acid-antibiotic conjugates. *Bioorg. Med. Chem. Lett.,* **24**(7): 1856-1861.

Kassab AE and Gedawy EM (2018). Novel ciprofloxacin hybrids using biology oriented drug synthesis (BIODS) approach: Anticancer activity, effects on cell cycle profile, caspase-3 mediated apoptosis, topoisomerase II inhibition, and antibacterial activity. *Eur. J. Med. Chem.,* **150**: 403-418.

Kloskowski T, Gurtowska N, Olkowska J, Nowak JM, Adamowicz J, Tworkiewicz J, Debski R, Grzanka A and Drewa T (2012). Ciprofloxacin is a potential topoisomerase II inhibitor for the treatment of NSCLC. *Int. J. Oncol.,* **41**(6): 1943-1949.

Lynch A, Harvey J, Aylott M, Nicholas E, Burman M, Siddiqui A, Walker S, Rees R (2003). Investigations into the concept of a threshold for topoisomerase inhibitor-induced clastogenicity. *Mutagenesis,* **18**(4): 345-353.

Nitiss JL (2009). Targeting DNA topoisomerase II in cancer chemotherapy. *Nat. Rev. Cancer,* **9**(4): 338-350.

Parasuraman S (2011). Prediction of activity spectra for substances. *J. Pharmacol. Pharmacother.,* **2**(1): 52-53.

Schoeffler AJ and Berger JM (2008). DNA topoisomerases: Harnessing and constraining energy to govern chromosome topology. *Q. Rev. Biophys.,* **41**(1): 41-101.

Sheppard JG and Long TE (2016). Allicin-inspired thiolated fluoroquinolones as antibacterials against ESKAPE pathogens. *Bioorg. Med. Chem. Lett.,* **26**(22): 5545-5549.

Shaharyar M, Ali MA and Abdullah MM (2007). Synthesis and antiproliferative activity of 1-[(sub)]-6-fluoro-3-[(sub)]-1,3,4-oxadiazol-2-yl-7-piperazino-1,4-dihydro-4-quinolinone derivatives. *Med. Chem. Res.,* **16**(6): 292-299.

Sharma PC, Jain A, Yar MS, Pahwa R, Singh J and Goel S (2015). Synthesis and antibacterial evaluation of novel analogs of fluoroquinolones annulated with 6-substituted-2-aminobenzothiazoles. *Arab. J. Chem.,* **8**(5): 671-677.

Shamsa F, Foroumadi A, Shamsa H, Samadi N, Faramarzi MA and Shafiee A (2011). Synthesis and in-vitro antibacterial activities of acetylanthracene and acetylphenanthrene derivatives of some fluoroquinolones. *Iran. J. Pharm. Res.,* **10**(2): 225-231.

Shafiee A, Emami S, Ghodsi S, Najjaria S, Sorkhia M, Samadi N, Faramarzi MA and Foroumadi A (2009). Synthesis and antibacterial activity of *N*-[2-(2-naphthyl) ethyl]piperazinyl quinolones. *J. Iran. Chem. Soc.,* **6**(2): 325-333.

Shavit M, Pokrovskaya V, Belakhov V and Baasov T (2017). Covalently linked kanamycin-ciprofloxacin hybrid antibiotics as a tool to fight bacterial resistance. *Bioorgan. Med. Chem.,* **25**(11): 2917-2925.

Sedghizadeh PP, Sun S, Junka AF, Richard E, Sadrerafi K, Mahabady S, Bakhshalian N, Tjokro N, Bartoszewicz M, Oleksy M, Szymczyk P, Lundy MW, Neighbors JD and Russell RGG (2017). Design, synthesis, and antimicrobial evaluation of a novel bone-targeting bisphosphonate-ciprofloxacin conjugate for the treatment of osteomyelitis biofilms. *J. Med. Chem.,* **60**(6): 2326-2343.

Vos SM, Tretter EM, Schmidt BH and Berger JM (2011). All tangled up: How cells direct, manage and exploit topoisomerase function. *Nat. Rev. Mol. Cell Biol.,* **12**(11): 827-841.

Wang R, Yin X, Zhang Y and Yan W (2018). Design, synthesis and antimicrobial evaluation of propylene-tethered ciprofloxacin-isatin hybrids. *Eur. J. Med. Chem.,* **156**: 580-586.

Wu CC, Li TK, Farh L, Lin LY, Lin TS, Yu YJ, Yen TJ, Chiang CW and Chan NL (2011). Structural basis of type II topoisomerase inhibition by the anticancer drug etoposide. *Science,* **333**(6041): 459-462.

Zahoor, AF, Yousaf M, Siddique R, Ahmad S, Naqvi SAR and Rizvi SMA (2017). Synthetic strategies towards the synthesis of enoxacin, levofloxacin and gatifloxacin based compounds: A review. *Synth. Commun.,* **47**(11): 1021-1039.