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Physicochemical, Pharmacokinetic and Toxicological Evaluation of 1-cyclopropyl-6-fluoro-N'-isonicotinoyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carbohydrazide: A *In Silico* Approache

MAHWISH AKHTAR[1*], TANVEER AHMED SIDDIQI[2], SYED INAYATULLAH[2], SYED AHMAD HASSAN[2], SABA NAZ[2]

1: Dow College of Pharmacy, Dow University of Health Sciences, Pakistan

2: Department of Mathematics, University of Karachi Karachi-75270, Pakistan

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ABSTRACT

The aim of the present research is to establish the physicochemical, pharmacokinetics and toxicological properties of a newly designed ciprofloxacin amide derivative by using in silico approach. This compound qualified all the drug likeness rule of Lipinski, Veber, Egan Muegge and Ghose. This novel designed derivative of ciprofloxacin ispredicted good physicochemical and pharmacokinetic properties (absorption, distribution, metabolism, elimination and toxicity). According to the predicted data the developed derivative owns drug-like properties and thenit is synthesized and developed a successful candidate for essential addition against antimicrobial studies.

Keywords: Ciprofloxacin analogue, computational study, drug likeness properties, physicochemical properties, pharmacokinetics

INTRODUCTION

Currently, in silico technique is one of the developing computational approaches that is utilized in drug discovery for evaluating the pharmacokinetics, ADME, and toxicity forecasting or predictions. In view of drug designing, it is a vital challenge to do small variation in a drug-like molecule without changing its effectiveness corresponding to target disease. Moreover, it is also important

to ensure that this drug-like molecule tends to its target efficiently [**1-3**].

The usage of computational information technology in drug discovery and advancing gives significant finding or potential for minimizing the hands-on experimental research work for compound choosing, understanding, and refining the achievement proportion. These priori findings of ADMET features may be useful for scientists to choose the active compound and discard those with high prospect of failure. The conclusive aim of the in silico ADMET features is to predict in vivo pharmacokinetics of a substantial drug molecule in the clinical trial. This needs a hybrid set of models for keeping all the intricated developments and their integration into a 'drug design' software package which yield the fusion of ADME features with those of pharmacological

activities, consistency, chemical tractability etc. to yield a molecule with the best set of activities [**4-8**].

In this era, antibiotic drugs resistance is one of the challenging issues in the world. For this purpose, fluoroquinolones are of our interest because of its high activity corresponds to various bacteria [**9**].

A form of fluoroquinolone (FQ) antibiotic is known as Ciprofloxacin which is frequently utilized for treating different types of infections, specifically urinary tract infections [**10, 11**]. Cipro occurs mostly as zwitter ion at physiological PH that gives significant hydrophilicity to the compound. In spite of this, Cipro shows efficient oral bioavailability (60-80%) which has actively transported in organic anion form [**12**].

The purpose of this research study is to design an innovative new compound by using current computational techniques that will obey the rules of drug-likeness i.e. Lipinski's Rule of Five, Veber Rules, Egan, Muegge Rule and Ghose Filters and improve pharmacokinetics parameters (absorption, distribution, metabolism and excretion).

MATERIALS AND METHODS

Tools

Dell computer operating system with Windows 10 (64 bit), Intel Core i7, CPU 290 GHz, 4.00 GB RAM.

Programs

Chem office Ultra Version 12.0, Marvin Sketch Version 5.5 are licensed software; Mcule property calculator (© 2016 mcule.com), pkCSM (product of University of Cambridge), ProTox II online tool (available from http://tox.charite.de/tox/), Molinspiration Property Engine (v2014.11, © Molinspiration Cheminformatics 2016), Chemicalize online tool (ChemAxon:

Atlassian Confluence 5.9.2, team collaboration software), Lazar and SwissADME (Swiss Institute of Bioinformatics- © 2013) are free online tools.

Methodology

The structure of CIN was design and draw by Chem office. The SMILE string of structure was generated by Marvin Sketch version 5.5. The drug likeness and physicochemical properties of CIN were calculated by Mcule, pkCSM, ProTox, Swiss ADME, Molinspiration and Chemicalize online tool as molecular weight (MW), the logarithm of octanol/water partition coefficient (LogP), number of rotatable bonds (Torsion), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), and polar surface activity (PSA). Bioactivity scoring of CIN was predicted by Molinspiration. The computational prediction of pharmacokinetics (ADME: absorption, distribution, metabolism, and excretion) and toxicity of CIN was performed by a pkCSM, preADMET and SwissADME online tool [**13, 14**]. To predicttoxicity (LD50) per oral on rodent and toxicity classification of compounds based on globally harmonized system (GSH) [**15**], pkCSM and Protox online tool was used

RESULTS AND DISCUSSION

The chemical structures

The chemical structures of CIN is shown in Figure 1.

Drug-like molecular and Physicochemical Parameters:

The consequences of drug-like molecular parameters of CIN predicted bythe help ofavailable online software and showed in table 1. Remarkably, CIN qualified all parameters of Lipinski's Rule of Five (MW < 500, log P < 5, H-bond donors < 5 and H-bond acceptors < 1), Veber Rules (rotatable bonds ≤ 10 and polar surface area ≤ 140), Egan (TPSA > 131.6Å or log *P* > 5.88), Muegge Rule (200 ≤ MW ≤ 500, HBD ≤ 5; HBA ≤ 10, -2 ≤ ClogP ≤ 5, # rings ≤ 7, # C ≥ 4, # Hetro. Atom ≥ 1, # Rot. Bonds ≤ 15) and Ghose Filters(MW 160-480, number of atoms 20-70, polar surface area ≤ 140, molar refractivity 40-130 and log P -0.4-5.6) and bioavailability score was 0.55. The compound structure feature also clear CMC-50 Like rules (general and for inflammatory, psychotic, hypertensive, neoplastic, infective class of drugs) and BBB rule. However, it did not qualify some of the parameters in MDDR Rules and WDI rule [**16-18**]. These predicted values evidently indicated that CIN possessed structural features and physicochemical properties that could

correlate with good oral bioavailability and distribution.

Bioactivity Study:

The predicted results of bioactivity score were constructed on Bayesian statistics [**19**] to compare chemical structure of active ligands on the specific target and inactive molecules, which could be clearly able to distinct drug-like molecules and non-drug inactive molecule. The biologically active molecules have higher value of bioactivity score. The synthesized derivative (CIN) run against six most important classes of drugs that were G-protein coupled receptor ligand, kinases inhibitor, ion-channel modulator, protease inhibitor, nuclear receptor ligand, enzyme inhibitor. The computed results indicate that CIN has excellent bioactivity with Enzyme inhibitor, kinase inhibitor and G-protein coupled receptor ligand and good bioactivity with rest of the enzyme inhibitor (table 2).

Pharmacokinetics:

The pharmacokinetics and toxicological properties of CIN are presented in tables 3 – 7.

Absorption

The predicted values of absorption parameters of CIN was analyzed by the help of online software tools. According to above prediction, The CIN have high water solubility and low buffer solubility. Furthermore, the predicted value of CIN showed that the molecule was well absorbed from human intestine (*in silico* permeability at Caco-2 was 1.225 (>0.90)) and very high human intestinal absorption that was 82-89 %. The skin permeability was very low that was -2.771 to -5.11 log Kp cm/h. The compound was found to be P-glycoprotein (Pgp) substrate (ATP-binding cassette transporter) means it works as a biological barrier for toxins and xenobiotics. Moreover, it was also predicted that CIN did not inhibit through P-glycoprotein-I and II.

Distribution

Volume of distribution represents total volume required by dose of a drug to be uniformly distributed to give the same concentration as in plasma. Higher values indicate that more of the drug is in tissues than the plasma [**20, 21**]. In case of CIN, volume of distribution in human is 0.254 log L/ kg (> -0.15) (table 4, 5; figure 2) mean it is highly distributed in human tissues. The values of BBB and CNS permeability were very low that was -1.107 and -3.486 log PS respectively, which indicates that CIN was unable to penetrate in brain as well as CNS.

Metabolism

The derivative (CIN) was screened against number of enzyme substrate and inhibitor that were CYP3A4 substrate, CYP2D6 substrate, CYP2C19 inhibitor, CYP1A2 inhibitor, CYP2C9 inhibitor, CYP3A4 inhibitor and CYP2D6 inhibitor. The CIN was substrate only with CYP2D6 substrate, unresponsive with rest of the substrate and inhibitor.

Excretion

The predicted excretion parameters of CIN were calculated by pkCSM. According to that, the total renal clearance rate of drug is very high that is 0.735 log ml/min/kg and non-substrate with Renal OCT2 substrate.

Toxicity

According to globally harmonized system of classification of labeling of chemicals, the compound's toxicity class was predicted to be 4 *i.e.* harmful if swallowed (300 mg/kg < LD50 ≤ 2000 mg/kg). The acute oral toxicity dose and chronic oral toxicity dose of rat were calculated to be 4336 mg/kg and 1.347 log mg/kg_bw/day, respectively. CIN produced very low acute oral toxicity against *T. pyriformis* (0.294 log µg/L), flathead Minnow fish (0.589 log mM), *Daphnia* (0.242952) and Medaka (0.121993). The CIN was not found to cause skin sensitization, hepatotoxicity, carcinogenic and cardiotoxicity. Furthermore, the compound showed medium risk to hERG; human ether-a-go-go gene. Although, it was found to be mutagenic.

Software predicted that CIN not produce serious effect because it was unable to bind with common toxicity targets that were beta-2 adrenergic receptor, adenosine receptor A2a, , androgen receptor, D3 dopamine receptor, flavin-containing amine oxidase A, estrogen receptor beta, estrogen receptor, glucocorticoid receptor, nuclear receptor subfamily 1 group I member 2, histamine H1 receptor, kappa-type opioid receptor, prostaglandin G/H synthase 1, cAMP-specific 3, MOR-1 and choriotropin-releasing factor receptor I, nuclear receptor subfamily 3 group C member 3.

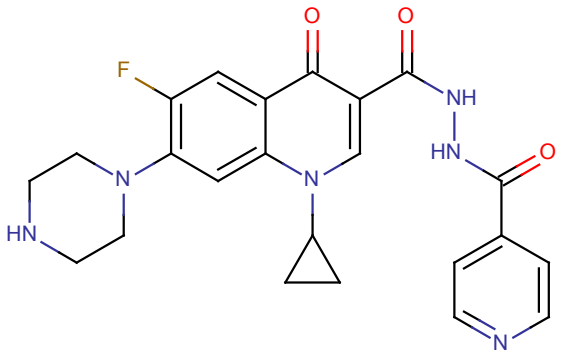

Figure 1: Structure of CIN

Table 1: Drug-like Molecular and Physicochemical Parameters of Derivative

Molecular properties	Mcule	pkCSM	ProTox II	Molinspiration	Chemicalize	Swiss ADME
molecular Mass (g/mol)	450.465	450.474	450.47	450.47	450.474	450.47
Total Atoms	56				56	
heavy Atoms	33		33	33	33	
Rings	5				5	
H-bond donor	3	3	0	3	3	3
H-bond acceptors	9	7	8	9	7	6
Rotatable bonds	7	4	7	5	5	7
Molecular Volume				388.48		
Surface area		188.122				
Polar surface area	108.36		108.36	108.36	106.67A2	108.36 A
Molar refractivity	126.7881				121.14cm3/mol	
Fraction of SP3 carbon					0.3	
log P	2.5305	1.3549		-1.58	0.36	
pKa					acidic 9.21, basic 8.57	

Table 2: Bioactivity Scoring of Derivative

Bioactivity	Molinspiration score
G-Protein coupled receptor ligand	0
Ion-channel modulator	-0.27
Kinase inhibitor	0.05
Nuclear receptor ligand	-0.51
Protease inhibitor	-0.21
Enzyme inhibitor	0.1

Table 3: Absorption Parameters of Derivative

Absorption parameters	Predicted value		
	pkCSM	preADMET	SwissADME
Water solubility	-2.818	11847.5 mg/ml	
Buffer solubility		0.176192	
Caco2 permeabilit	1.225	20.2135	
MDCK cell permeability		0.745117	
Human intestinal absorption	82.58%	92.162249	High
Skin permeability	-2.771	-4.85308	(-8.30 cm/s)
P-glycoprotein substrate	yes		yes

P-glycoprotein I inhibitor	yes	no	
P-glycoprotein II inhibitor	no	no	

Table 4: Distribution Characteristics of Derivative

Distribution Parameters	pkCSM	preADMET	Lazar
Volume of distribution (human)	0.254		
Plasma protein binding		33.155999	
Fraction unbound in plasma	0.227		
Central nervous system permeability	-3.486		
Blood-brain barrier permeability	-1.107	0.0238811	0.0999

Table 5: Predicted Solubility and Distribution of Drug Molecule in Different Body Fluids

pH	logD	Solubility [mg/ml]
1.7	-3.34	450.47
4.6	-2.41	450.47
6.5	-1.41	32.84
7.4	-0.57	4.38
8	-0.05	1.32

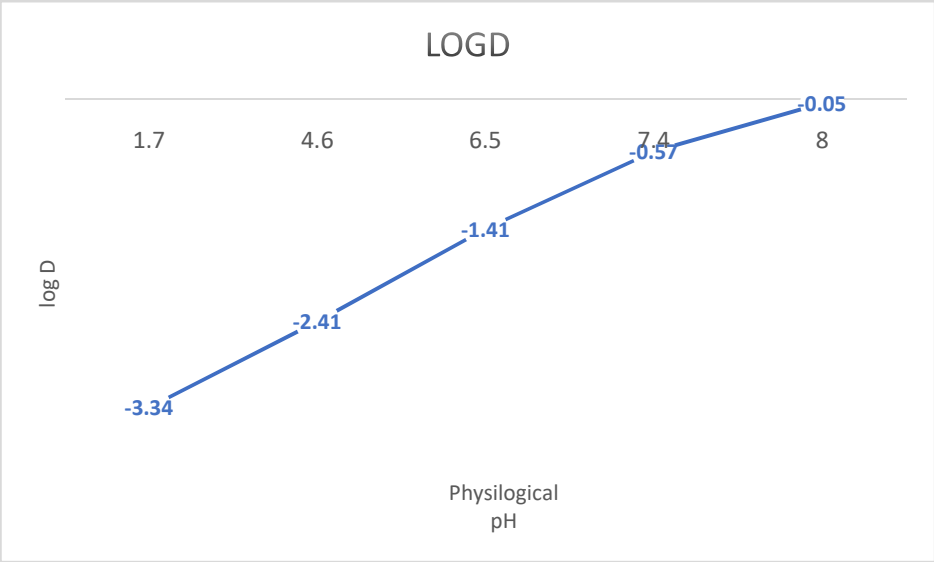

Figure 2: Predicted Drug Distribution of CIN at Different Body Fluids

Table 6: Metabolism Parameters of Derivatives

Metabolism Parameters	pkCSM	Swiss ADME	preADMET
CYP2D6 substrate	No	Yes	No
CYP3A4 substrate	Yes	Yes	Weakly
CYP1A2 inhibitior	No	No	
CYP2C19 inhibitior	No	No	Inhibitor
CYP2C9 inhibitior	No	Yes	Inhibitor
CYP2D6 inhibitior	No	Yes	No
CYP3A4 inhibitior	No	Yes	No

Table 7: Toxicity parameters of derivatives

Toxicity parameters	Predicted value			
	pkCSM	preADMET	ProTox II	LAZAR
Toxicity class			4	
Acute oral toxicity (rat- LD50)	2.734 mpl/kg		4336mg/kg	
Chronic oral toxicity (rat)	1.347 log mg/kg-bw/day			
Maximum recommended daily dose				0.0371 (mmol/kg-bw/day), 16.7 (mg/kg_bw/day)
AMES toxicity	no	MUTAGEN		
AMES (TA100_10RLI)		negative		
AMES (TA100_NA)		positive		
AMES (TA1535_10RLI)		negative		
AMES (TA1535_NA)		negative		
Kazius-Bursi Salmonella mutagenicity				
Carcinogenicity				0.158
T. pyriformis toxicity	0.294			
Minnow toxicity	0.589	0.308294		0
Daphnia toxicity		0.242952		0.222 (mmol/L), 99.9 (mg/L)
Medaka toxicity		0.121993		
Hepatotoxicity	yes			
Skin sensitization	no			
hERG-I inhibitor	no	medium_risk		
hERG-II inhibitor	yes	medium_risk		
Max. tolerated dose (human)	0.018 log mg/kg_bw/day			
Mutagenicity				0.378

CONCLUSION

The present research aimed to establish the physicochemical, pharmacokinetics and toxicological properties of a newly designed ciprofloxacin amide derivatives by in silico approaches. The physicochemical properties, bioactivity, pharmacokinetics and toxicity were determined. The compound qualified all the drug likeness rule of Lipinski, Veber, Egan Muegge and Ghose. The designed derivatives of ciprofloxacin were predicted to high absorption from human intestine which was >62% bioavailability, 0.65 log mL/min/kg renal clearance and elimination via dehalogenation (phase-I reactions) and conjugation with glutathione and cysteine (phase-II reactions). The predicted bioactivity of designed ciprofloxacin derivative was 0.2 with enzyme inhibitor, predicted to have good ion-channel modulator and enzyme inhibitory activities, and non-toxic to liver, skin and heart (toxicity class 4 and LD50 2.7 mol/kg or 4336mg/kg).

However, the derivative was not mutagenic and nor carcinogenic, but it may be hepatotoxic. According to the above data the derivative owns drug-like properties, later it

may be synthesized and developed a successful candidate for essential addition against antimicrobial studies.

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