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Research Article

In silico Analysis of the Cathepsin K Binding Affinity and Pharmacokinetics of 2-Cyanopyrimidine Derivatives

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Abstract

Introduction: Cathepsin K is being targeted for the treatment of various human disease including osteoporosis and cancer. In the present study, a compound, which contains a 2-cyanopyrimidine scaffold with optimal substituents that can interact with S2 and S3 subsites of cathepsin K, was derivatized.

Objective: To determine the *in silico* binding affinity, the toxicity and physicochemical properties of 1-[2-(3-biphenyl)-4-methylvaleryl)] amino-3-(2pyridylsulfonyl) amino-2-propanone and its derivatives.

Methods: UCSF chimera was used for macromolecule preparation and Autodock Vina was used for calculating binding affinities. PyMOL and UCSF Chimera were used to analyze the postdock binding interactions of the derivatives. Osiris property explorer was used to predict the toxicity and certain selected physicochemical properties of the derivatives.

Results: The substitution of the phenoxy moiety with lipophilic substituents was found to increase the binding affinity, unlike substitutions made on the cyanopyrimidine moiety. The derivatives were found to have high binding affinity and were non-toxic, but showed low drug likeness and drug scores.

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INTRODUCTION

In silico methods are being used extensively in drug development owning to their potential of reducing the drug discovery cost and the increased time efficiency to unveil new potentially active compounds [1,2]. Cathepsin K is a key enzyme in the process of bone resorption and its inhibition is a new therapeutic target for the treatment of osteoporosis [3]. In addition, there is now good evidence that cathepsin K contributes to stroma degradation and angiogenesis during tumor progression and thus promotes the growth and metastasis of tumor cells [4].

It has been reported that 2-cyanopyrimidine were one of the novel scaffolds for the design of non-peptidic cathepsin K inhibitor with R_2 group and R_1 group moieties that can possibly interact with S2 and S3 subsites of cathepsin K respectively [5].Another research showed that aryloxycarboxylic acid as P3 moiety can produce micromolar inhibition of cathepsin K in aminophenoxyacetic acid based inhibitors [6]. A research also showed that t-butyl moiety is an optimal P2 substituent in ketoamide series [7]. In present study t-butane and phenoxy acetic acid were virtually condensed with 2-cyanopyrimidine. So, a novel lead having a cyanopyrimidine scaffold with aryloxycarboxylic acid and t-butyl substitution, which were expected to interact with S2 and S3 pockets respectively, was generated. Accordingly, the binding affinity of2-cyano-6-(1'-aryloxycarbonyl 1'-t-butyl-amino)-pyrimidine and some of its derivatives were predicted by molecular docking against the crystal structure of cathepsin K.

Failure of promising lead(s) to exhibit desirable ADME/T profile is now regarded as the major reason for the late-stage drug attrition rate. According to a recent report, poor pharmacokinetics (39%) and preclinical toxicity (11%) were the major reasons for failures in the drug development, in addition to the lack of efficacy, adverse effects in man and commercial reasons. In addition to the experimental evaluation of ADME/T, *in silico* predictions of these properties have gained popularity in pharmaceutical industries [8]. As a result, the physicochemical properties and toxicology of each derivative were also computed.

MATERIALS AND METHODS

Preparation of macromolecule

The 3D crystal structure of human cathepsin K in complex with 1-[2-(3-biphenyl)-4-methylvaleryl)] amino-3-(2pyridylsulfonyl) amino-2-propanone (PDB code: 1BGO) was retrieved from the RCSB protein data bank [9]. The dock prep tool of UCSF chimera version 1.10.1 for Mac was used to prepare the receptor for docking [10]. Eventually, the macromolecule was saved in pdbqt format using Python Prescription (PyRx) 0.8 for Mac [11] that contains a hydrogen atom in all polar residues of the macromolecule. The quality of the prepared molecule was analyzed using Ramachandran plot and VERIFY 3D.

Ligands preparation

The 2D chemical structures of the derivatives (Table 1) were prepared using ChemBio Draw 14.0.0.117 for Mac [12]. The 2D chemical structures were converted into the respective 3D structures using the Open Babel of PyRx 0.8 virtual screening tool for Mac. After energy minimization of each derivative using Uff force field of the Open Babel, the derivatives were converted into autodock ligands.

Docking validation

The ligand from the active site of the crystal structure of cathepsin K was removed using UCSF Chimera for Mac. After the ligand was redocked using PyRx 0.8 autodock vina [13], the alignment between the docked ligand and the ligand from the crystal structure was done using MacPyMOL [14].

Molecular docking

Docking was performed using PyRx autodock Vina [13]. The results were quantified in terms of free binding energy (ΔG). The highest binding energy values corresponding to the RMSD value of zero were considered as the binding affinity value of the ligands.

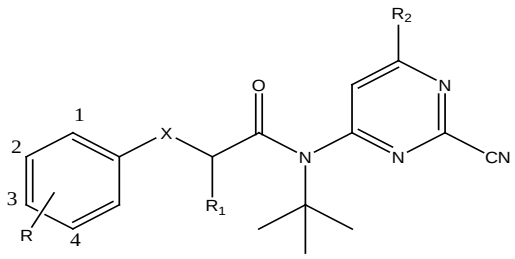
Osiris property explorer

The Osiris Property Explorer is an integral part of Actelion's in-house substance registration system. It lets you draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid. Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red. Whereas a green color indicates drug conform behavior [15].

RESULTS AND DISCUSSION

Ramachandran plot, which is a commonly used indicator of the quality of the macromolecule model, of prepared macromolecule was obtained using RAMPAGE [16]. The percentage of residues in the favored region and in allowed region was 96.2% and 3.8%

Table 1: The 2D chemical structures of the lead compound and its derivatives.



Compounds	R	X	R_1	R_2
Lead compound	H	O	H	H
Compound 1	2-CF_3	O	H	H
Compound 2	3-CF_3	O	H	H
Compound 3	4-CF_3	O	H	H
Compound 4	3t-butyl	O	H	H
Compound 5	3-phenyl	O	H	H
Compound 6	3-cycloheptyl	O	H	H
Compound 7R	3t-butyl	O	α-methyl	H
Compound 7S	3t-butyl	O	ß-methyl	H
Compound 8	3t-butyl	O	α-ethyl	H
Compound 9	3t-butyl	O	α-propyl	H
Compound 10	3t-butyl	O	α-butyl	H
Compound 11	3t-butyl	O	α-isobutyl	H
Compound 12	3t-butyl	NH	α-ethyl	H
Compound 13	3t-butyl	NH	α-ethyl	Formic acid
Compound 14	3t-butyl	NH	α-ethyl	Methyl ethanoate
Compound 15	3t-butyl	NH	α-ethyl	Methyl propionate
Compound 16	3t-butyl	NH	α-ethyl	Acetic acid
Compound 17	3t-butyl	NH	α-ethyl	Propionic acid
Compound 18	3t-butyl	NH	α-ethyl	α-methyl propionic acid

respectively. Moreover, the number of residues in outlier region was zero (Figure 1).

The Structural Analysis and Verification Server (SAVES) were also used for checking and validation of the protein structure [17]. In VERIFY3D result, 97.67% of the residues had an averaged 3D-1D score >=0.2. This value is higher than the pass value, which is at least 80% of the amino acids with a score >=0.2 in the 3D-1D profile.

Docking validation

The docking validation showed that the X-ray crystallographic conformer was nearly identical with the docked conformer, as deducted from the alignment of the two structures with RMSD value of 1.46 Å [Figure 2].

Analyzing the docking result

The docking of the lead compound gave -6.4 Kcal/mol free energy of binding [Table 2]. The substitution of the phenoxy at 2, 3 or 4 positions with trifluoromethyl group led to an increase in binding affinity. The substitution of the phenoxy at position 3 with t-butyl, phenyl or cycloheptyl groups greatly enhanced the affinity. Particularly, Compound 6 that contains a cycloheptyl group showed the lowest binding free energy (-7.3 Kcal/mol). Those results can be translated as the bulkier the substituent is at position 3 of the phenoxy moiety, the higher the binding affinity will be. The crystal structures ofsome derivatives [Figure 3] showedthat the spacious nature of the binding site of the cathepsin K crystal structure.

The residues that are found to be involved in hydrogen bonding interactions and hydrophobic interactions are given in Table 3. The table explains that hydrophobic interactions are the main intermolecular force of interactions responsible for the binding affinity of the cyanopyrimidine derivatives.

The second pattern of substitution focused on the space between the phenoxy and the 2-cyanopyrimidine moieties. Although an asymmetric center is created in substitutions of such kind, a significant difference in the binding affinity of enantiomers was not observed. This was demonstrated with almost no difference in the binding affinity between compound 7S (-6.7 Kcal/mol) and 7R (-6.6 Kcal/mol). R_1 substituent generally decreased the binding affinity, which suggests that the spacer may be sterically demanding.

The replacement of the ether with a secondary amine does

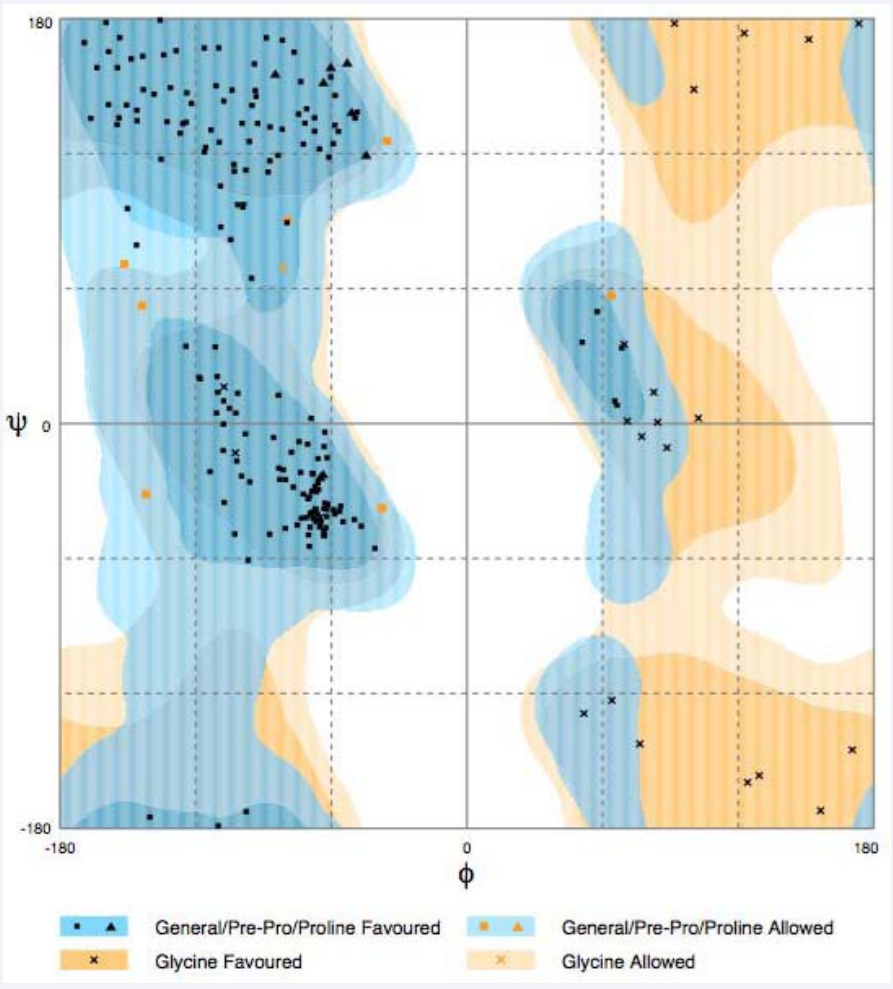


Figure 1 Ramachandran plot for the analysis of ψ and ϕ torsion angles for all residues on the macromolecule prepared for docking.

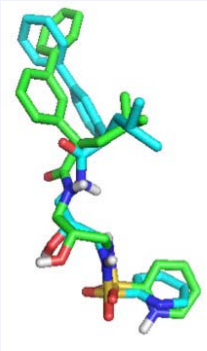


Figure 2 The alignment of the docked conformer on the x-ray crystallographic conformer in the active site of cathepsin K.

Table 2: The predicted binding affinity, toxicity and physico-chemical properties.

Compounds	Binding affinity (Kcal/mol)	Mutagenicity	Tumorogenicity	Irritant effect	Reproductive toxicity	cLog *P*	Solubility	Drug likeness	Drug score
Lead	-6.4	---	---	---	---	2.29	-3.25	-2.36	0.46
C1	-7.0	---	---	---	---	3.14	-4.03	-10.2	0.36
C2	-6.8	---	---	---	---	3.14	-4.03	-8.96	0.36
C3	-6.5	---	---	---	---	3.14	-4.03	-10.2	0.36
C4	-7.1	---	---	---	---	3.87	-4.41	-3.46	0.34
C5	-6.7	---	---	---	---	3.95	-5.34	-2.27	0.3
C6	-7.3	---	---	---	---	4.77	-5.29	-6.35	0.24
C7R	-6.7	---	---	---	---	4.23	-4.79	-2.66	0.31
C7S	-6.6	---	---	---	---	4.23	-4.79	-2.66	0.31
C8	-6.1	---	---	---	---	4.69	-5.06	-1.4	0.31
C9	-6.6	---	---	---	---	5.14	-5.33	-3.71	0.23
C10	-6.7	---	---	---	---	5.6	-5.6	-7.47	0.2
C11	-5.5	---	---	---	---	5.36	-5.49	-2.02	0.23
C12	-6.1	---	---	---	---	4.4	-5.1	-1.53	0.32
C13	-6.3	---	---	---	---	3.94	-5.14	-1.58	-
C14	-6.2	---	---	---	---	4.78	-6.08	-1.17	0.25
C15	-5.6	---	---	---	---	5.24	-6.35	-3.05	0.18
C16	-6.8	---	---	---	---	3.94	-5.1	-1.59	0.31
C17	-6.3	---	---	---	---	4.4	-5.37	-2.69	0.25
C18	-6.6	---	---	---	---	4.61	-5.53	-1.7	0.25

Table 3: Summary of the residues interacting with cyanopyrimidine derivatives.

Compounds	Hydrogen bonding interaction	Hydrophobic interactions
Lead compound	V149	S141, Y143, V149, Y150, Y151, N175, A197, K200
Compound 1	G19	Q19, G23, H162, Q143, W184, W188
Compound 2	T14	R8, V13, P15, V16, N190, K191, Y193 L195
Compound 3	Y193	R8, G11, V13, P15, V16, L45, E186, K191, Y193
Compound 4	Q19, C25, N161	Q19, C25, Q143, N161, H162, W184
Compound 5	V16	R8, V13, P15, V16, G186, K191, Y193
Compound 6	Q19, G64, 234H_2O	Q19, C63, Q143, N161, W184, W188
Compound 7R	G64	Q19, G64, G23, Q143, N161, W184
Compound 7S	Q19, 234H_2O	G23, C25, Q143, H162, W184, W188
Compound 8	234H_2O, 231H_2O	G23, G66, A134, A137, Q143, N161, H162, A163, W184

Compound 9	---	S141, K147, G148, V149, Y150, Y151, N175, A197, K200
Compound 10	Q19	Q19, S138, Q143, N161, H162, W184,
Compound 11	N161, 231H_2O	G23, G64, G66, Y67, H162, L160, N161
Compound 12	Y193	R8, K9, V13, P15, V16, E186, K191, Y193
Compound 13	Q19, G20, W184	N18, Q19, G20, C25, S138, N161, H162, W184
Compound 14	Q19, C25 (2*)	N18, Q19, G21, G23, C25, G65, A137, H162, W184
Compound 15	Y89	K17, N18, Q19, G20, F28, P50, E84, Y89, V90
Compound 16	Q143, Y145, G189	Q143, F144, Y145, N187, W184, W188, G189
Compound 17	N47, E84, E186	P15, V16, K17, N18, N47, E84, Y89, G185, E186
Compound 18	V16, N47, E84, E186 (2*)	P15, V16, K17, N18, F28, P50, E84, Y89, V90, E186
* Indicates the number of hydrogen bonds		

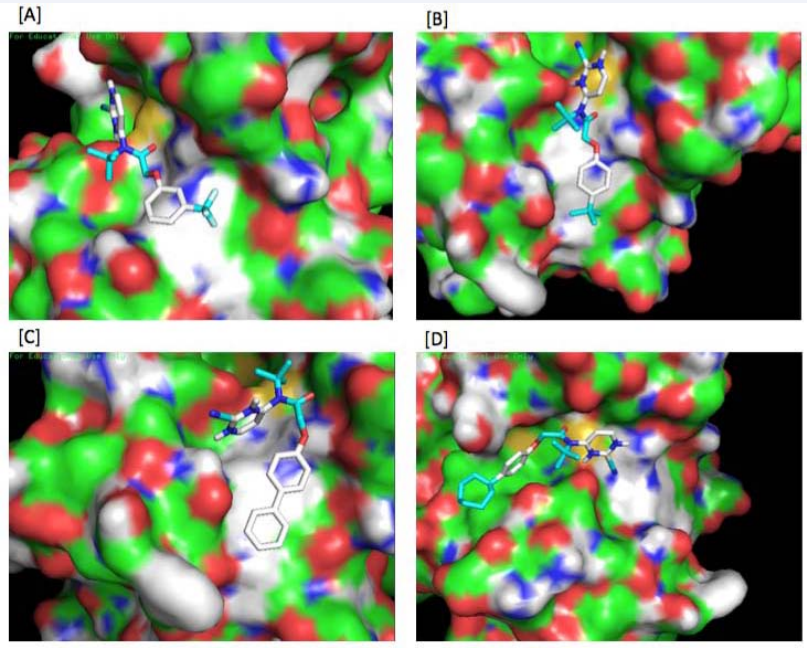


Figure 3 The binding of [A] compound 1, [B] compound 4, [C] compound 5 and [D] compound 6.

not produce a significant change in binding affinity, which is revealed by a nearly similar binding affinity between compound 8 (-6.3 Kcal/mol) and compound 12 (-6.1 Kcal/mol).

The substitution at position 4 of 2-cyanopyrimidine with alkyl groups having good water solubility was investigated as S1 pocket was found to accommodate polar aliphatic amino acids. However, such kind of substitutions was predicted to be not favorable. This can be clearly observed from the decrease in binding affinity when the size of the substitution was increased from methyl ethanoate as in compound 14 (-6.2 Kcal/mol) to methyl propionate (-5.6 Kcal/mol). This may be due to a steric clash with the active site of cathepsin K, which may reduce an entry into the deep pocket. Carboxylic acid containing substituents at position 4 of cyanopyrimidine has decreased the affinity to the active site of cathepsin K.

CONCLUSIONS AND RECOMMENDATIONS

The derivatives showed a potential as a cathepsin K inhibitor. Increasing the size lipophilic substituents of the phenoxy moiety was predicted to increase the binding affinity. In contrary, substitution of cyanopyrimidine was not in favor of binding affinity. All ligands were also predicted to be safe from toxicities like mutagenicity and tumorogenicity. An additional research on the synthesis and determination of IC_{50}, however, is required.

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