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IDENTIFICATION OF A POTENT INHIBITOR AGAINST CATHEPSIN-K FOR OSTEOPOROSIS: A STRUCTURE BASED VIRTUAL SCREENING APPROACH

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ABSTRACT

Cathepsin K (Cat K) is a lysosomal cysteine protease which involves in degradation of bone may lead to severe bone fragility in Osteoporosis patients. Cat K is the primary enzyme involved in osteoclastic bone resorption. Inhibition of Cat K represents a potential drug target for Osteoporosis. Odanacatib (CID 10152654) is a known potent, reversible nonpeptidic biaryl inhibitor for Cat K. The PubChem Database was screened for similar potent drug like compounds as Odanacatib. Virtual Screening and Docking studies were performed for these molecules against Cat K protein using PyRx Virtual Screening Tool and AutoDock Vina. The docking results showed that the compounds CID42633343, CID42634247, CID42634755, CID42634578 and CID44447660 were having highest binding affinity values like -10, -8.9, -8.8, 8.6 and 8.6. The present study indicates that the lead molecules have to be evaluated further for better potential lead molecules.

KEYWORDS : **Cat K, Virtual Screening, Docking, SMILES, H-bond.**

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INTRODUCTION

Osteoporosis is a bone disease and a major public health problem. The characteristics feature of Osteoporosis is bone loss mediated by osteoclasts and insufficient rebuilding of bone matrix by osteoblasts. Bone consists of up to 90% type-I collagen fibers, which provide the scaffold for mineral apposition. During Osteoporosis bone loses its mineral as well as organic matrix content due to the activity of osteoclasts. The collagenases of the matrix metalloproteinase (MMP) family have been considered [1] as the main culprit proteases for the degradation of collagen. During bone resorption, multinucleated osteoclasts [1] form lacunae on the surface of the bone, into which protons and proteases are secreted. This acidic environment is suitable for proteolytic degradation of the collagen component. Cat K has high collagenase [2, 3] activity at the acidic pH, which is required to degrade the collagen matrix. Several studies have shown that Cat K deficiency [4] leads to an increase in bone mineral density (BMD). Recent reports on the inhibition of Cat K have focused on derivatives of aldehydes [5], amino methyl ketones [6, 7, 8], hydroxymethyl ketones [9], ketobenzoxazoles [10] and most recently nitriles[11, 12, 13]. It is clear that Cat K represents a critical bone resorbing protease and the race was on to develop highly selective Cat K inhibitors for the treatment of Osteoporosis. In this study, Odanacatib (Mk-0822) is used as the template in database searching based on the structure similarity. PubChem Database was used in the database searching for finding out novel and potent active compounds with low toxicity by using advanced bioinformatics tools.

MATERIALS AND METHODS

1. Preparation of the protein structure

The X-ray crystal structure of Cat K complexed with the ligand was obtained from the Protein Data Bank (1TU6) with a resolution of 1.76 Å [14]. The atomic coordinates of the protein was separated and its geometry [15] optimized with Argus Lab 4.0.1. For docking with Auto Dock Vina, default parameters were applied to the protein. The enzyme Cat K structure is showninFigure1.

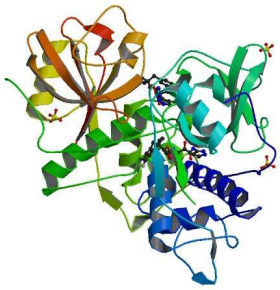


Figure 1
Cathepsin K Complex molecule

2. Preparation of the ligand structure

The chemical structure of Odanacatib (Mk-0822), which has got high potency for Cat K (IC_{50} = 0.2nm) is shown in Figure 2. It is a potent, reversible nonpeptidic biaryl inhibitor for Cat K. Therefore, Odanacatib is used as the template in PubChem database searching based on the structure similarity. The dataset contains 85 chemical structures. All the atomic coordinates were converted to pdbqt format using Open Babel GUI © 2006 (developed by Chris Morley) [16].

Figure 2
Odanacatib

3. Virtual screening

The Odanacatib drug molecule was used as a search query to retrieve molecules with novel chemical structures. PyRx Virtual Screening Tool was used to search the database. The retrieve molecules were selected for docking studies and also to study the interactions with active site of Cat K.

4. Molecular docking studies

For the docking of ligands into protein active site and to estimate the binding affinities of docked compounds an advanced molecular docking program AutoDock Vina (1.1.2) was used in this study. The rotational bonds of the ligands were treated as flexible while those of the protein were kept rigid. Grid boxes were fixed around an active site of the protein. Genetic Algorithm (GA) was used for searching; the scoring functions for the interactions were calculated and docking evaluation was carried.

5. Structural analysis and Visualization

Protein – ligand interaction was analyzed and visualized by PyMol (0.99rc6).

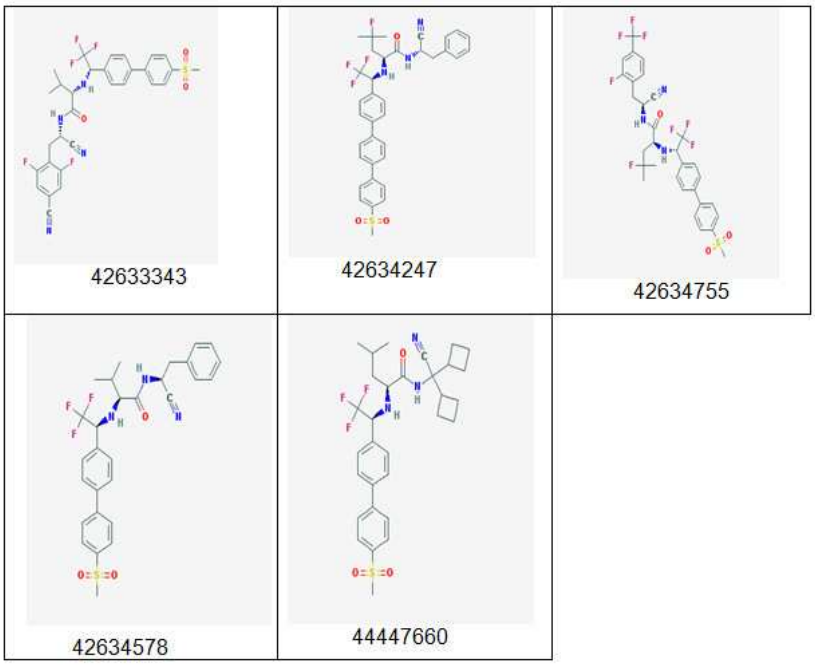
RESULTS AND DISCUSSION

Cat K is an attractive target for Osteoporosis disease. Although several inhibitors of Cat K have been clinically validated for the treatment of Osteoporosis during the past years, the search for new active compounds against Cat K is still considerably challenging. Our work aimed at using molecular docking and virtual screening to filter millions of compounds to identify new anti Cat K inhibitors.

1. Virtual Screening result

85 compounds from PubChem [17], all structural analogs of Odanacatib, were selected using the search algorithm of the website and accessing the Odanacatib chart in PubChem through the option ‘‘search similar structures.’’ This option uses a locally developed simplified molecular input line entry specification (SMILES) string comparison method to identify related structures and perform structure similarity searches. All structures are converted into SMILES strings, and a substring-matching program (similar to BLAST) is used to identify similar structures. The scoring scheme is based simply on the number of character matches for the longest matching substring [17] . PyRx Virtual Screening Tool was used to screening the compounds. The retrieve molecules were selected for docking studies, to study the interactions with active site of Cat K. The screening molecules were listed in Table 1.

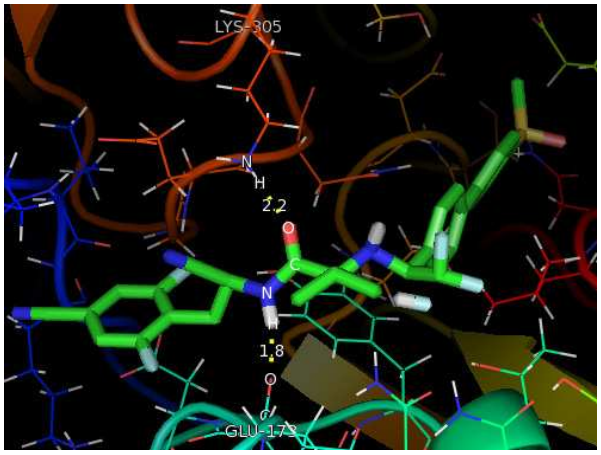
Table 1
Chemical structures of the best binding affinity molecules



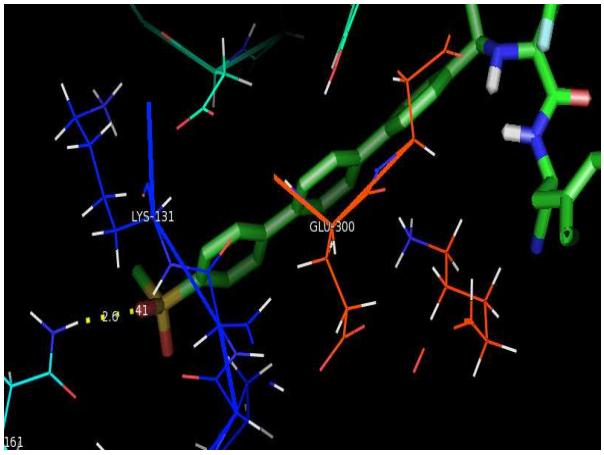
2. Docking result

Three-dimensional structure information of the target protein was taken from the PDB entry 1TU6; with a resolution of 1.76Å [14]. Processing of the protein included the deletion of the ligand and the solvent molecules as well as the addition of hydrogen atoms. AutoDock Vina was used for the docking [18] studies. The docking scores are listed in Table 2. In the ligand and protein docking calculations, hydrogen bonds (H-bond) are supposed to make important contributions to the interactions[19, 20, 21, 22] between the ligand and protein. The docking results indicate there is one H-bond for Odanacatib ligand to the 1TU6, which is between a CN of ligand (Odanacatib) and OC of ASN 174 (3.2Å) of protein. There are two H-bonds between 42633343 ligand and 1TU6. The first H-bond is NH of the ligand to CO of GLU 173 (1.8Å), the second H-bond is OC of ligand to NH of LYS 305 (2.2Å) of protein. There is one H-bond between SO of 42634247 ligand to HN of ASN161 (2.6 Å) of protein. There are two H-bonds between 42634755 ligand and protein. One H-bond is NH of the ligand to OC of LYS305 (1.9 Å) of protein and the second H-bond is NH of ligand to OC of GLU 173 (2.1 Å) of protein. There is one H-bond between NH of 42634578 ligand to OC of ASN301 (2.3Å) of protein. There are two H-bonds between 44447660 ligand and protein. One H-bond is NH of the ligand to OC of GLU173 (2.4Å) of protein and the second H-bond is NH of ligand to OC of LYS305 (2.1Å) of protein. These findings would be helpful for future rational drug design. The interactions are shown in Table 3.The graphical view ligand and protein interactions are shown in Figure 3.

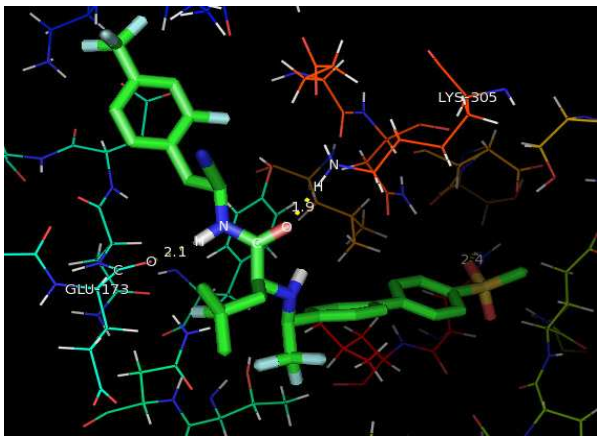
42633343



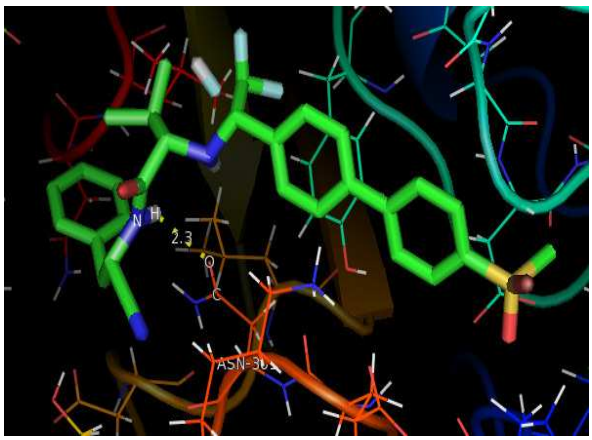
42634247



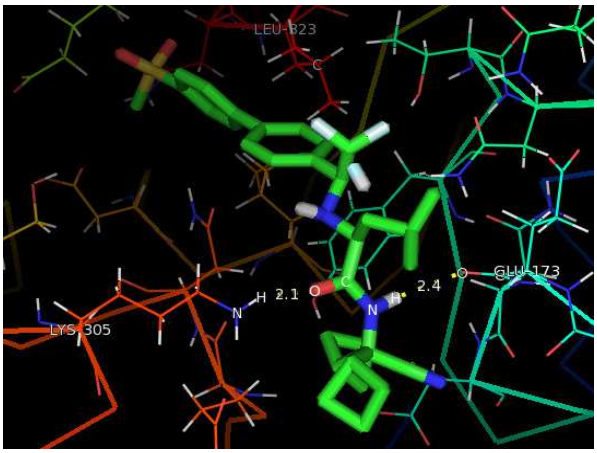
42634755



42634578



44447660



Odanacatib

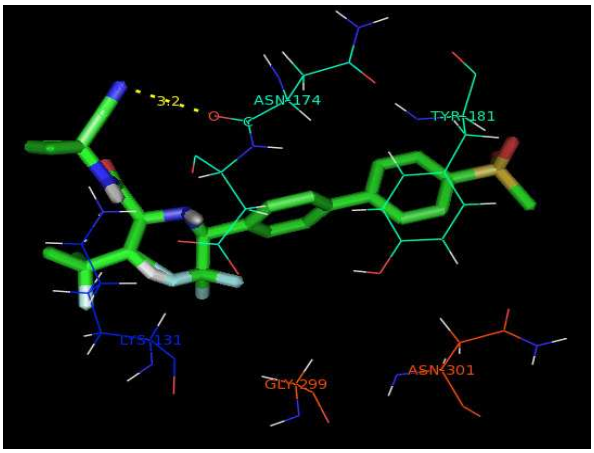


Figure 3
Binding of ligands at the active site of Cat K protein

Table 2
Docking ligands show their binding affinity values

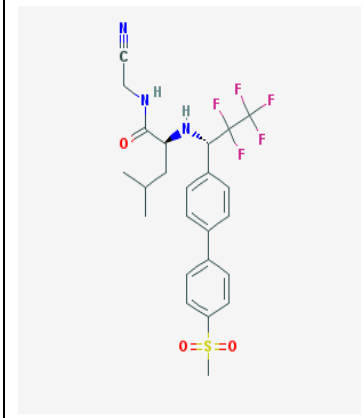
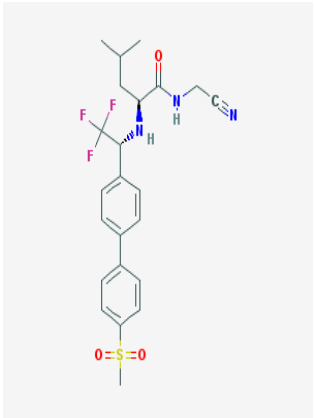
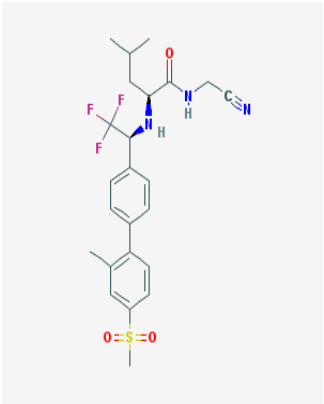
S. No.	Ligand	Binding Affinity
1	1TU6energymini_Odanacatib	-8.1
2	1TU6energymini_42633343	-10
3	1TU6energymini_42634247	-8.9
4	1TU6energymini_42634578	-8.6
5	1TU6energymini_42634755	-8.8
6	1TU6energymini_44447660	-8.6

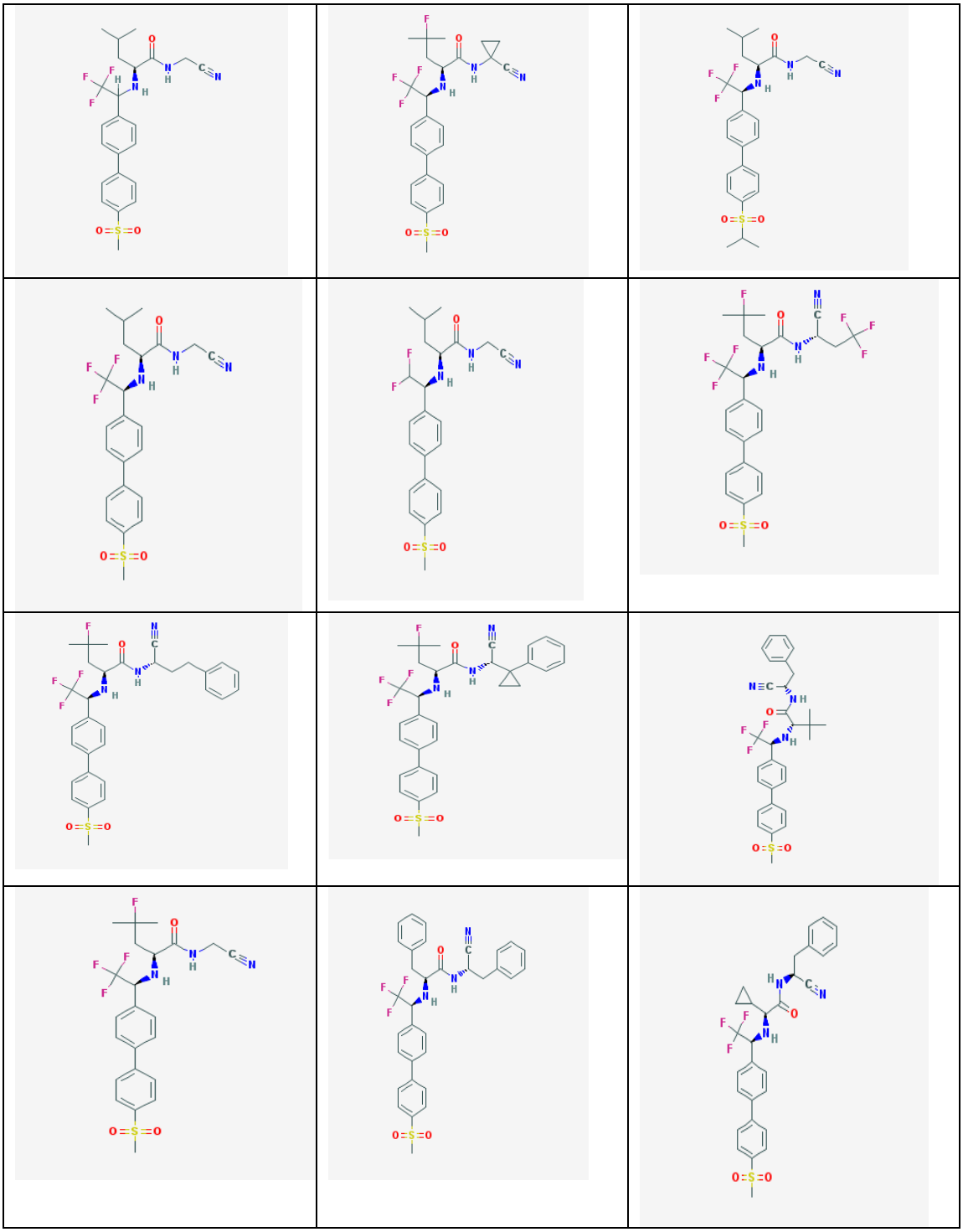
Table 3
Hydrogen bonding interactions of ligands, against the structure of Cat K protein

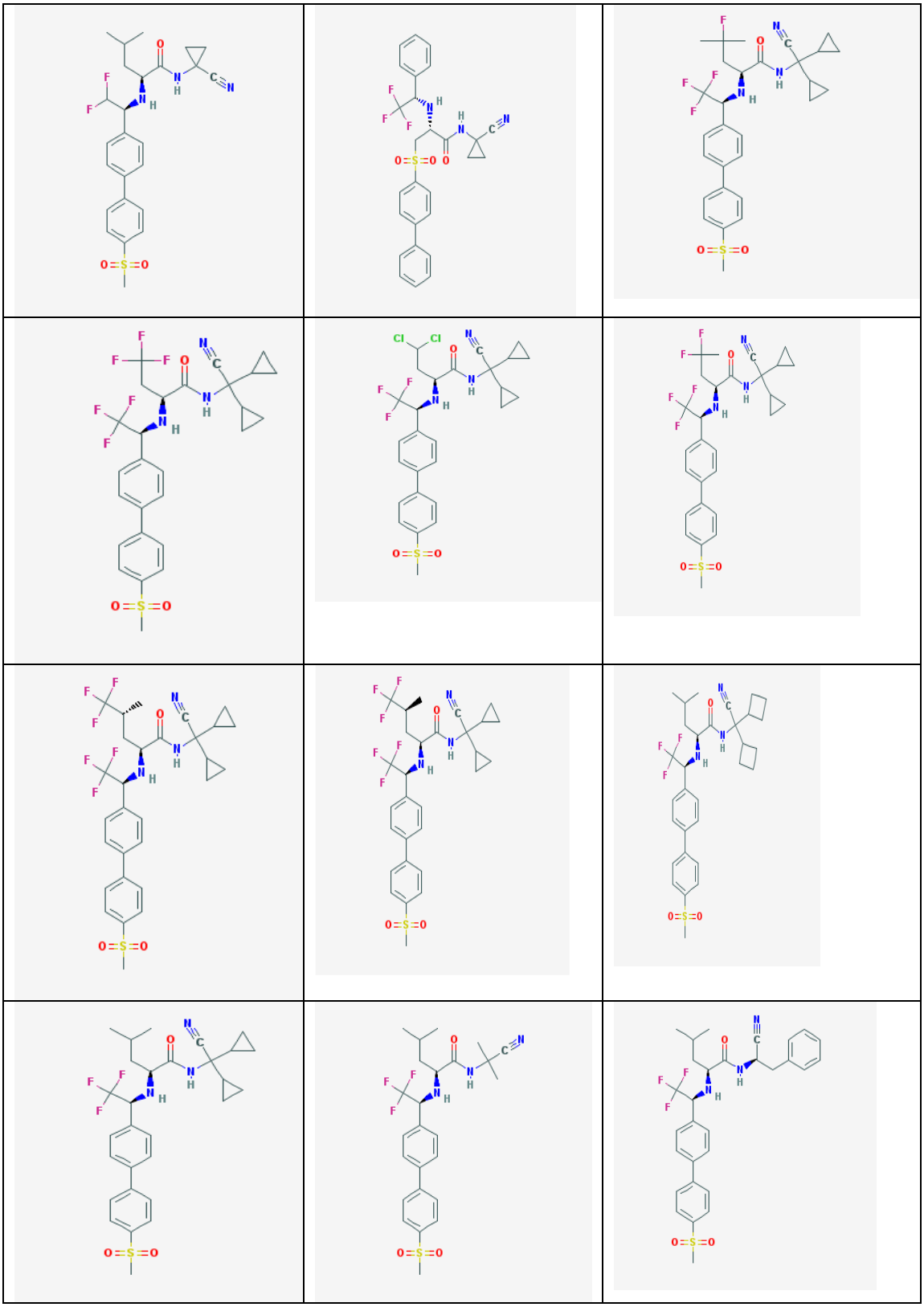
S.No.	PubChem Compounds(CID)	H-bonding interactions		Residues	Binding Affinity (K/cal)	H-bond distance(Å)
		Ligand	Protein			
1	42633343	NH---	---CO	GLU173	-10	1.8
		OC---	---NH	LYS305		2.2
2	42634247	SO---	---HN	ASN161	-8.9	2.6
3	42634755	NH---	---OC	LYS305	-8.8	1.9
		NH---	---OC	GLU173		2.1
4	42634578	NH---	---OC	ASN301	-8.6	2.3
5	44447660	NH---	---OC	GLU173	-8.6	2.4
		NH---	---OC	LYS305		2.1
6	Odanacatib	CN----	---OC	ASN174	-8.1	3.2

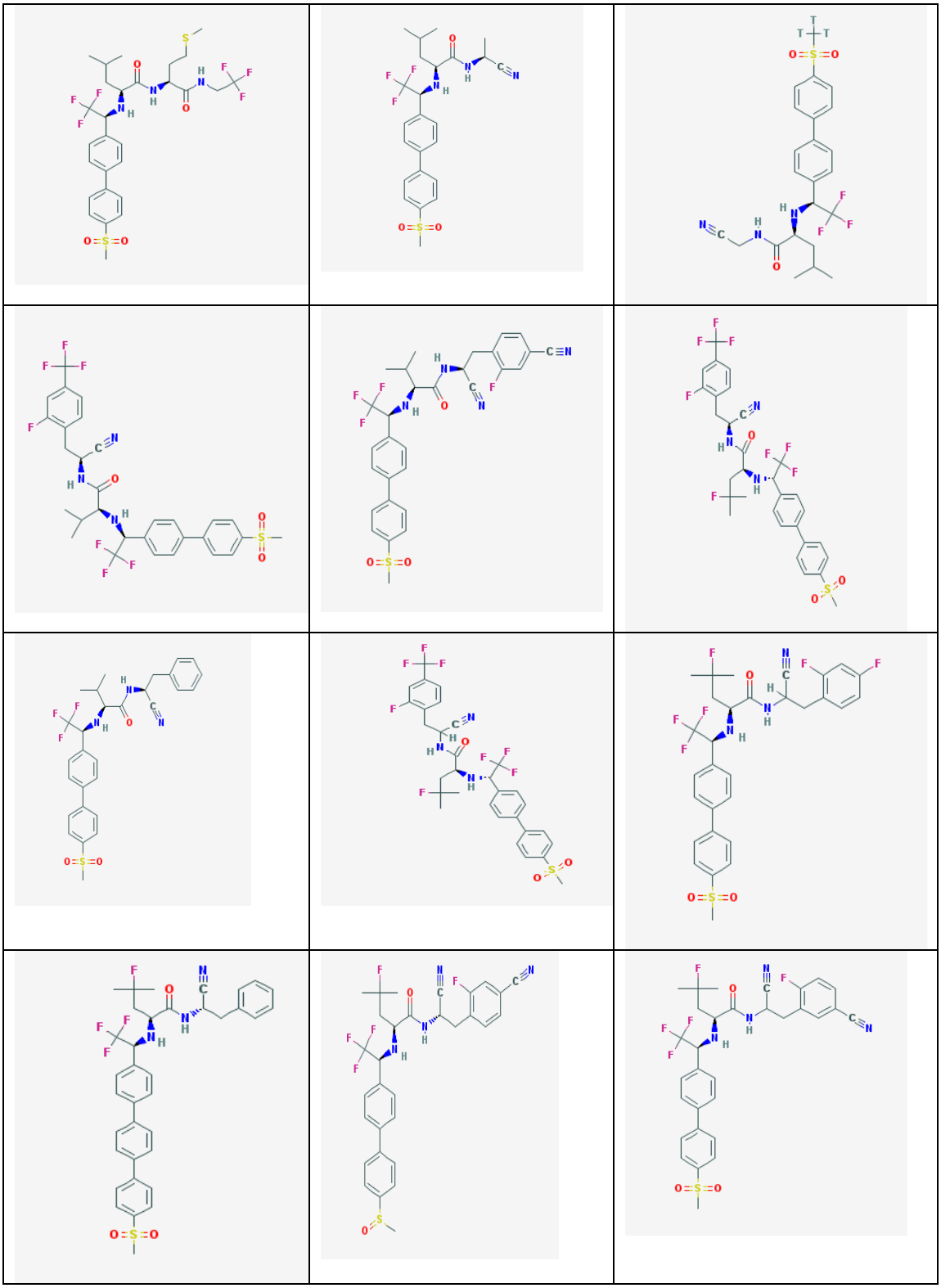
Screening Molecules

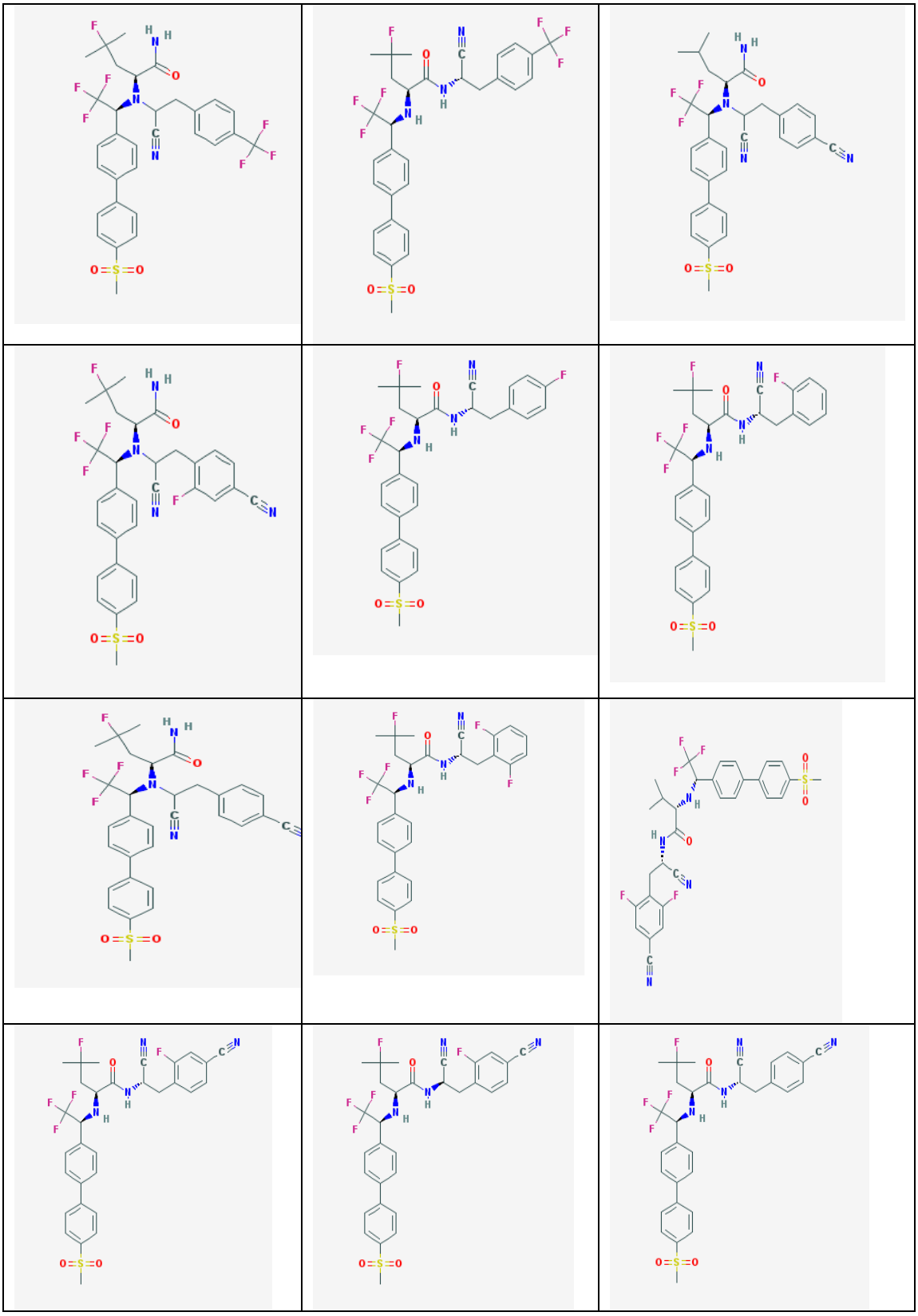
Table4
Chemical structures of the screening compounds.

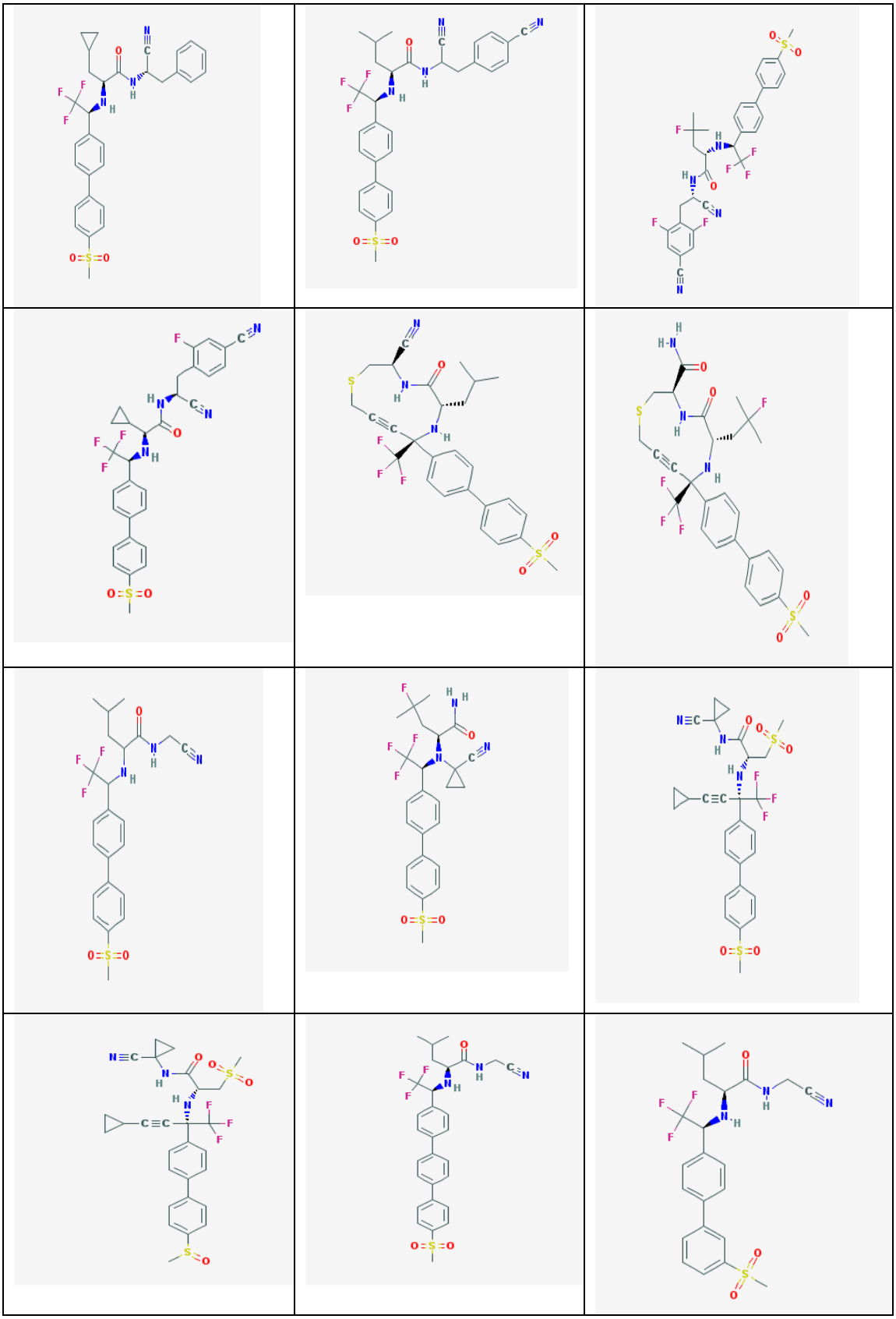




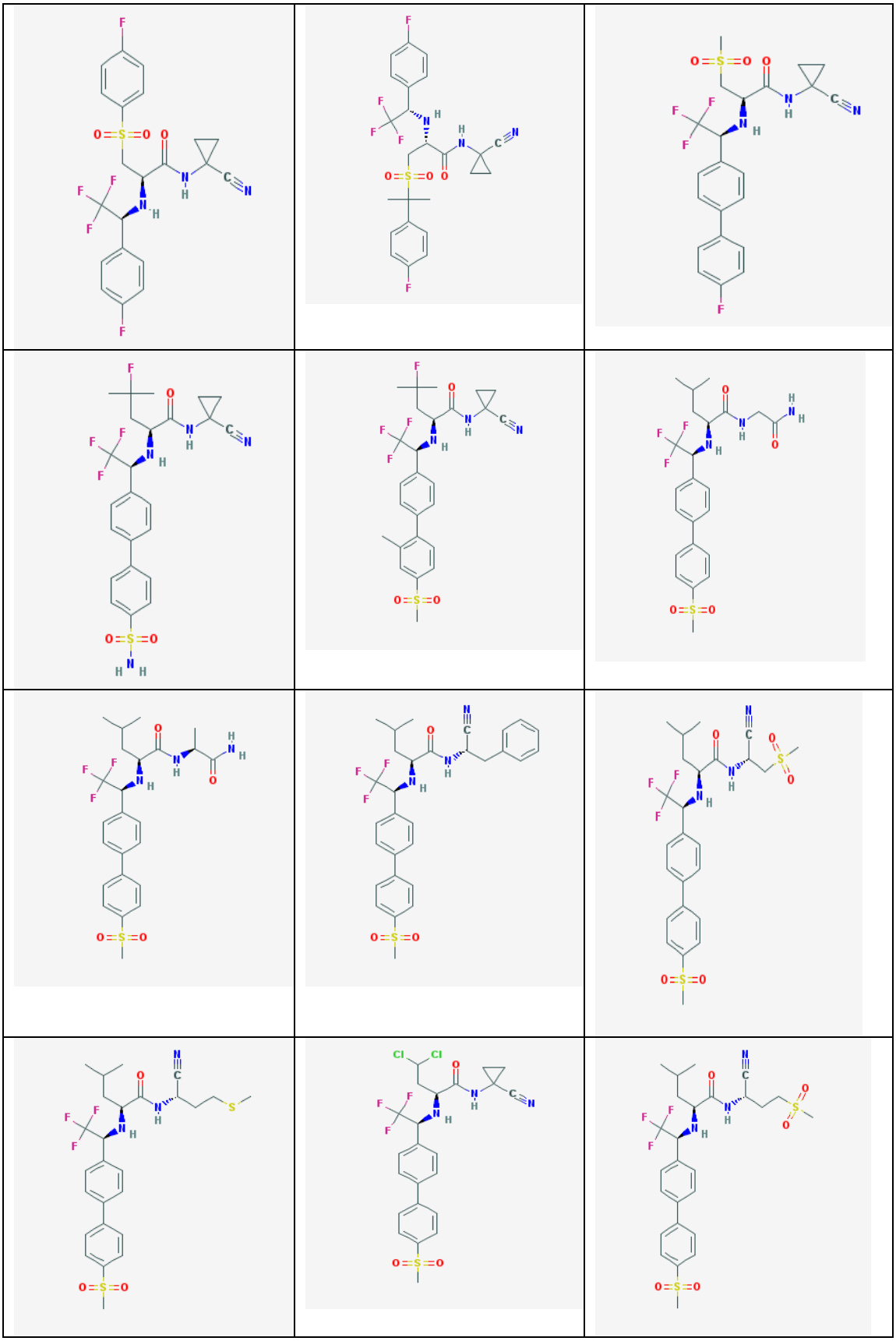












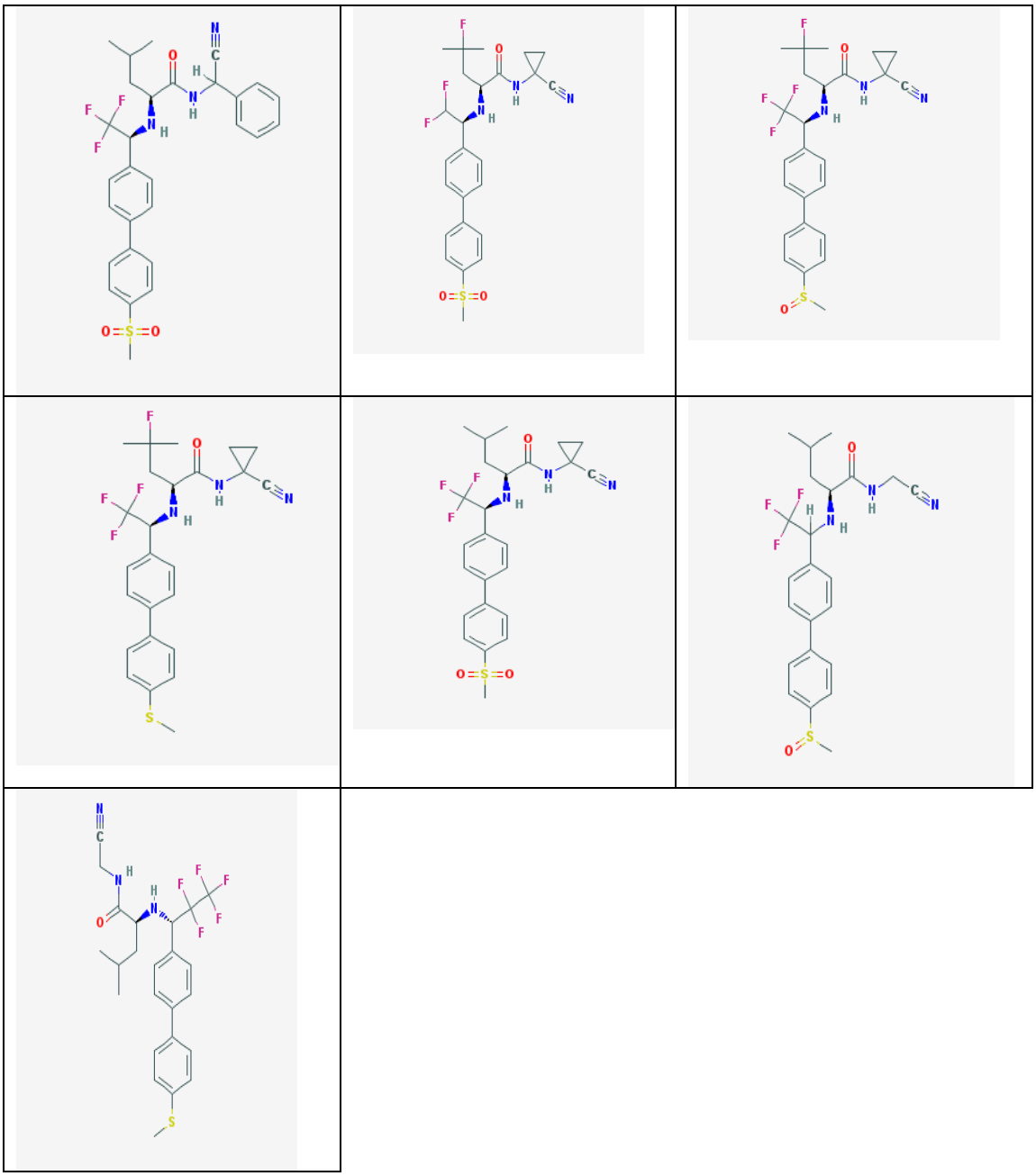


Table 5
Various ligands show their binding affinity values.

Ligand	Binding Affinity
1TU6energymini_Odanacatib	**-8.1**
1TU6energymini_9913088	-6.6
1TU6energymini_10152443	-6.8
1TU6energymini_10152654	-6.9
1TU6energymini_10174190	-8
1TU6energymini_10217728	-6.8
1TU6energymini_10238886	-6.7

1TU6energymini_10239534	-6.8
1TU6energymini_10289119	-6.4
1TU6energymini_10310741	-6.4
1TU6energymini_11283555	-7
1TU6energymini_11496895	-8.1
1TU6energymini_11540740	-6.8
1TU6energymini_11670661	-6.9
1TU6energymini_15985697	-7.4
1TU6energymini_15985699	-7.2
1TU6energymini_15985700	-7.1
1TU6energymini_15985701	-6.9
1TU6energymini_15985753	-7.3
1TU6energymini_15985754	-7.6
1TU6energymini_16661368	-6.8
1TU6energymini_16661369	-6.9
1TU6energymini_16666339	-6.5
1TU6energymini_16666340	-6.9
1TU6energymini_23648280	-6.7
1TU6energymini_23648288	-6.9
1TU6energymini_23648288	-7
1TU6energymini_23648289	-7.1
1TU6energymini_23648303	-6.9
1TU6energymini_23648308	-6.6
1TU6energymini_24900649	-5.6
1TU6energymini_24900650	-6.5
1TU6energymini_25020135	-7.3
1TU6energymini_25119462	-6.9
1TU6energymini_25134765	-6.2
1TU6energymini_25134766	-6.6
1TU6energymini_42632968	-8.4
1TU6energymini_42632969	-7.1
1TU6energymini_42633146	-8
1TU6energymini_42633150	-7.8
1TU6energymini_42633339	-8.5
1TU6energymini_42633340	-7.9
1TU6energymini_42633341	-8.4
1TU6energymini_42633343	**-10**
1TU6energymini_42633522	-8.1
1TU6energymini_42633692	-7.9
1TU6energymini_42633693	-8
1TU6energymini_42633695	-7.1
1TU6energymini_42633698	-6.3
1TU6energymini_42633881	-8.2

1TU6energymini_42633882	-6.3
1TU6energymini_42634064	-8.5
1TU6energymini_42634066	-8.1
1TU6energymini_42634247	**-8.9**
1TU6energymini_42634576	-7.6
1TU6energymini_42634577	-8
1TU6energymini_42634578	**-8.6**
1TU6energymini_42634755	**-8.8**
1TU6energymini_42635119	-8.3
1TU6energymini_42635298	-8.3
1TU6energymini_44442257	-7.8
1TU6energymini_44442268	-7.3
1TU6energymini_44447657	-7.3
1TU6energymini_44447658	-8
1TU6energymini_44447659.	-7.9
1TU6energymini_44447660	**-8.6**
1TU6energymini_44447661	-7.6
1TU6energymini_44447662	-7.1
1TU6energymini_44447663	-7.1
1TU6energymini_44447664	-7
1TU6energymini_44447665	-7.3
1TU6energymini_44447666	-7.5
1TU6energymini_49848860	-7.1
1TU6energymini_51003522	-7.2
1TU6energymini_52941283	-6.1
1TU6energymini_52941414	-8.1
1TU6energymini_52942931	-6.5
1TU6energymini_52943722	-6.1
1TU6energymini_52943841	-7.4
1TU6energymini_52946267	-7.5
1TU6energymini_52947761	-7.2
1TU6energymini_53320190	-7.6

CONCLUSIONS

In an attempt work, we have searched for novel and potent anti Cat K inhibitors through Virtual Screening on PubChem database based on structural similarity of ligand (Odanacatib). The newly identified cysteine protease enzyme Cat K is an important target in drug design for therapeutic intervention of Osteoporosis disease. Because the function of Osteoporosis is excessive bones resorption, the inhibition of Cat K may prevent the resorption. Among screened compounds CID 42633343, CID42634247, CID42634755, CID42634578 and CID44447660 have the highest binding affinity compare to Odanacatib. Therefore, it may be suggested that best lead compounds are a hopeful drug molecule like Odanacatib against Osteoporosis.

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