Discovery of dual inhibitors of KPC-3 and KPC-15 of Klebsiella pneumoniae – a ligand and structure based virtual screening study

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ABSTRACT

Background: Development of carbapenem resistance against *Klebsiella pneumoniae* is a situation of grave concern and require urgent attention. Among the KPC produced by *K. pneumoniae*, KPC-3 and KPC-15 play a major role in development of resistance to carbapenems.

Materials and methods: KPC-2 structure was taken and then mutated to obtain the structure of KPC-3 and KPC-15. The binding sites of KPC-3 and KPC-15 were predicted by the COACH server. Drug like ligands from ZINC were then screened by ligand-based drug screening (LBVS) by keeping Relebactam as template. The top 50,000 selected ligands were then screened by structure based virtual screening (SBVS) using *idock*. The consensus weighted rank was computed for identifying ligands with dual inhibitory property. Relebactum was kept as a comparator for SBVS and the similarity search was conducted between the identified top 3 ligands against Relebactam. The ADMET properties were explored using admetSAR.

Results: Based on consensus weighted ranks, top 3 ligands with dual inhibitory property are- ZINC76060350 (consensus weighted rank - 1.5), ZINC05528590 (2), ZINC72290395 (3.5). All the top 3 dual inhibitor have a good probability of passing through the blood brain barrier. The RDKit and Morgan fingerprint scores between Relebactam and top three ligands were 0.24, 0.22, 0.23 and 0.26, 0.19, 0.25 respectively (showing only 20% similarity). Therefore, the three identified ligands may independently be effective in inhibiting the activity of KPC-3 and KPC-15.

Conclusion: The suggested ligands could be taken forward for the development of new drug against a multi-resistant- *Klebsiella pneumoniae* infections.

Introduction

Carbapenems antibiotics like imipenem and meropenem has been one of the best available antibiotics which are used as the 1st line of therapy in case of severe infection caused by extended-spectrum beta (b)-lactamases (ESBL) producing Enterobacteriaceae [1–3]. The activity of carbapenem could be augmented by addition of beta lactamase inhibitors. An example of this is imipenem which showed a significant improvement in activity against various species of Enterobacteriaceae following addition of Relebactam [as assessed by decrease in the minimum inhibitory concentration (MIC)] [4]. Having said this, Carbapenem-resistant *Enterobacteriaceae* is a grave concern against this potent drug and it is spreading in fast pace all around the globe with increase in morbidity and mortality associated with healthcare-associated infection (HAI). The mechanism involved in the development of resistance to carbapenem are multi-fold ranging from (i) modifications in the outer membrane permeability, (ii) the up-regulation of the efflux system corresponding to the hyperproduction of AmpC β lactamases (cephalosporinases) or ESBL's, and (iii) the production of an enzyme which degrades the carbapenem (carbapenemases) [5,6]. Among the Carbapenem-resistant Enterobacteriaceae, the most common bacteria is *K. pneumoniae* [7]. This drove our attention towards infection and resistance caused by *Klebsiella pneumonia* and development of a drug against this entity.

Klebsiella pneumonia, a gram-negative nosocomial bacteria is responsible for both community-acquired and HAI [8]. The carbapenem act by binding to the penicillin binding proteins (PBP) of the bacteria thereby resulting in the lysis of the bacterial cell wall. This in return instigate the bacterial cell to produce carbapenemase which binds with the carbapenems to hydrolyse the antibiotics containing beta-lactams [9]. Carbapenemases (KPC-2 & its variant KPC-3 and KPC – 15) production by K. pneumoniae is mainly responsible for the resistance against carbapenem [10–13]. Based on molecular classification, the carbapenemases are divided into classes A to D. The active site of classes A and C contain serine where's class B contain zinc (metal). Class A and C are therefore referred to as serine (non-metallo) β-lactamases whereas class B are referred to as the metallo β-lactamases [8]. The class A β-lactamases comprises of carbapenemases such as NMC, IMI, SME, GES and, KPC (variants from KPC-2 to KPC-17) families. Class D consists of β-lactamases which are of oxacillinase variety, whereas, class B metallo β-lactamases contains IMP, GIM, VIM, SPM and SIM [14].

KPC-3 and KPC-15 are two of the most frequently found carbapenemases in regions of U.S. and they hydrolyse a wide variety of β-lactam antibiotics namely penicillin's, cephalosporin's and carbapenem's [8,15]. Various reports indicate that KPC-15 also shows resistance to almost 18 conventional antimicrobial agents, including carbapenem antibiotics [12].

With the current advancement in bioinformatics, Computer Aided Drug Discovery (CADD) has grown into a big tree with its root deeply engraved in the garden of drug discovery. CADD enables drug discovery at fraction of cost and empowers academic setup to venture into this massive ocean. Within CADD, Virtual screening (VS) is an in-silico technique for screening a large library of drugs against disease of interest. VS can be broadly classified into two categories- (i) ligand-based virtual screening (LBVS) (screening using active compounds as templates), and (ii) structural-based virtual screening (SBVS) (docking of ligand against protein of interest), both complementing each other. ADMET characteristics have a crucial effect on the development of a potential drug [16]. The availability of online ADMET prediction servers such as admetSAR, gives a fair assumption on the ADMET of the identified compound without having to conduct any experiment [17]. With these in the background, we aspired to identify dual inhibitor of KPC-3 and KPC-15 by employing LBVS followed by SBVS.

Material and Methodology

A personal workstation - HP-Z640 working on Ubuntu 18.04.2 LTS with a configuration of Intel Xeon(R) CPU E5-2640 v3@2.60 GHz x 16 (hyperthreaded cores), 64 GB RAM, 500 GB HDD, and Quadro K620/PCLe/SSE2 graphics card was used for conducting all the studies. Open source in-silico offline tools and webservers (or) proprietary tools with appropriate academic licences were used for conducting the experiments and their mention/ reason for selection have been given in the respective section of methodology. Broadly the identification of active ligands against KPC-3 and KPC-15, can be divided into 9 steps namely: 1. retrieval of ligands; 2. ligand based virtual screening; 3. accession of target protein; 4. preparation of receptor for docking; 5. active site prediction; 6. structure based virtual screening; 7. analysis of docking results; 8. structure similarity search; 9. determination of ADMET properties.

1. Retrieval of the ligands

"Drug-like" molecules subset within the ZINC12 database (http://zinc.docking.org) was used for retrieval of ligands. The downloaded library consisted of around 10 lakh ligands in 5 set in mol2 format (2 lakh ligands in each set). These being "Drug-like" molecules by default obey Lipinski's Rule of Five as well as follow certain criteria of drug-likeness namely high potency, ligand efficiency, lipophilic efficiency and, bioavailability.

2. Ligand-based virtual screening

Ligand-based virtual screening uses the information of known active ligand for prediction. It does not require the knowledge of target protein structure for screening [18]. The ligand-based tool known as LiSiCA was used to screen all 5 sets of ligands retrieved from the previous step. LiSiCA uses a clique algorithm to find two- and three- dimensional similarities between pairs of ligands as supplied in mol2 atom types [19]. Relebactam being an already known inhibitor of KPC was taken as the reference active ligand for both KPC-3 and KPC-15 structure [20]. The following input parameters were used while running LiSiCA (i) dimension (d) was 3, (ii)-m=0.5 for Rigorous screening, (iii)-c=10000 for a maximum number of output files. LiSiCA. The resulted output files contained 10000 ligands per set giving a pool of 50000 compounds. These resulted output files obtained from LiSiCA (mol2) were converted into pdbqt format using python script supplied with MGLtools (prepare_ligand4.py). These top 50000 ligands were taken for SBVS using *idock*.

3. The accession of target protein

RCBS protein data bank, a global archive of protein and nucleic acids structures in 3D format was used for retrieving the protein target structure. As 3D structure of KPC-3 and KPC-15 was not available in RCBS protein data bank (at the time of screening - April 2019) we took the structure of KPC-2 carbapenemase from *K. pneumoniae* (PDB ID 2OV5) and then decided to mutate the structure to arrive at KPC-3 and KPC-15. It was a non-liganded X-ray crystallographic structure of 1.85 □ resolution. Chain A of protein was separated out using pymol [21]. This file was exported for the

further mutation to obtain KPC-3 (H272Y) and KPC-15 (A119L and G146K) [12] using Swiss PDB Viewer [22] in PDB format.

4. **Preparation of the receptor for docking**

MGLTools inside AutoDock was used for preparing the receptor. The water molecules of the target protein were deleted for computational convenience of bio-energies and for clearing the active binding pockets which could potentially distort the pose search. The AutoDock uses the United Atom force field of AMBER which only uses polar hydrogens. Therefore, we merged the non-polar hydrogen and added only polar hydrogen to get correct ionization state and tautomer [23,24]. In addition, it also helps in reducing the number of atoms to be modelled explicitly during docking. Finally, the Kollman United Atom charges were assigned to the protein structure. The modified protein was then converted into pdbqt structure.

5. **Active site prediction**

COACH server was used for the estimation of the active site of the protein (Yang et al., 2013a). The PDB file of KPC-3 and KPC-15 was uploaded as a query in COACH server and it took approximately 10 hours to predict the active site coordinates. The summary of COACH is computed based on the results of TM-SITE, S-SITE, COFACTOR, FINDSITE, ConCavity. The final summary consisted of residue-specific binding probability, template clustering results and predicted bound ligands. It also provides consensus binding residues between the uploaded protein and the PDB hit along with their respective C-score values and cluster size.

6. **Structure-based virtual screening**

In SBVS, a library of ligands is docked into an identified docking site of the protein so that there occurs biophysical interaction between them and bio-energies are computed using algorithms. In order to perform this, though there are many docking algorithms available, AutoDock have always stood apart among academicians. Within the available algorithms of AutoDock, it has been shown that AutoDock Vina is better as compared to that of AutoDock 4 for virtual screening, by decreasing the time for computation as well as giving equally good estimates [25]. Infact, it has been shown that the prediction accuracy of AutoDock Vina is as good as other paid tools in the market. *idock* is an improvement over the AutoDock Vina and is preferred for 3 important reasons: it enables the process of multi-threading in a multi-core computer; permits the storage of details pertaining to grid maps along with the receptor in the working memory - thereby avoiding repetitive detection for each ligand; enables the

detection and avoidance of ligands having inactive torsions. All the above factors significantly improves and reduces the time required for ligand-protein docking [26,27]. It is available as an open-source tool in both online and offline version. We used the offline version of *idock*. Behind *idock* is an algorithm based on regression and optimization theorems giving an estimate of the biophysical interaction in terms of binding energy (or) idock score. The lower the binding energy, the more is the interaction between the ligand and protein, and this helps in ranking and identifying the active ligand. In addition, conformation of the ligand at the docked site and the plausibility of the given structure of the ligand are taken into consideration during docking. A shell script was coded for repeating the process of docking and the final outputted results were ranked based on binding energy for further analysis. The dual inhibitors of both KPC-3 and KPC-15 were identified based on consensus weighted rank. Consensus weighted rank was calculated by dividing the mean of the ranks of the compound observed in docking against each protein by the predicted number of proteins inhibited by the molecules. Based on the initial results of docking of Relebactam KPC-3 and KPC-15, we took a cut-off of -6 kcal/mol binding energy (both KPC-3 and KPC-15) for identifying a molecule as inhibitor of a protein (any ligand showing a binding energy of less than -6 kcal/mol was taken as inhibitor). The lower the consensus weighted rank more potent is the dual inhibitor.

7. Analysis of the docking results

PymoL was used for the preparation of complex PDB file of protein and identified ligands. It was also used for analysing the docking position using the same. Ligplot+ was used for studying the interaction between the ligand and protein and for identifying the bond length [28].

8. Structure similarity search

Structure similarity between relebactam and top 3 identified ligands (dual inhibitors) were made using python package RDKit [29]. The canonical SMILES were used as an input query and the depicted 2D structures were converted into a 3-D structures using distance geometry for structure comparison. RDKit performs structure comparison using 2 main fingerprints (i) RDKit fingerprint, for substructure fingerprinting where the atom types are set by atomic number and aromaticity; bond types based on atom types and bond types (ii) Morgan fingerprint, is a reimplementation of the extended connectivity fingerprint (ECFP), which takes into account the neighbourhood of each atom for similarity fingerprint.

9. Determination of ADMET properties

The unsuitable pharmacokinetics and toxicity shown by efficacious compounds leads to the failure of drug candidate in clinical trials. Therefore, it forms a critical process in the evaluation of drug-likeness of abstained small molecules. Since we have taken "drug-like" molecules, they automatically show suitable absorption, distribution, metabolism, excretion and toxicity (ADMET) characteristics. However, we employed admetSAR to further study the ADMET properties of the identified lead ligands. The 40 endpoints of ADMET properties were identified using admetSAR tool which takes the input in form of canonical SMILES. [17].

Results and Discussion

1. Identification of Ligand Binding Sites

The COACH and COFACTOR were used to identify the active sites of KPC-3 and KPC-15 of Klebsiella pneumoniae via the COACH server. The results obtained from the COACH showed a close relation with 2jbfA (chain A of PDB ID 2JBF) with a C-score (confidence score of the predicted result) value of 1.00 for KPC-3, while for KPC-15, the close relation was observed with 4xuzA (chain A of PDB ID 4XUZ) with a C-score value of 0.87. It ranges between 0-1 (inclusive of the endpoints), where the higher C-score value indicates a more reliable prediction. The hydrolysis of the substrate increases with the increase in the interaction between the substrate and the beta-lactamase. For both KPC-3 and KPC-15, the COFACTOR represented a close match with 2ov5A (chain A of PDB ID 2OV5) with a TM score (template modelling score - estimates the protein structure similarity) equals to 1.00 and root mean square deviation (RMSD) equals to 0.00 Å. With a smaller value of RMSD, a higher resolution of the protein structure is observed [30]. Similar to C-score, TM also ranges between 0-1 (inclusive of the endpoints) where a higher value indicates a similarity between structures. The predicted putative ligand binding site residues in KPC-3 are- Cys39, Ser40, Lys43, Trp75, Ser100, Asn102, Leu137, Asn140, Thr186, Lys204, Thr205, Gly206, Thr207 while in KPC-15 are- Cys39, Ser40, Lys43, Pro74, Trp75, Ser100, Asn102, Leu137, Asn140, Lys204, Thr205, Gly206, Thr207, Cys208, Val210. The final COACH results, (i.e.,) the predicted active site coordinates for both KPC-3 and KPC-15 were- x = 55.891, y = -21.594, z = -4.513.

2. **Virtual Screening and Analysis of Docking Results**

The top 3 potential inhibitor of KPC-3 as predicted by *idock* are ZINC05528590 (ligand 1a, -6.41 kcal/mol), ZINC76060350 (ligand 2a, -6.37 kcal/mol), ZINC72290395 (ligand 3a, -6.33 kcal/mol) (Table 1). The best ranked compound, ZINC05528590, IUPAC name- (5S)-5-hydroxy-5-methyl-1-oxa-3,4-diazaspiro[5.5]undecan-2-one, has a molecular weight of 200.23g/mol with H-bond donor of 3 and H-bond acceptor of 4. The top 3 potential inhibitor of KPC-15 as predicted by *idock* are ZINC76060350 (ligand 1b, -6.39 kcal/mol), ZINC22689973 (ligand 2b, -6.38 kcal/mol), ZINC05528590 (ligand 3b, -6.34 kcal/mol) (Table 2). ZINC76060350 with an IUPAC name of (3R)-1-[(1R)-cyclohex-2-en-1-yl]-3-(5-methyl-1H-pyrazol-3-yl)piperidine having molecular weight of 245.36g/mol, showed best docking score and having 1 & 2 H-bond donor and acceptor respectively. Based on consensus weighted ranks, top 3 ligands with dual inhibitory property are- ZINC76060350 (ligand 1c, consensus weighted rank - 1.5), ZINC05528590 (ligand 2c, consensus weighted rank - 2), ZINC72290395 (ligand 3c, consensus weighted rank - 3.5) (Table 3). The binding of the ligand 1c with the active site of KPC-3 and KPC-15 along with the hydrogen bond interaction with bond length are shown in figure 1a and figure 1b respectively. The Ligplot+ images of the top 3 dual inhibitors of KPC3 and KPC15 are denoted in figure 2. All the selected compounds showed an overall high binding affinity towards the target protein i.e. (the minimum binding energy of the 10th rank ligand against KPC-3 was -6.22 kcal/mol and KPC-15 was -6.17 kcal/mol).

The analysis of 40 essential ADMET endpoints of all the five potential compounds are listed in table 4. In absorption and distribution, all the top-five compounds can pass through the blood brain barrier with the maximum permeability probability of 0.99 shown by 2 compounds- ZINC76060350, ZINC72361341. All the 5 ligands can pass through human intestinal absorption [31] with the minimum probability of 0.92. The good human oral bioavailability is seen with almost all ligands with a maximum probability of 0.89 seen with ZINC71781573. Plasma protein binding shows the probability value of 1.00 by all the ligands which can be modified by addition or deletion of side chain in further stages of development of compound. The UGT (UDP-glucuronosyltransferases) were analysed to investigate the metabolically liable site for lead optimization [32]. Only one ligand showed a UGT catalysed metabolism- ZINC05528590. There are no interactions seen with estrogen binding receptors and androgen-binding receptors for majority of the

compounds. No hepatotoxicity is seen out of the five compounds. An important factor to be analysed during a development of a drug is human either-a-go-go inhibition, where hERG (the human *Ether-à-go-go*-Related Gene) is a gene coordinating the electrical activity of the heart [33]. Compound which have a positive hERG have a potential to cause prolongation of the QT. Two of the five compounds show toxicity against human either-a-go-go inhibition with a maximum probability value of 0.79. This is not a cause of concern as computational model of hERG inhibition and hERG inhibition does not equate to QT prolongation risk, hence for this property confirmation by invitro and invivo studies needs to be undertaken [34]. The carcinogenicity potential is not seen in all the five compounds. The biodegradation, eye-corrosion and eye-irritation is absent for all the compounds.

3. **Structure similarity result**

Based on a previous study, Relebactam was identified as a potential inhibitor for KPC-2 showing a dependent relation with imipenem (Papp-Wallace et al., 2018; Zhanel et al., 2018). While in that study, Relebactam activity against KPC-3 and KPC-15 were not assessed, we checked for the activity of Relebactam against the KPC-3 and KPC-15 and compared the binding energy with that of binding energy of top 3 dual inhibitors identified in this study. Our study revealed that ZINC76060350, ZINC05528590, ZINC72290395 gave docking binding energy (Table 3) similar to that of Relebactam (docking energy of Relebactam for KPC-3 and KPC-15 were -6.49 and -6.31 respectively). We then assessed the three identified ligands against Relebactam for structural similarity. (i) The RDKit fingerprint scores between Relebactam and ligand-1c, 2c and 3c were 0.24, 0.22, 0.23 respectively and (ii) Morgan fingerprint score between Relebactam and ligand-1c, 2c and 3c were 0.26, 0.19, 0.25 respectively. This shows that the structure of all 3 identified ligands are different from Relebactam, showing only 20% similarity (approximately), and therefore may independently be effective in inhibiting the activity of KPC-3 and KPC-15. The cons of the study are the non-validation of the identified ligands in an in-vitro study for its activity against multi-resistant *K. pneumoniae* and in addition, non-employment of some CADD techniques such as molecular dynamics and computational combinatorial chemistry to optimize the identified ligands further. We are planning to undertake these studies in near future.

Conclusion

The inhibition of KPC-3 and/or KPC-15 affects the activity of beta-lactamases which can be used as an advantage for the treatment of infections caused by carbapenemase secreting *K. pneumoniae* showing resistance to major group of antibiotics. According to the results obtained, we suggest that ZINC76060350, ZINC05528590 and ZINC72290395 are the potential dual inhibitors of KPC-3 and KPC-15. The suggested ligands could be taken forward for the development of new drug against a multi-resistant- *Klebsiella pneumoniae* infections.

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Figure Legends:

Figure 1: A) Interaction of KPC-3 to ZINC05528590 with a binding energy of -6.41 kcal/mol. B) Interaction of KPC-15 to ZINC76060350 with a binding energy of -6.39 kcal/mol. The top part of the figure shows the surface representation of the protein and ligand in ball & stick appearance whereas the bottom part of the figure is the zoomed in binding site with helical representation of the protein and ball & stick appearance of the ligand. The dotted yellow line represents the hydrogen bond interaction between the ligand and the protein. In case of KPC-3 there are 2 hydrogen bonds (2.3 Å and 3.2 Å), while incase of KPC-15 there is 1 hydrogen bond (2.3 Å) interaction.

Figure 2: Predicted 2-D structure representation of the interaction of top-3 ligands having combined inhibiting potential of KPC-3 and KPC-15 proteins of *K. pneumoniae* residue using ligplot+ (A) Ligand-1 (ZINC76060350) docked at the active binding pocket of the KPC-3 protein and KPC-15 protein (B) Ligand-2 (ZINC05528590) docked at the active binding pocket of the KPC-3 protein and KPC-15 protein (C) Ligand-3 (ZINC72290395) docked at the active binding pocket of the KPC-3 protein and KPC-15 protein. Dotted green colour shows H-bonding. The ball-and-stick represents the ligand-protein side chains, where the purple colour shows the ligand bonding with its protein. The non-bonded protein residues interaction with ligand is depicted by spoked arcs. When the two structural models are superposed, the equivalent 3D positions of the potential protein residues are indicated by red circles and ellipses. [35].

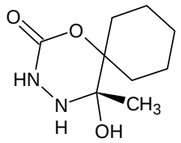
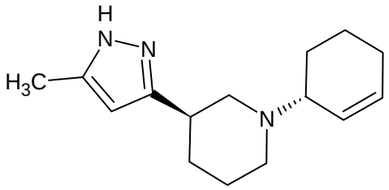
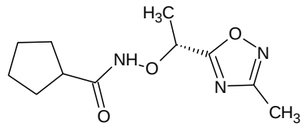
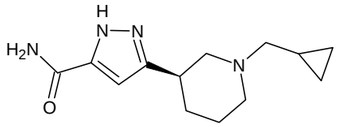
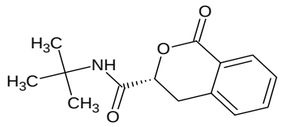
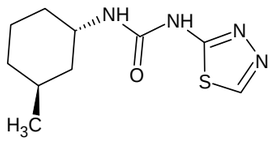
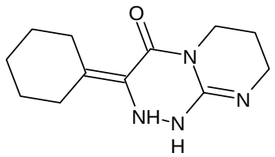
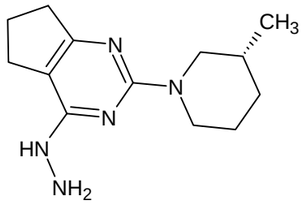
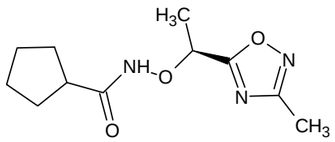
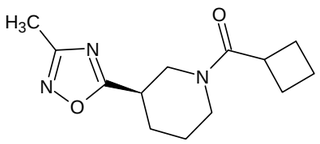
KPC-3 Potential Inhibitors					
Ligand ID	**Ligand-1a**	**Ligand-2a**	**Ligand-3a**	**Ligand-4a**	**Ligand-5a**
Structure					
ZINC ID	ZINC05528590	ZINC76060350	ZINC72290395	ZINC72361341	ZINC44718159
Binding energy (kcal/mol)	-6.41	-6.37	-6.33	-6.32	-6.29
Ligand ID	**Ligand-6a**	**Ligand-7a**	**Ligand-8a**	**Ligand-9a**	**Ligand-10a**
Structure					
ZINC ID	ZINC43127801	ZINC71781573	ZINC57328571	ZINC72290396	ZINC72348513
Binding energy (kcal/mol)	-6.29	-6.28	-6.28	-6.22	-6.22

Table 1: The top 10 potential inhibitors against KPC-3 are reported here according to the lowest binding energy. The Ligand-1a is the best compound seen with the value of -6.41, ID: ZINC05528590, IUPAC name- (5S)-5-hydroxy-5-methyl-1-oxa-3,4-diazaspiro[5.5]undecan-2-one

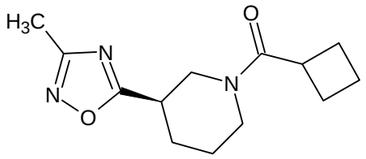
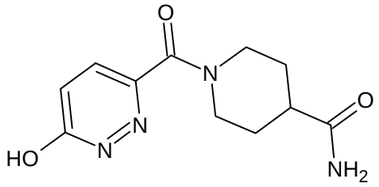
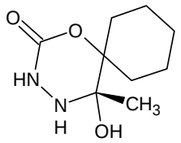
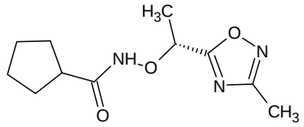
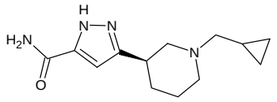
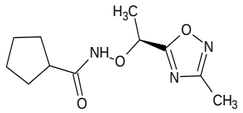
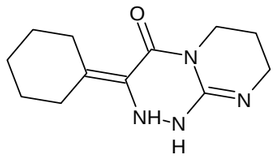
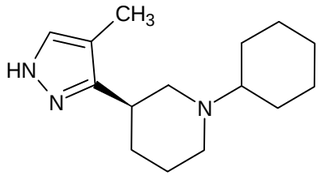
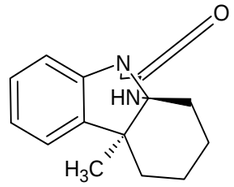
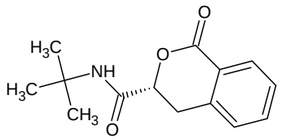
KPC-15 Potential Inhibitors					
Ligand ID	**Ligand-1b**	**Ligand-2b**	**Ligand-3b**	**Ligand-4b**	**Ligand-5b**
Structure					
ZINC ID	ZINC76060350	ZINC22689973	ZINC05528590	ZINC72290395	ZINC72361341
Binding energy (kcal/mol)	-6.39	-6.38	-6.34	-6.28	-6.23
Ligand ID	**Ligand-6b**	**Ligand-7b**	**Ligand-8b**	**Ligand-9b**	**Ligand-10b**
Structure					
ZINC ID	ZINC72290396	ZINC71781573	ZINC72381181	ZINC00517667	ZINC44718159
Binding energy (kcal/mol)	-6.19	-6.18	-6.18	-6.17	-6.17

Table 2: The top 10 potential inhibitors against KPC-15 are reported here according to the lowest binding energy. The Ligand-1b is the best compound seen with the value of -6.39, ID: ZINC76060350, IUPAC name-(3R)-1-[(1R)-cyclohex-2-en-1-yl]-3-(5-methyl-1H-pyrazol-3-yl)piperidine.

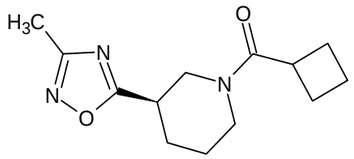
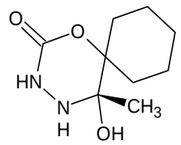
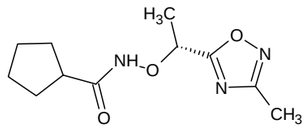
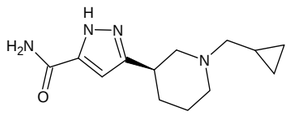
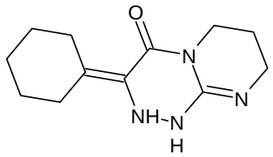
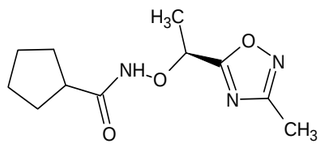
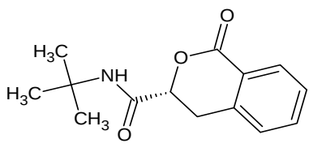
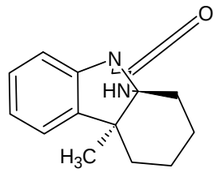
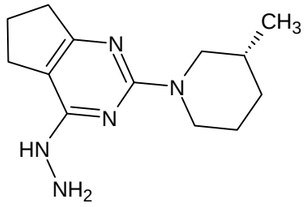
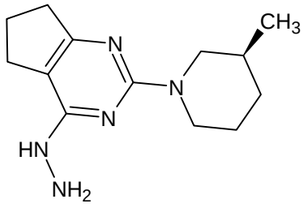
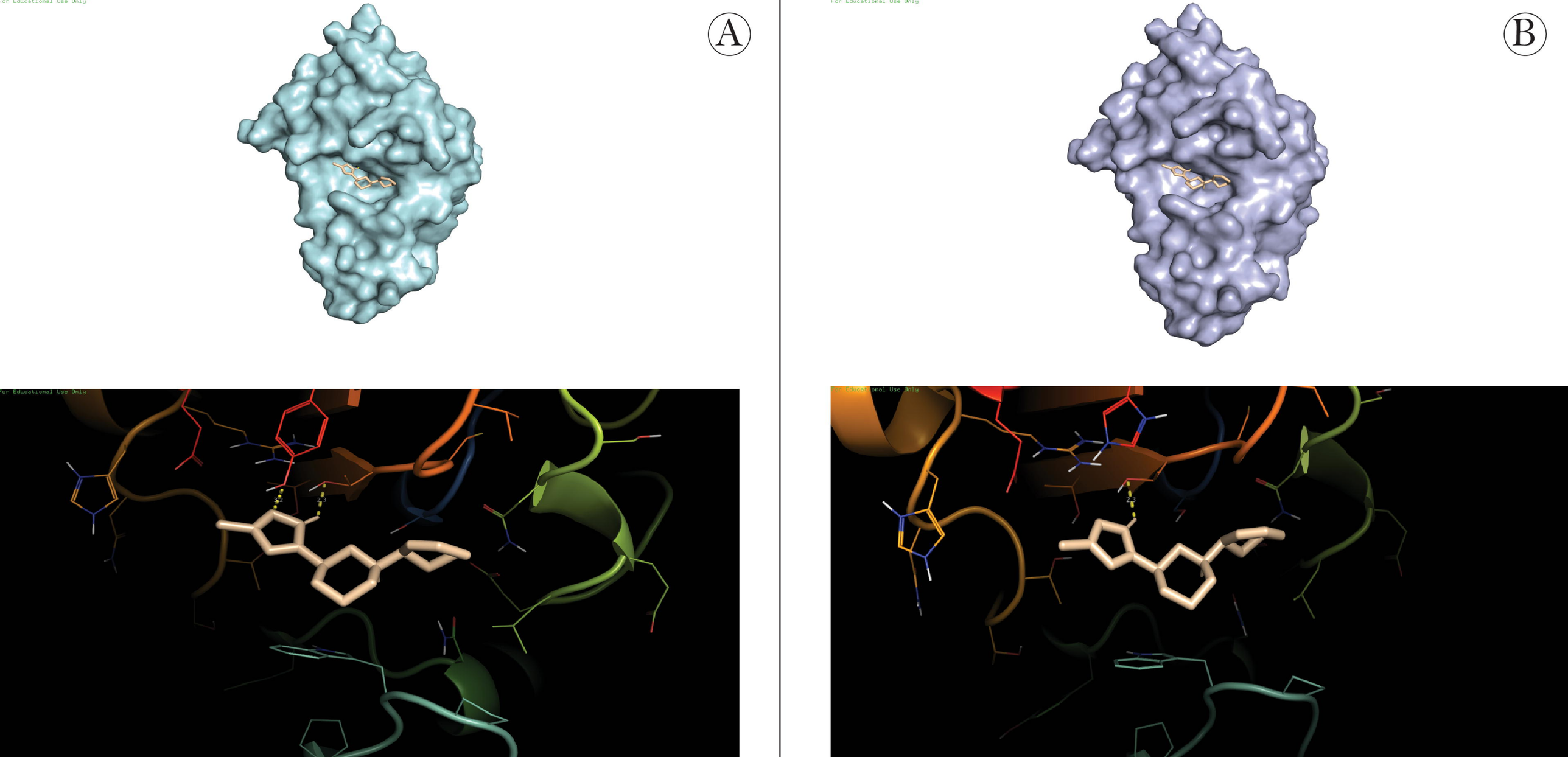
Dual Potential Inhibitors of both KPC-3 and KPC-15					
Ligand ID	**Ligand-1c**	**Ligand-2c**	**Ligand-3c**	**Ligand-4c**	**Ligand-5c**
Structure					
ZINC ID	ZINC76060350	ZINC05528590	ZINC72290395	ZINC72361341	ZINC71781573
Consensus weighted rank	1.5	2	3.5	4.5	7
Binding energy against KPC-3 (kcal/mol)	-6.37	-6.41	-6.33	-6.32	-6.28
Binding energy against KPC-15 (kcal/mol)	-6.39	-6.34	-6.28	-6.23	-6.18
Ligand ID	**Ligand-6c**	**Ligand-7c**	**Ligand-8c**	**Ligand-9c**	**Ligand-10c**
Structure					
ZINC ID	ZINC72290396	ZINC44718159	ZINC00517667	ZINC57328571	ZINC57328576
Consensus weighted rank	7.5	7.5	10	11	12.5
Binding energy against KPC-3 (kcal/mol)	-6.22	-6.29	-6.22	-6.28	-6.22
Binding energy against KPC-15 (kcal/mol)	-6.19	-6.17	-6.17	-6.1	-6.12

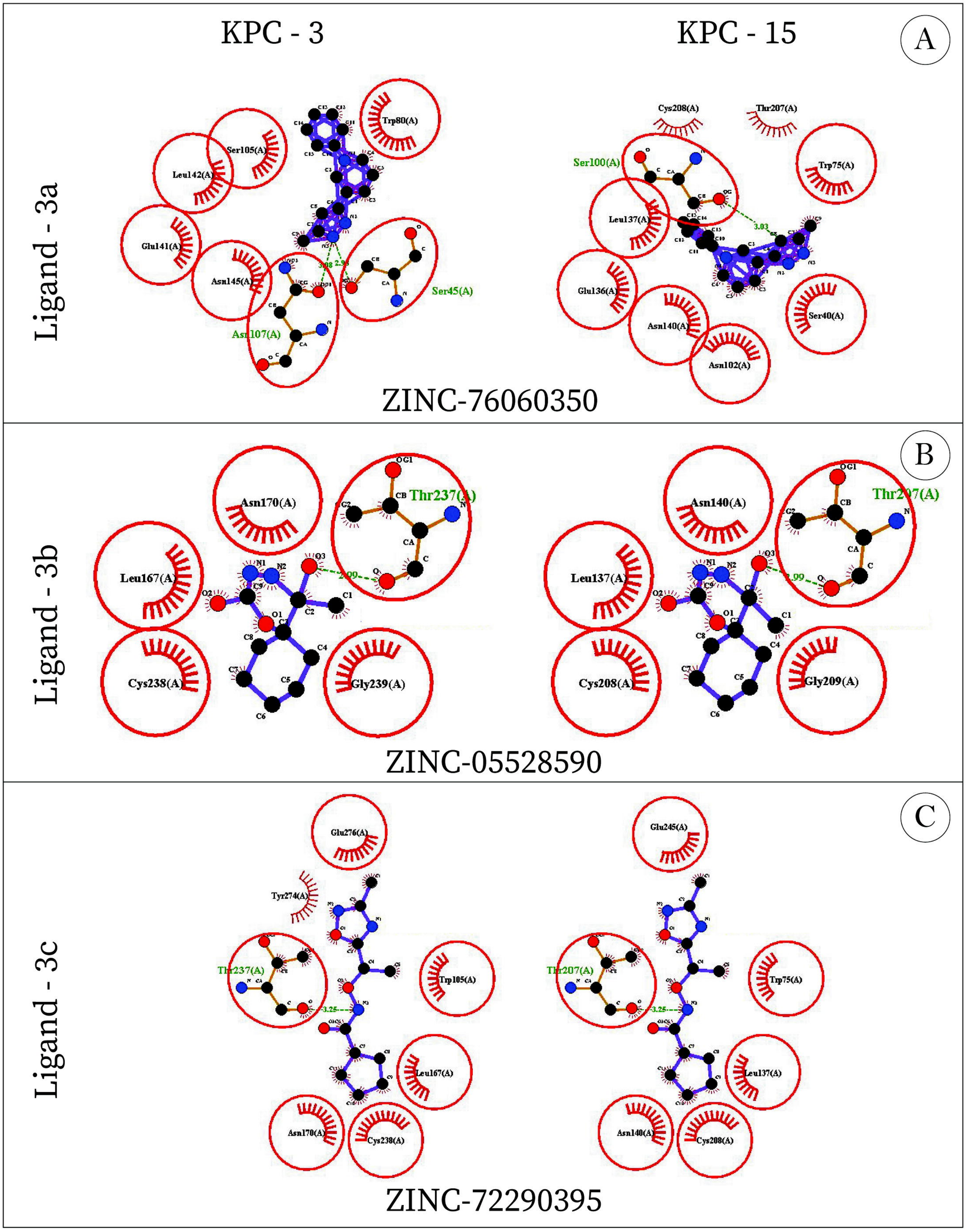
Table 3: The top 10 potential inhibitors against KPC-3 and KPC-15 are reported here according to the lowest binding energy. The Ligand-1c is the best compound seen with consensus weighted rank of 1.5, ID: ZINC76060350, IUPAC name- (3R)-1-[(1R)-cyclohex-2-en-1-yl]-3-(5-methyl-1H-pyrazol-3-yl)piperidine.

	ZINC76060350		ZINC05528590		ZINC72290395		ZINC72361341		ZINC71781573	
	Values	Probability	Values	Probability	Values	Probability	Values	Probability	Values	Probability
Absorption and Distribution										
Blood Brain Barrier	+	0.99	+	0.97	+	0.57	+	0.99	+	0.98
Human Intestinal Absorption	+	0.98	+	0.92	+	0.99	+	0.99	+	0.95
Human oral bioavailability	+	0.67	+	0.66	+	0.61	-	0.60	+	0.89
Plasma protein binding	1.04	1.00	0.87	1.00	0.71	1.00	0.59	1.00	0.64	1.00
Metabolism										
UGT catalyzed	-		+		-		-		-	
CYP1A2 inhibition	-	0.5	-	0.83	-	0.56	-	0.76	+	0.51
CYP2C19 inhibition	+	0.64	-	0.76	-	0.60	-	0.69	-	0.58
CYP2C9 inhibition	-	0.8	-	0.84	-	0.81	-	0.82	-	0.53
CYP2C9 substrate	-	0.8	-	0.59	-	0.81	-	1.00	-	0.60
CYP2D6 inhibition	+	0.57	-	0.91	-	0.92	+	0.54	-	0.78
CYP2D6 substrate	+	0.37	-	0.80	-	0.88	-	0.73	-	0.85
CYP3A4 inhibition	-	0.94	-	0.99	-	0.98	-	0.93	-	0.56
CYP3A4 substrate	+	0.53	-	0.53	+	0.52	-	0.54	-	0.54
P-glycoprotein substrate	-	0.57	-	0.96	-	0.79	-	0.51	-	0.86
Non-specific Interaction										
BSEP inhibitior	-	0.56	-	0.99	-	0.92	-	0.66	-	0.86
BRCP inhibitior	-		-		-		-		-	
Estrogen receptor binding	-	0.63	-	0.70	-	0.95	-	0.72	-	0.53
Glucocorticoid receptor binding	-	0.64	-	0.79	-	0.61	-	0.65	-	0.47
Thyroid receptor binding	-	0	-		-		-		-	
Androgen receptor binding	-	0.81	-	0.58	-	0.66	-	0.86	-	0.64
PPAR gamma	-	0.72	-	0.83	-	0.71	-	0.54	-	0.60
Aromatase binding	-	0.89	-	0.59	-	0.57	-	0.73	+	0.58
OATP1B1 inhibitior	+	0.94	+	0.92	+	0.95	+	0.95	+	0.96
OATP1B3 inhibitior	+	0.93	+	0.94	+	0.94	+	0.94	+	0.94
OATP2B1 inhibitior	-	0.85	-		-	0.85	-	1.00	-	0.86
OCT1 inhibitior	-		-		-		-		-	
OCT2 inhibitior	-	0.57	-	0.78	-	0.80	-	0.68	+	0.53
MATE1 inhibitior	-	0.94	-	0.98	-	0.88	-	0.72	-	0.89
P-glycoprotein inhibitior	-	0.87	-	0.98	-	0.90	-	0.96	-	0.94
CYP inhibitory promiscuity	+	0.86	-	0.98	-	0.54	-	0.70	+	0.70
Toxicity										
Hepatotoxicity	-	0.8	-	0.70	-	0.53	-	0.55	-	0.55
Human either-a-go-go inhibition	+	0.79	-	0.70	-	0.50	+	0.73	-	0.60
Micronuclear	+	0.68	+	0.65	+	0.80	+	0.71	+	0.69
Ames mutagenesis	-	0.79	-	0.75	-	0.67	-	0.70	-	0.67
Caco-2	+	0.73	+	0.68	-	0.60	-	0.71	+	0.80
Carcinogenicity (binary)	-	0.92	-	0.93	-	0.83	-	0.94	-	0.93
Other parameters of pharmaceutical interest										
Water solubility(log S)	-1.85	NA	-3.08	NA	-1.75	NA	-1.81	NA	-3.07	NA
Biodegradation	-	0.77	-	0.68	-	0.57	-	0.78	-	0.68
Eye corrosion	-	0.94	-	0.98	-	0.98	-	0.98	-	0.98
Eye irritation	-	0.92	-	0.97	-	0.95	-	0.92	-	0.61

Table 4: 40 ADMET endpoints of the top-five dual inhibitors of KPC-3 and KPC-15, including absorption, distribution, metabolism, non-specific interactions, toxicity and other parameters of pharmaceutical interest, as investigated using admetSAR.

NA –stands for Not Applicable


A
B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KPC - 15
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Leu142(A)
Glu141(A)
Asn145(A)
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Ser45(A)
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Trp75(A)
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Glu136(A)
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Asn102(A)
Ser40(A)
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B
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Gly209(A)
ZINC-05528590
C
Ligand - 3c
Glu276(A)
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