

Molecular modeling and prediction of binding mode and relative binding affinity of Art-Qui-OH with *P. falciparum* Histo-Aspartic Protease (HAP)

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Abstract:

The relative binding affinity in terms of $\Delta\Delta G_{\text{bind-cald}}$ value of the antimalarial compound artemisinin-quinine hybrid is primarily derived and is discussed in this article with reference to the $\Delta G_{\text{bind-cald}}$ values of two known inhibitors Pepstatin-A and KNI-10006 complexed with HAP enzyme. The $\Delta G_{\text{bind-cald}}$ value for KNI-10006 and Pepstatin-A is -14.10 kcal/mol and -13.09 kcal/mol respectively. The MM-GB/SA scoring results in the relative binding energy ($\Delta\Delta G_{\text{bind-cald}}$) of the hybrid molecule with respect to Pepstatin-A as 2.43 kcal/mol and 3.44 kcal/mol against KNI-10006. The overall binding mode of Art-Qui-OH resembles that of Pepstatin-A binding in HAP active site. We suggest here that the $\Delta\Delta G_{\text{bind-cald}}$ value & proposed binding mode of the Art-Qui-OH for HAP enzyme should be considered for further structure-based drug design effort.

Key Words: HAP, Art-Qui-OH, $\Delta\Delta G_{\text{bind-cald}}$, ΔG_{score}

Background:

The enzyme Histo-aspartic protease (HAP) is one of the 10 plasmepsins (PMs) identified in the genome of *Plasmodium falciparum*, the parasite responsible for the most virulent and widespread form of malaria [1]. It is one of the 4 PMs residing in the food vacuole of the parasite that are involved directly in the process of haemoglobin degradation making them potential targets for novel antimalarial therapy [2]. Despite its high sequence identity (60%) with other PMs (PMI, PMII, and PMIV), which are typical pepsin-like aspartic proteases, the active site of HAP contains several significant deviations from the pepsin standard. The particular substitutions are Asp32, which together with Asp215 creates the catalytic dyad in classic aspartic proteases, is replaced by a histidine, giving this enzyme its name. In addition, substitutions are found in the functionally important flexible loop called the "flap" (residues 70–83), and it participates in enzyme catalysis. The substitutions include the strictly conserved Tyr75 and the highly conserved Val/Gly76,

which are replaced by Ser and Lys, respectively [1]. The flap plays a major role in all pepsin-like aspartic proteases, changing its conformation upon ligand binding. Despite its unusual active-site amino acids, HAP seems to be a reasonably active enzyme [2]. The recombinant HAP enzyme, cleaves at the penta-peptide substrate sequence Met1-Phe2-Leu3-Ser4-Phe5 that corresponds to the 32-36 residues of human haemoglobin α -chain. This fragment is located in the region joining two α -helices. It contacts the β -chain in haemoglobin tetramer and is hidden inside the globular protein. Upon haemoglobin dissociation the 32-36 fragment is exposed to solvent and becomes available to proteolytic enzyme [3]. Enzymatic activity data indicate that HAP is most active as a monomer. The dominant monomer form showed a K_m of 2.0 μM and a turnover number, K_{cat} , of 0.0036 s^{-1} using the internally quenched fluorescent synthetic peptide substrate [4]. However, the available biochemical as well as structural data does not

provide an unambiguous answer about the exact nature of the catalytic mechanism of this unusual enzyme.

Although the overall level of sequence similarity suggests that HAP should have a pepsin-like fold [5] the predicted details of the active-site architecture and, consequently, the mode of enzymatic activity have been subjects of considerable disagreement. In the model published by Andreeva *et al.* (2004), HAP was postulated to function as a trypsin-like serine protease, with the catalytic triad consisting of Ser35, His32, and Asp215 [6]. But recent structural studies disapproved this hypothesis [1]. On the other hand, Bjelic and Aqvist postulated a reaction mechanism of HAP that would assign a direct catalytic role only to Asp215, whereas the role of His32 would be to provide critical ~10,000-fold stabilization along the reaction path [7]. It has become clear that experimental structural evidence is necessary to validate or disapprove these predictions.

The unique properties of HAP make it an especially attractive protein to target for antimalarial drug development [2]. HAP apparently has a high affinity for aspartic protease inhibitor Pepstatin-A (Figure S1), with a K_i value 0.081nM similar to those of other plasmepsins. Pepstatin-A binds competitively in the active site of Histo-Aspartic Protease (HAP) and makes hydrogen bonds with Asp215 and His32 [1]. KNI compounds are peptidomimetic inhibitors containing flexible and asymmetric functional groups and are proposed to be high affinity inhibitors of vacuolar plasmepsins [8]. Many KNI compounds utilize a common molecular scaffold containing an allophenylnorstatine moiety followed by a thioproline ring. One of them, KNI-10006 (Figure S2) was subsequently shown to be a potent inhibitor of HAP with an IC_{50} of 0.69 μ M [9]. Recent X-ray crystallographic data supports that another members of this class, KNI-10395, is also a potent inhibitor of HAP [10].

Recently, in malaria chemotherapy hybrid molecules with dual functionality and/or targets have been developed through a rational drug design approach. This is termed as "covalent bitherapy" [11]. There are numerous advantages of employing hybrid molecules in malaria therapy. Hybrid drugs whose toxicity as single entities is already known may be less expensive to develop, than new single entities or their combinations. This may also results in a lower risk of drug-drug adverse interactions, compared to other approaches to designing multi-component drugs [12]. Art-Qui-OH (Figure S3) is a novel & potent antimalarial hybrid compound with IC_{50} values of 8.95nM and 9.59nM after 48 hrs of *in vitro* incubation for *Plasmodium falciparum* chloroquine sensitive 3D7 and chloroquine resistant FcB1 strains [13]. Additionally, the artemisinin-quinine hybrid compound, because of the coupling of the lipophilic fast acting, rapidly cleared artemisinin with the higher half-life, slower acting polar quinine base is likely to have a higher half-life *in vivo* and *in vitro*.

The hybrid drug promises to be clinically effective against MRD (multi-drug resistant) malaria because the chance of parasites simultaneously developing resistance as a result of genetic mutations to two drugs with different modes of action is likely to be lower than the chance of developing resistance to single drug. So in view of the reported antimalarial synergism between artemisinin/other endoperoxides and quinine [14], as

a proof-of-concept we planned to evaluate *in silico* the binding mode and relative binding affinity of Art-Qui-OH with the Histo-Aspartic Protease (HAP) of *P.falciparum*. The reason of choosing HAP as the drug target is that, HAP is the most divergent vacuolar plasmepsin, with no counterpart in other characterized species of *Plasmodium* [15]. It is the observed divergence of HAP from mammalian proteolytic enzymes that is the most interesting point here, because it would be even more valuable if the drug hybrid were also active on other species of *Plasmodium*. Moreover, regarding inhibition of HAP enzyme only one compound is so far been reported (KNI-10006) while for Plm-I and Plm-II, a number of very potent inhibitor have been synthesized [15].

Methodology:

Preparation of protein

The X-ray structure of HAP-Pepstatin-A complex (PDB ID: 3FNT) and HAP-KNI-10006 complex (PDB ID: 3FNU) have been used as the initial structure in the preparation of Art-Qui-OH binding site. Hydrogen atoms were added to the model using Maestro interface (version 8.5; Schrodinger LLC, New York) based on an explicit all atom model. The multi step Schrodinger's protein preparation tool (PRep) has been used for final preparation of protein. The complex obtained was minimized using OPLS-2005 force field with Polack-Ribiere Conjugate Gradient (PRCG) algorithm. The minimization was stopped either after 5000 steps of minimizations or after the energy gradient converged below 0.001 kcal/mol.

Ligand preparation

The structure of Art-Qui-OH hybrid was collected from published data [16]. We used ISIS Draw 2.3 software for sketching the structure and converting to its 3D representation by using ChemSketch 3D viewer of ACDLABS 12.0. LigPrep was used for final preparation of ligands for docking. LigPrep is a utility of Schrodinger software suit that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers and steric isomers and perform a geometry minimization of ligand. Ligprep utility produces a number of structures from each input structure with various ionization states, tautomers, stereo chemistries, and ring conformations.

The program automatically generated all possible stereoisomers (default value of 32 was used) for each ligand. Furthermore, a unique low-energy ring conformation for each stereoisomer with correct chirality was generated with the help of Ligprep. All the structures were subsequently subjected to molecular mechanics energy minimization using Impact (version 5.6, Schrodinger Inc.) with default settings: maximum cycles 100, conjugate gradient minimizer, initial step size 0.05, maximum step size 1.0, gradient criteria 0.01. The partial atomic charges were assigned to the molecular structures using the 2005 implementation of the OPLS-AA force field. These energy minimized structures were used for Glide (grid-based ligand docking with energetic) docking.

Molecular Docking of Art-Qui-OH to HAP (Histo-Aspartic Protease)

The "Extra Precision" (XP) mode of Glide docking [17] (version 4.5, Schrodinger Inc.) has been used to perform all docking calculations using the OPLS-AA 2005 force field. In this work

the bounding box of size 10 Å × 10 Å × 10 Å was defined in Histo-Aspartic Protease and centred on the mass centre of the crystallographic KNI-10006 and Pepstatin-A to confine the mass centre of the docked ligand. The scale factor of 0.4 for van der Waals radii was applied to atoms of protein with absolute partial charges less than or equal to 0.25. Five thousand poses per ligand were generated during the initial phase of the docking calculation, out of which best 1000 poses per ligand were chosen for energy minimization. The dielectric constant of 4.0 and 1000 steps of conjugate gradient minimizations were included for energy minimization protocol. Upon completion of each docking calculation, 100 poses per ligand were generated and the best docked structure was chosen using a Glide Score (Gscore) function. Glide Score is a more sophisticated version of ChemScore [18] with force field-based components and additional terms accounting for solvation and repulsive interactions. The choice of the best pose is made using a model energy score (E_{model}) that combines the energy grid score, Gscore, and the internal strain of the ligand.

Post-scoring with MM-GB/SA

The pre-positioned structure of Art-Qui-OH, KNI-10006 and Pepstatin-A with Histo-Aspartic Protease from Glide docking were used to study the association of these ligands with the receptor (PDB ID: 3FNT). For each ligand, the pose with the lowest Glide score was rescored using Prime/MM-GBSA approach. This approach is used to predict the free energy of binding for the set of ligands to the receptor. The docked poses were minimized using the local optimization feature in Prime and the energies of the complex were calculated using the OPLS-AA force field and generalized-Born/surface area (GB/SA) continuum solvent model. The binding free energy (ΔG_{bind}) is then estimated using equation [19] as follows:

$$\Delta G_{\text{bind}} = E_{R:L} - (E_R + E_L) + \Delta G_{\text{solv}} + \Delta G_{\text{SA}} \quad (\text{Equation 1})$$

where $E_{R:L}$ is energy of the complex, $E_R + E_L$ is sum of the energies of the ligand and unliganded receptor, using the OPLS-AA force field, ΔG_{solv} (ΔG_{SA}) is the difference between GBSA solvation energy (surface area energy) of complex and sum of the corresponding energies for the ligand and unliganded protein. Corrections for entropic changes were not applied in this type of free energy calculation.

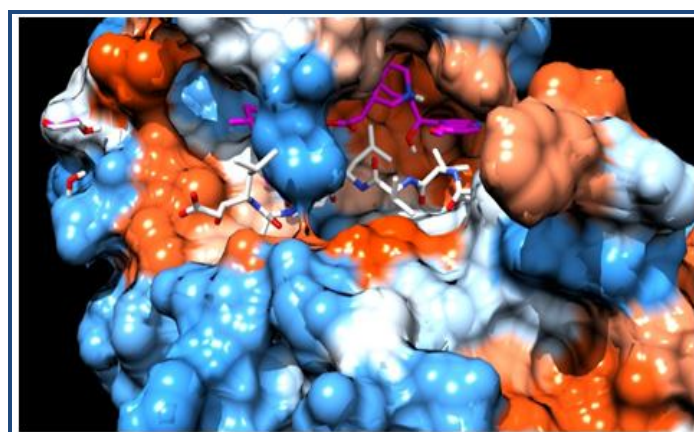


Figure 1: Overlapped docking poses of Pepstatin-A, and Art-Qui-OH obtained from Glide docking onto binding site of Histo-Aspartic Protease. (Magenta= Art-Qui-OH, Silver= Pepstatin-A).

Discussion:

Molecular docking methods are widely used by academic institutes and pharmaceutical industries to study drug-target interactions in order to understand the basic electronic/steric features required for therapeutic action and to design new drug candidates with improved activities. These docking calculations provide insight into interactions of ligands with amino acids in the binding pocket of a target and to predict the corresponding binding affinities of ligands [20]. **Table 1 (see supplementary material)** presents the Glide XP docking results of KNI-10006, Pepstatin-A, and Art-Qui-OH against Histo-Aspartic protease. Their Glide score values among the ligands vary in between -7.460 and -8.777 kcal/mol. The relative docking scores (ΔG score) of Art-Qui-OH with reference to Pepstatin-A and KNI-10006 is 1.298 kcal/mole and 1.317 kcal/mol respectively. These results demonstrate that binding mode of Art-Qui-OH resembles the binding mode of Pepstatin-A in HAP active site [21].

The docked complexes were rescored with Prime-MM/GBSA approach and the relative binding energy ($\Delta \Delta G_{\text{bind-cald}}$) of Art-Qui-OH was calculated using Pepstatin-A and KNI-10006 as reference **Table 2 (see supplementary material)**. The drop in calculated relative binding energy $\Delta \Delta G_{\text{bind-cald}}$ value (2.43 kcal/mol) of Art-Qui-OH with reference to Pepstatin-A revealed a strong binding affinity (binding mode resembles to Pepstatin-A) (**Figure 1**) when compared to $\Delta \Delta G_{\text{bind-cald}}$ value of 3.44 kcal/mol for KNI-10006 (**Figure 2**) [21]. Although more computationally demanding, the MM-GB/SA scoring generally yields far superior correlations with experimentally scoring functions [22-25].

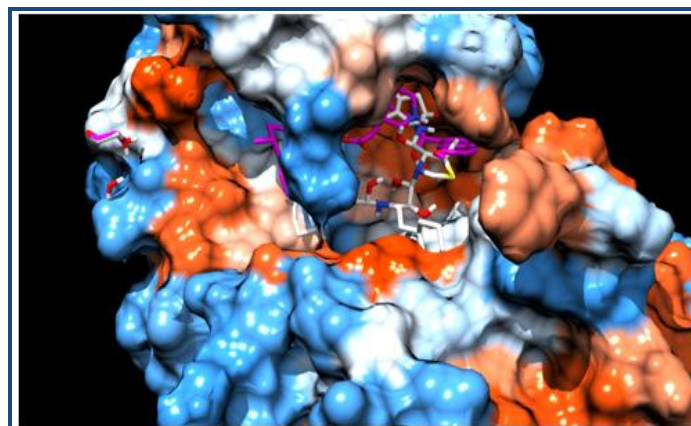


Figure 2: Overlapped docking poses of KNI-10006, and Art-Qui-OH obtained from Glide docking onto active site of Histo-Aspartic Protease (Magenta= Art-Qui-OH, Silver=KNI-10006)

Validation of the docking method by reproducing the crystallized HAP-KNI10006 and HAP-Pepstatin-A Complex

The original crystal structures of HAP-Pepstatin-A complex (PDB ID: 3FNT) & HAP-KNI-10006 (PDB ID: 3FNU) complex were used to validate the Glide-XP docking protocol. This was done by moving the crystallized ligand outside of active site and then docking it back into the active site. The top 6 configurations after docking were taken into consideration to validate the result. The root mean square deviation (RMSD) for each configuration in comparison to the co-crystal of Pepstatin-A was 0.000-1.248 Å **Table 3 (see supplementary material)**, whereas the RMSD value calculated from KNI-10006 of each

configuration was 0.000-1.531 Å **Table 4** (see **supplementary material**). The best docked structures, which are the configurations with the lowest G_{score} , were compared with the crystal structure as shown in the table. These docking results illustrate that the best docked KNI-10006 and Pepstatin-A complex agrees well with its crystal structure.

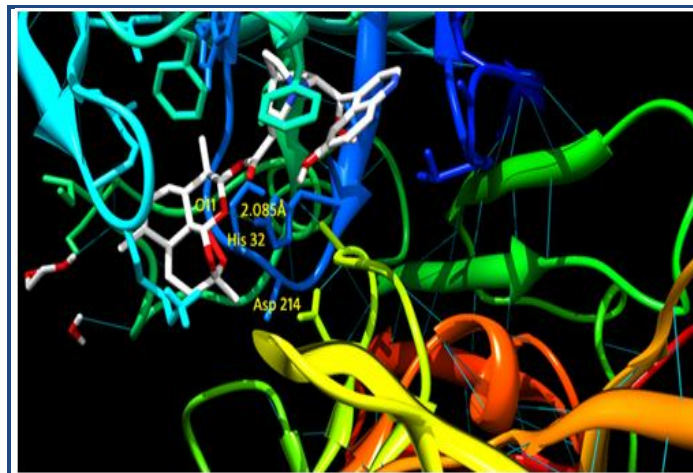


Figure 3: Illustrates the H- bond interaction of His32 and O11 atom of Art-Qui-OH

Inhibitor (Art-Qui-OH) binding to HAP

Pepstatin-A was noted to bind in an extended conformation, with the statine hydroxyl positioned between Asp215 and His32. The binding mode of Pepstatin-A in the active site of HAP indicates that Asp215 and His32 are very likely involved in the catalytic reaction. The KNI-10006 inhibitor predominantly interacts within the enzyme in the flap area. Unlike KNI-10006, the mode of binding of Art-Qui-OH hybrid is drastically different and the hybrid interacts in the active site of the enzyme with O11 atom of artemisinin moiety forming an H-bond with His32 (**Figure 3**). The O11 atom of artemisinin and His 32 N \times 2 H-bond distance is 2.085 Å. This revealed a strong binding affinity between ligand and binding site residue. No interaction has been observed in the binding pose of Art-Qui-OH with the functionally important flexible loop called the “flap” (residue 70-83), which changes its conformation upon ligand binding.

The replacement of Asp by a His residue in the enzyme active site and some other replacements indicate the possibility of a catalytic mechanism differing from that of aspartic proteases. However, up to now, there are no available data on HAP specificity, which could confirm its catalytic activity. Experimental study revealed the stabilizing role of His 32 residue in the substrate catalytic reaction of HAP enzyme. Additionally, Bjelic and Aquist suggested that the positive charge on His32 provides a critical stabilization (by a factor of ~10, 000) to the water/hydroxide nucleophile as well as developing negative charge on the substrate during catalysis. There is also supportive evidence to state that mutation of His 32 to alanine disrupts a hydrogen bonding network that is critical for proper positioning of the Asp 215 residue [26]. Moreover on the basis of recent crystal structure of HAP-KNI-10395 complex (3QVI) it has also been postulated that His32 might be directly involved in providing the electrophilic component for the catalytic mechanism [10].

Conclusion:

In this manuscript we have presented two lines of evidence that (i) Art-Qui-OH binds at or near the Pepstatin-A binding site of Histo-Aspartic Protease (ii) The $\Delta\Delta G_{bind-cald}$ of the hybrid molecule is 2.43 kcal/mol for Pepstatin-A in comparison to $\Delta\Delta G_{bind-cald}$ value of 3.44 kcal/mol for KNI-10006. We suggest that the molecular interactions described here for the binding mode and relative binding affinity of the Art-Qui-OH inhibitor for HAP enzyme should be considered for further structure-based design effort.

Competing interests:

The authors have declared that no competing interests exist.

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Supplementary material:

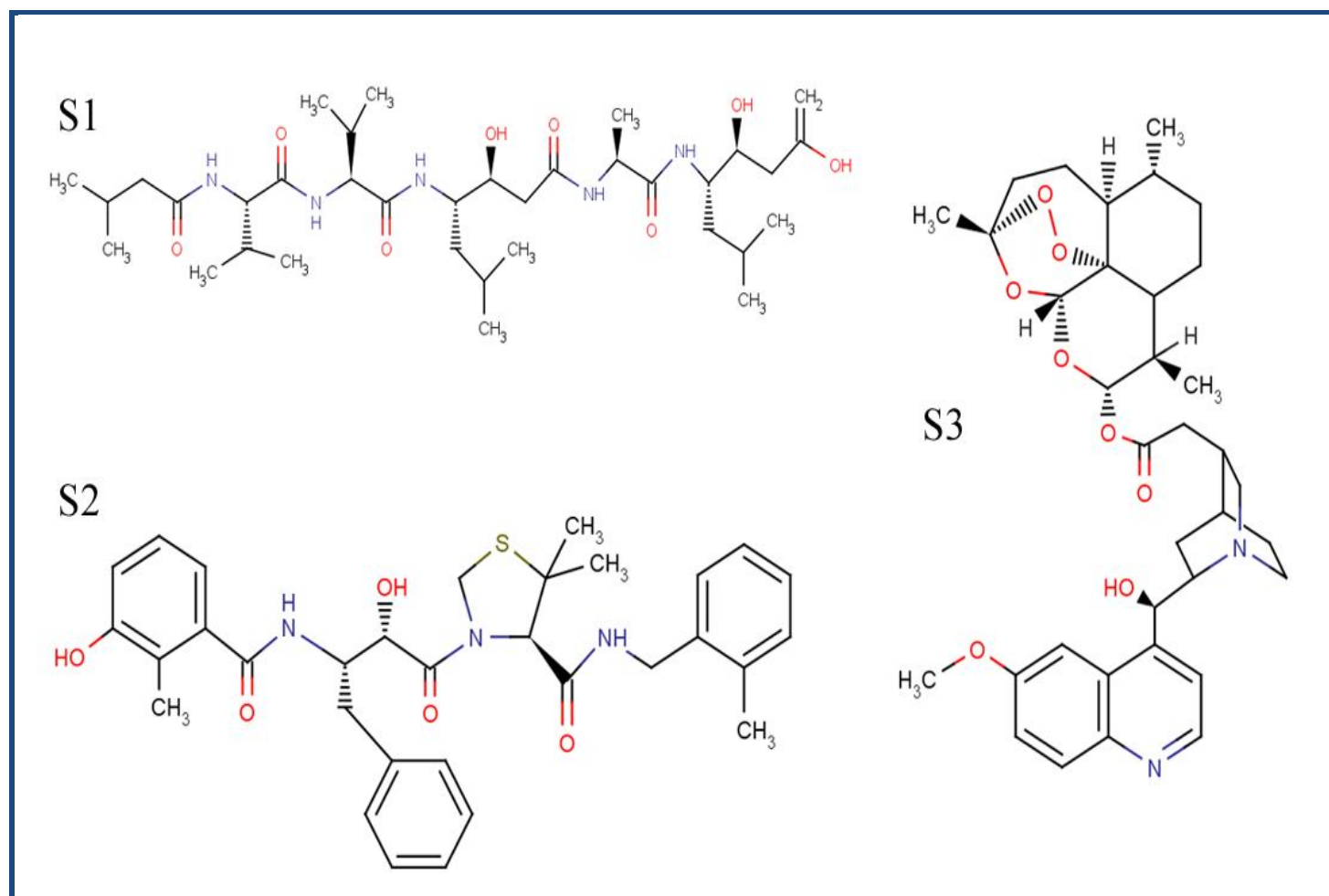


Figure S1: 2D structure of Pepstatin-A; **Figure S2:** 2 D structure of KNI-10006; **Figure S3:** 2D structure of Art-Qui-OH

Table 1: ΔG_{score} for Pepstatin-A, KNI-10006, Art-Qui-OH Hybrid

Receptor	Ligand	GlideScore _(Gscore)	Glide energy	GlideE _{model}	ΔG_{score}
HAP	KNI-10006	-8.777	-40.436	0.000	1.317
HAP	Pepstatin-A	-8.758	-19.222	0.000	1.298
HAP	Art-Qui-OH	-7.460	-32.824	-44.216	-

$$\Delta G_{\text{score}} = G_{\text{score- Art-Qui-OH}} - G_{\text{score- Pepstatin-A/KNI-10006}}$$

Table 2: $\Delta G_{\text{bind}}^{\text{exp}}$ & $\Delta G_{\text{bind}}^{\text{calc}}$ for Pepstatin-A, KNI-10006, Art-Qui-OH Hybrid

Receptor	Ligand	$\Delta G_{\text{bind}}^{\text{exp}a}$	$\Delta G_{\text{bind}}^{\text{calc}b}$	$\Delta\Delta G_{\text{bind-calc}}(\text{kcal/mol})$
HAP	KNI-10006	-14.38 ^b	-14.10	3.44
HAP	Pepstatin-A	-13.75 ^c	-13.09	2.43
HAP	Art-Qui-OH	-	-10.66	-

All the energy parameters are expressed in kcal/mol

$$\Delta\Delta G_{\text{bind-calc}} = \Delta G_{\text{bind-calc}}^{\text{Art-Qui-OH}} - \Delta G_{\text{bind-calc}}^{\text{Pepstatin-A/KNI-10006}}$$

^aThe $\Delta G_{\text{bind}}^{\text{exp}}$ is calculated using the relationship $\Delta G_{\text{bind,exp}} = 2.303RT \log Ki$; ^bValue from Ref [Nezami *et al*, 2003]; ^cValue from Ref [Banerjee *et al*, 2002]

Table 3: RMSD and docking score value from the docking simulation of 6 lowest configurations of co-crystal Pepstatin-A with Histo-Aspartic Protease (3FNT)

Configuration	GlideScore	$\Delta G_{\text{Score}}^a$	RMSD ^b (Å)
1	-8.758	0	1.132
2	-8.405	0.35	0.000
3	-8.369	0.38	1.217

4	-7.970	0.78	0.000
5	-7.922	0.83	1.112
6	-7.773	0.9	1.248

^a Δ GScore= $E_i - E_{lowest}$; ^bRMSD, RMSD between docked poses corresponding to each configuration.

Table 4: RMSD and dockings core value from the docking simulation of 6 lowest configurations of co-crystalKNI-10006 with Histo-Aspartic Protease (3FNU)

Configuration	GlideScore	Δ GScore ^a	RMSD ^b (Å)
1	-8.777	0	1.384
2	-8.527	0.25	0.000
3	-8.079	0.69	0.433
4	-7.956	0.82	1.304
5	-7.917	0.86	0.589
6	-7.897	0.88	1.531

^a Δ GScore= $E_i - E_{lowest}$; ^bRMSD, RMSD between docked poses corresponding to each configuration.