Indian Journal of Chemistry
Vol. 59B, May 2020, pp. 669-676

Prediction of agonist, partial agonist and full antagonist of *H. pylori TlpB* utilizing molecular docking

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Received 9 June 2019; accepted (revised) 24 January 2020

Helicobacter pylori infections are one of the major issues that produce gastric and duodenal ulcers due to chronic gastritis. Deforestation and global warming may cause ecological imbalance followed by climatic change due to enhanced temperature. This may contribute to abdominal discomfort and gastritis specifically in case of in taking a lot of non-vegetables, fast and junk foods, oily and spicy foods. *H. pylori*, which is asymptomatic for almost 80% of people's gastrointestinal tract (G.I.T.), may be stimulated due to chronic gastritis. It has been associated with colorectal polyps and cancer, if not treated well. Therefore, attention has been paid to predict some urea compounds as *H. pylori* antagonists utilizing structure-based molecular docking. Earlier reports of such work do not exist.

Keywords: *Helicobacter pylori*, TlpB gene, molecular docking, agonist, partial agonist, full antagonist

Helicobacter pylori infection is a major health concern worldwide. *Helicobacter pylori* is a Gram-negative, microaerophilic bacterium having the ability to colonize and it can grow in human gastric epithelial tissue and mucus. It can cause peptic ulcers, duodenal ulcers, and chronic gastritis and it is also involved in the development of gastric cancer[1]. Marshall BJ and Warren JR have identified *H. pylori* in 1984[2]. 10 years later, the International Agency for Research on Cancers classified *H. pylori* as carcinogenic to humans[3]. The acid environment of the stomach is crucial for *H. pylori* to survive in the presence of urea. H. pylori cannot survive in the normal environment of the stomach in the presence of urea because of the subsequent increase in *p*H rather than ammonia toxicity. *H. pylori* N6 strain survived well in solutions with *p*H values ranging from 4.5 to 7.0 in the absence of urea[4] but At low *p*H values (*e.g.* 3.5), the addition of urea increases survival[5]. There are four chemoreceptor genes (*tlpA*, *tlpB*, *tlpC*, and *tlpD*) expressed in *H. pylori* strain SS1 and strain KE26695. Matthew A and co-workers experimentally found that *tlpA*, *tlpC*, and *tlpD* mutants colonize mice to near wild-type levels whereas *tlpB* mutants were defective for colonization of highly permissive C57BL/6 interleukin-12 (IL-12) ($p40^{-/-}$)-deficient mice. *p*H taxis, like motility and urease activity, is crucial for colonization and insistence in the gastric mucosa, and thus TlpB function might represent a novel target for the inhibition of *h. pylori* bacterium[6]. Sweeney EG and co-workers were experimentally found that urea and the urea binding site residues of *H.pylori* chemo-receptor TlpB play critical roles in the ability of *H. pylori* to measure the acid concentration. The signaling model predicts that protonation events at Asp114, affected by changes in *p*H, dictate the stability of TlpB through urea binding[7].

Therefore it was postulated that urea derivatives may act as a competitive inhibitor of *H. pylori* chemoreceptor TlpB. It is also important to note that there is no direct inhibitor of *H. pylori* TlpB studied yet. Therefore in the present study, we have attempted to search urea compounds utilizing PubMed, PubChem, Sigma-Aldrich, HMDM, Wikipedia, chemical book, *etc*. These compounds have been docked and the mode of binding in term of interacting residues was analyzed in comparison to urea hydroxyurea and acetamide which are shown as agonist because these ligands interacting with ASP114, responsible for protonation where stabilization of TlpB. Hence in quest of urea derivatives which are may interact with *H. pylori* TlpB active cavity having a lack of interaction with

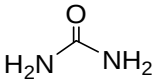
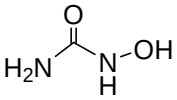
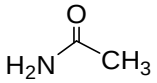
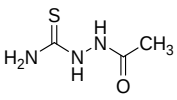
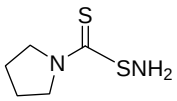
ASP114 may become an antagonist. These have been performed in the present study utilizing structure-based molecular docking.

Materials and Methods

Many 38 urea derivatives were obtained from Pub Chem, Sigma-Aldrich databases and several kinds of literature. The chemical structures of these derivatives were drawn utilizing 2D ChemDraw. 2D structures were transformed into 3D views which were fully optimized considering Marck molecule force field (MMFF) using a value of 0.01 as dielectric constant using chem3D ultra for making them energetically stable[8]. The crystal structure of *H. pylori* chemoreceptor TlpB (PDB ID: 3UB6) co-crystallized with urea was selected as the receptor for the docking studies[7]. Molecular docking helps to study interactions between ligand and receptor to identify active binding residues of target proteins. It helps to obtain the best geometry of ligand-receptor complex so that the energy of interaction between ligand and receptor is minimum. The minimum energy of interaction is called a score represented by different scoring functions. The ligand with minimal score could be screened as potential bioactive lead having a maximal affinity towards receptor, yielding a relative rank ordering of the docked compounds with respect to affinity. Prediction of affinity is referred to as scoring[9-11].

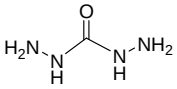
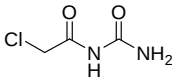
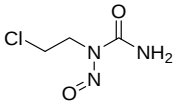
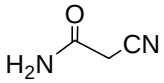
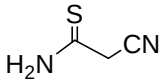
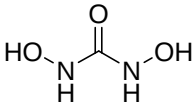
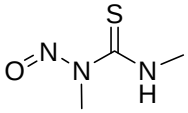
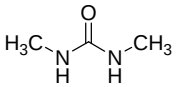
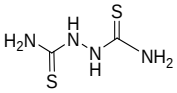
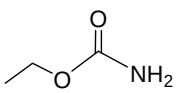
The protein was downloaded from the Brookhaven protein data bank[12]. The downloaded protein was prepared by removing all water molecules and hydrogen atoms in the H-depleted target molecule were added. All the optimized ligands (Table I) were docked into the active binding cavity of *H. pylori* chemoreceptor TlpB target gene using Argus Lab 4.0.1 freeware[13, 14]. The co-crystal urea was taken as reference for creating the ligand binding site considering grid resolution (angle) of 0.4 degrees as default value. ArgusLab allows free rotation of the ligand inside the cavity so as to generate a number of 150 poses.

Table I — Urea compounds along with their mode of interactions in the cavity of H. *pylori* chemo-receptor TlpB

Compd	Compd Name	Compd Structure	CAS Number	Mode of interaction	Docking scores (kcal/mol)	Predicted nature of the ligand
		Crystal structure analysis of *H. pylori* chemo-receptor TlpB				
1	Urea		57-13-6	LYS116, TYR153, ASP114, TYR140	-5.130	Full agonist
2	Hydroxyurea		127-07-1	LYS116, TYR153, ASP114, TYR185	-6.257	Full agonist
3	Acetamide		60-35-5	LYS116, TYR153, ASP114	-5.627	Partial agonist
		Urea compounds docked against H. *pylori* chemo-receptor TlpB				
4	1-Acetyl-3-thiosemicarbazide		2302-88-7	LYS116, ASP114, TYR185, MET102	-7.275	Partial agonist
5	Ammonium pyrrolidono di thio caboxylate		5108-96-3	LYS116, ASP114, TYR153, TYR185, TYR183, TYR136, VAL103, VAL116, MET155	-6.265	Partial agonist

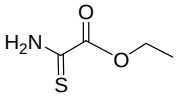
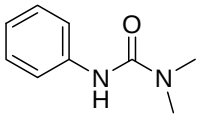
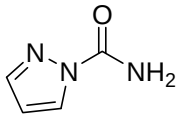
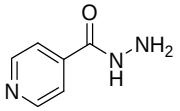
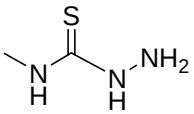
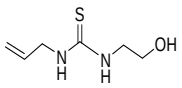
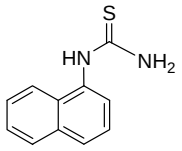
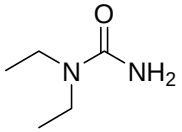
(*Contd.*)

Table I — Urea compounds along with their mode of interactions in the cavity of H. *pylori* chemo-receptor TlpB (*Contd.*)

Compd	Compd Name	Compd Structure	CAS Number	Mode of interaction	Docking scores (kcal/mol)	Predicted nature of the ligand
		Urea compounds docked against H. *pylori* chemo-receptor TlpB				
6	Carbohydrazide		497-18-7	LYS116, ASP114, TYR185, TYR101, TYR183, ASN94, PRO156	-7.475	Partial agonist
7	Chloro-acetylurea		4791-21-3	LYS116, ASP114, TYR185, VAL116, PRO115	-7.356	Partial agonist
8	1-(2-Chloroethyl)-1-Nitrosourea		2365-30-2	LYS116, ASP114, TYR140, TYR153, MET102, THR183	-6.040	Partial agonist
9	2-Cyanoacetamide		107-91-5	LYS116, ASP114, TYR140, ASN117	-5.537	Partial agonist
10	2-Cyano-thioacetamide		7357-70-2	LYS116, TYR153, ASP114, TYR136	-6.490	Partial agonist
11	1,3-di-hydroxyurea		686-68-0	LYS116, ASP114, TYR185, LYS157	-6.106	Partial agonist
12	1,3-dimethyl-1-Nitrosothiourea		79645-01-5	LYS116, TYR153, ASP114, TYR185, MET102, VAL103, PRO156	-5.239	Partial agonist
13	1,3-di-methylurea		96-31-1	LYS116, ASP114, TYR185, LYS157, MET102	-6.200	Partial agonist
14	2,5-dithiobiurea		142-46-1	LYS116, TYR153, ASP114, MET102, VAL116, ASN117	-6.386	Partial agonist
15	Ethyl carbamate		51-79-6	LYS116, TYR153, ASP114, TYR185	-6.601	Partial agonist

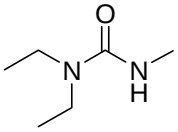
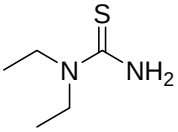
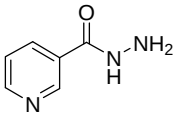
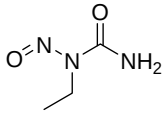
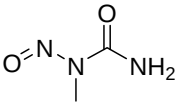
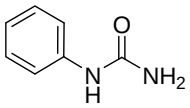
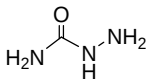
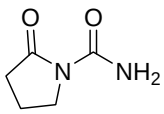
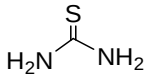
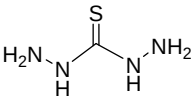
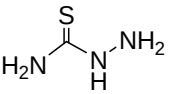
(*Contd.*)

Table I — Urea compounds along with their mode of interactions in the cavity of H. *pylori* chemo-receptor TlpB (*Contd.*)

Compd	Compd Name	Compd Structure	CAS Number	Mode of interaction	Docking scores (kcal/mol)	Predicted nature of the ligand
		Urea compounds docked against H. *pylori* chemo-receptor TlpB				
16	Ethylthio-xamate		16982-21-1	LYS116, TYR153, ASP114, TYR185, TYR136, MET155, PRO156	-7.708	Partial agonist
17	Fenuron		101-42-8	LYS116, ASP114, THR183, VAL116, MET155, PRO115, PRO156	-6.953	Partial agonist
18	1H-Pyrazole-1-Carboxamide		931-08-8	LYS116, ASP114, TYR185, MET102, VAL103, VAL113, PRO115	-5.948	Partial agonist
19	Isoniazid		54-85-3	ASP114, TYR185, TYR136, MET102	-7.305	Partial agonist
20	4-methyl-3-thiosemicarbazide		6610-29-3	LYS116, ASP114, TYR185, MET102	-7.317	Partial agonist
21	N-Allyl-N'-(2-hydroxyethyl) thiourea		105-81-7	LYS116, ASP114, TYR136, MET155, VAL103, VAL116	-6.944	Partial agonist
22	1-Napthylthiourea		86-88-4	LYS116, ASP114, TYR153, TYR185, TYR183, PRO115, LYS157	-8.145	Partial agonist
23	N,N-Diethylurea		634-95-7	LYS116, TYR153, ASP114, TYR185, TYR136, MET155, VAL103, PRO156	-5.970	Partial agonist

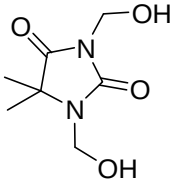
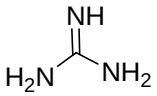
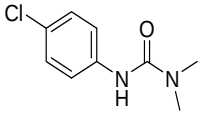
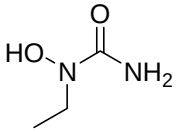
(*Contd.*)

Table I — Urea compounds along with their mode of interactions in the cavity of H. *pylori* chemo-receptor TlpB (*Contd.*)

Compd	Compd Name	Compd Structure	CAS Number	Mode of interaction	Docking scores (kcal/mol)	Predicted nature of the ligand
		Urea compounds docked against H. *pylori* chemo-receptor TlpB				
24	N,N-Diethyl-N'-methylurea		39499-81-5	LYS116, ASP114, VAL103, VAL116, PRO115, PRO156,	-6.315	Partial agonist
25	N,N-diethyl-thiourea		7204-46-8	LYS116, ASP114, MET102 MET155, VAL116, PRO156	-6.012	Full antagonist
26	Nicotinic Hydrazide		553-53-7	LYS116, TYR153, ASP114, TYR185, MET102	-8.641	Partial agonist
27	N-Nitroso-N-ethylurea		759-73-9	TYR153, ASP114, TYR140	-5.427	Partial agonist
28	N-Nitroso-N-methylurea		684-93-5	LYS116, ASP114, TYR185, MET102, VAL116, PRO115	-5.204	Partial agonist
29	N-Phenylurea		64-10-8	LYS116, TYR153, ASP114, TYR185	-8.348	Partial agonist
30	Semicarbazide		57-56-7	LYS116, ASP114, TYR185	-6.485	Partial agonist
31	Squamolone		40451-61-0	LYS116, TYR153, ASP114, PRO115	-5.99	Partial agonist
32	Thiourea		62-56-6	LYS116, ASP114, TYR185, LYS157	-5.115	Partial agonist
33	Thio-carbohydrazide		2231-57-4	LYS116, ASP114, TYR185, LYS157, PRO156	-7.368	Partial agonist
34	Thio-semicarbazide		79-19-6	LYS116, ASP114, TYR185, VAL116, MET102	-6.370	Partial agonist

(*Contd.*)

Table I — Urea compounds along with their mode of interactions in the cavity of H. *pylori* chemo-receptor TlpB (*Contd.*)

Compd	Compd Name	Compd Structure	CAS Number	Mode of interaction	Docking scores (kcal/mol)	Predicted nature of the ligand
		Urea compounds docked against H. *pylori* chemo-receptor TlpB				
35	DMDM Hydantoin		6440-58-0	LYS154, VAL131, SER130, MET115, TYR136, ASN117	-5.163	Full antagonist
36	Guanidine		50-01-1	TYR185, LYS157, MET102	-5.124	Full antagonist
37	Monuron		150-68-5	TYR136, VAL131, MET155, ASN117, PRO156, PRO163, GLN129	-6.206	Full antagonist
38	N-ethyl-N-hydroxyurea		7433-42-3	LYS116, TYR153, TYR185, PRO156	-5.666	Full antagonist

Results and Discussion

The best complex pose with minimal interaction energy has been taken into consideration for a better explanation of the mode of interaction between the ligand and active amino acid residues of the receptor protein.

The present investigation deals with the docking of 38 urea derivatives with *H. pylori* chemoreceptor TlpB. The detailed results of interactions are given in Table I. Docked complexes provide an insight into the activity patterns of various 38 urea derivatives in terms of hydrogen-bond, sulfur-bond, and hydrophobic interactions.

Molecular docking of 38 urea derivatives was carried out to predict its agonistic and antagonistic activity. Urea, hydroxyurea, and acetamide, were co-crystallized with TlpB (PDB IDs: 3UB6, 3UB9, 3UB7). It was shown that urea produces H-bond interactions with LYS116, TYR153, TYR140, and ASP114. Hydroxyurea produces the same mode of binding whereas acetamide does not interact with TYR140 because of possessing a methyl group. Carbonyl group of urea, hydroxyurea, and acetamide produce H-bonding with LYS116 while both amino groups produce H-bonding with ASP114 where one amino is also produced H-bonding with TYR140. Methyl group present in acetamide does not produce any interaction which is shown in Figure 1, Figure 2 and Figure 3.

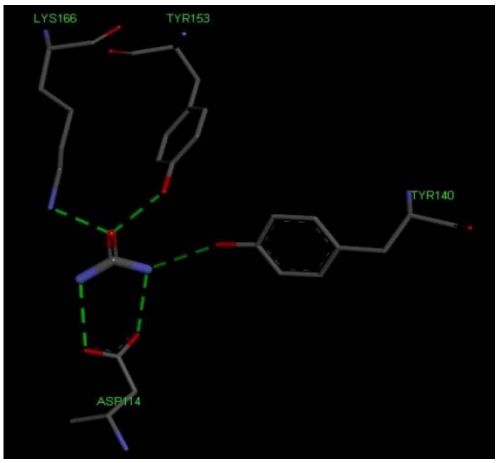


Figure 1 — Agonistic interaction between ligand (Urea) and *H. pylori* TlpB.

It was reported that urea is an agonist. As the mode of binding of hydroxyurea is similar to that of urea, the hydroxyurea may also act as agonist whereas acetamide may categorize as a partial agonist. Compounds 4-34 may interact with LYS116 and ASP114 which are common amino acids for partial agonist hydroxyurea. Therefore these compounds may be placed in the category of partial agonist.

Compounds no. 35-38 enter into the same cavity of TlpB and may produce antagonistic activity because they do not capture ASP114 which is crucial for *p*H sensing long life of H. pylori. Compound no. 35 produces H-bond interaction with ASN117, LYS154, SER130, and VAL131 while hydrophobic interaction with MET115 and TYR16. Compound no. 36 produces H-bond interaction with LYS157, MET102, and TYR185. Compound no. 37 produces H-bond interaction with ASN117 and GLN129 while hydrophobic interaction with PRO156, PRO163, TYR136, VAL131 and S-bonding with MET155. Compound no. 38 produces H-bond interaction with LYS116, TY153, TYR185 and PRO156 showing in Figure 4, Figure 5, Figure 6 and Figure 7.

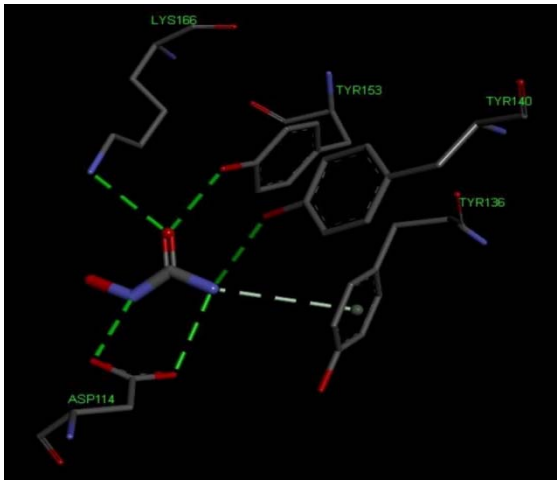


Figure 2 — Partial-agonistic interaction between ligand (Hydroxyurea) and *H. pylori* TlpB.

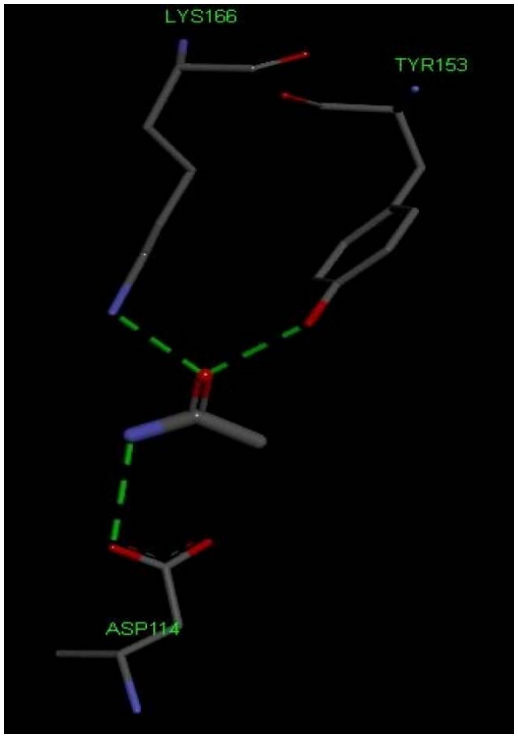


Figure 3 — Partial-agonistic interaction between ligand (Acetamide) and *H. pylori* TlpB.

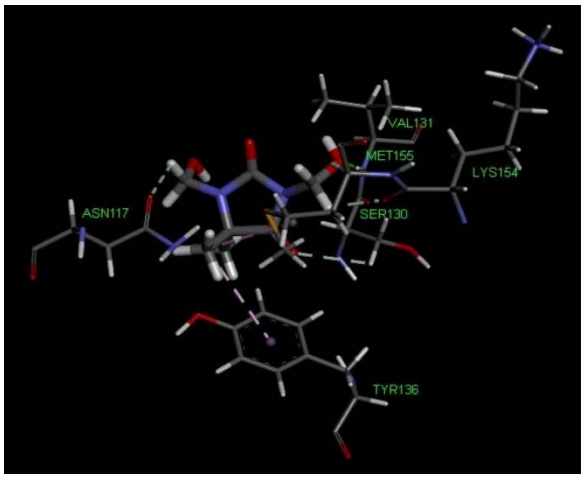


Figure 4 — Antagonistic interaction between ligand (DMDM hydantoin) and *H. pylori* TlpB.

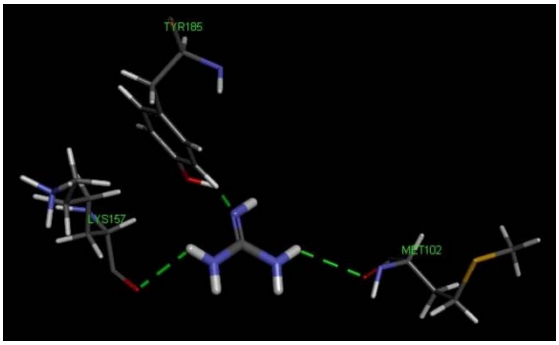


Figure 5 — Antagonistic interaction between ligand (Guanidine) and *H. pylori* TlpB.

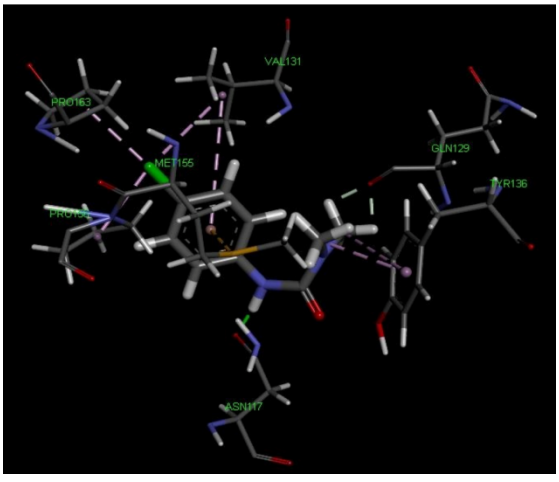


Figure 6 — Antagonistic interaction between ligand (Monuron) and *H. pylori* TlpB.

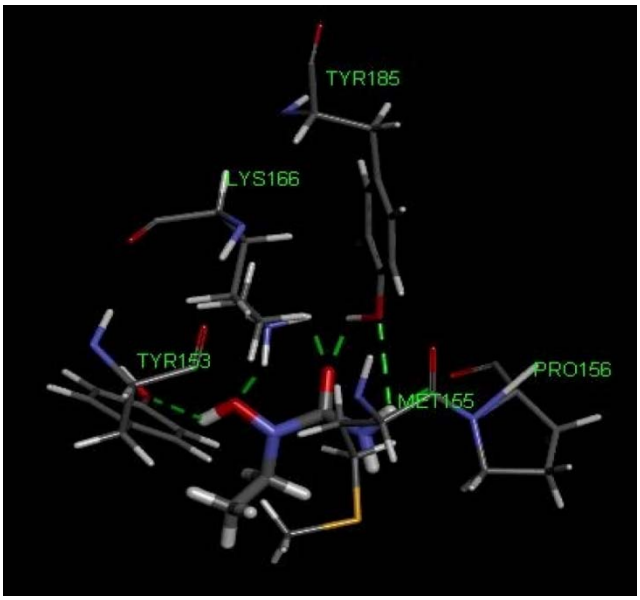


Figure 7 — Antagonistic interaction between ligand (N-ethyl-N-hydroxyurea) and *H. pylori* TlpB.

Conclusion

ASP114 is *p*H sensing amino acid which helps for *H. pylori* life in GIT. Compound numbers 35-38 when docked with *H. pylori* TlpB cavity, it causes the breakdown of ASP114 amino acid from the active binding site. Therefore these compounds may act as an antagonist. Compound numbers 3-34 show mode of binding similarity with urea. The captured amino acids are LYS116, ASP114, TYR140, TYR153, MET102, THR183. Therefore these compounds may become partial agonist and may potentiate *H. pylori* life in GIT. The binding energy range of agonist is -6.257 to -5.123 Kcal/mol. The binding energy range of full antagonist is -6.206 to -5.124 Kcal/mol. Therefore it is concluded that the full antagonist such as DMDM hydantoin, Guanidine, Monuron, and N-ethyl-N-hydroxyurea may produce competitive inhibition of target *H. pylori* TlpB cavity because they produce similarity in mode of binding as well as the energy of interaction.

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