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Investigation of Antiparasitic Properties of Benzimidazole Derivatives Against Amebiasis

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Abstract: Entamoeba histolytica is one of the common causes of infection in humans around the world. It causes clinically significant infection due to the fact that it causes morbidity and mortality. There is a need for new and safe drugs in the treatment of amebiasis. In this study, the activity of proton pump inhibitors against this parasite were investigated. Pantoprazole, lansoprazole, omeprazole, esomeprazole and rabeprazole were examined in detail. Initially, related drugs are optimized at M062X/6-31+G(d) level in water. Then, 3JS5, 3IDO and 3ILY were minimized at OPLS3e method. The docking calculations were performed and it is found that pantoprazole could be a significant candidate in the inhibiting of Entamoeba histolytica. Then, the interaction between pantoprazole and the target parasite were examined in the range of 0 – 100 nanoseconds (ns). The interaction energies in each one ns were calculated. As a result, the interaction was found as stronger than 88 ns. Pantoprazole was clinged to Entamoeba histolytica to inhibiting it.

Keywords: *Proton Pump Inhibitors, Parasite, Entamoeba Histolytica, Docking, In Silico*

Highlights

- *Antiparasite properties of benzimidazole derivative was investigated.*
- *In silico analyses were performed.*
- *Molecular dynamic calculations were done in the range of 0 – 100 ns.*

1. Introduction

Amoebiasis is a parasitic disease caused by the enteric protozoan Entamoeba histolytica. It is one of the common causes of infection in humans around the world. It causes clinically significant infections in both developed and developing countries. Concordantly to this, it is an important cause of morbidity and mortality all over the world. Clinically, it can lead to a clinical picture ranging from mild diarrhea and abdominal pain to fulminant colitis and even perforation. In addition to these, it can also cause hepatic abscess and chronic diarrhea [1-3].

Metronidazole, a nitroimidazole compound, plays an important role in the treatment of amebiasis. Its high efficacy, cheapness and oral intake make metronidazole the main therapeutic drug used against amoebiasis. However, long-term use can cause side effects including nausea, vomiting, dry mouth, metallic taste in the mouth, abdominal pain and headache. In addition to these unpleasant side effects, there are also problems with alcohol intolerance and use during pregnancy and breastfeeding. As well, it is stated that metronidazole has carcinogenic effects in animals. Moreover, even after a successful treatment, recurrent drug use may occur due to the recurrence of the disease [3,4].

For these reasons, there is a need for new and safe drugs in the treatment of amebiasis. However, very

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high costs remain a serious obstacle to developing a new drug. Whereas, determining a new indication for a drug or compound whose preclinical and clinical results are known before, greatly reduces the costs and time required to bring a drug to the market. Therefore, review and evaluation for this purpose the compounds whose bioactivity and toxicity are known stands as a wise approach.

Considering these data and facts, we wanted to evaluate the antiamoebic efficacy of proton pump inhibitors, which are imidazole derivatives and have been used for the treatment of acid-peptic diseases for years and have no obvious side effect profile [5]. Thus, we wanted to make a preliminary study whether proton pump inhibitors can be indicated in the treatment of amoeba.

2. Computational Method

Proton pump inhibitors which are (RS)-6-(Difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl) methylsulfinyl]-1H-benzo[d]imidazole (pantoprazole), (RS)-2-([3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl)-1H-benzo[d]imidazole (lansoprazole), 5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl) methanesulfinyl]-1H-benzimidazole (omeprazole), (S)-(−)-5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-3H-benzoimidazole (esomeprazole) and (RS)-2-([4-(3-Methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl)-1H-benzo[d]imidazole (rabeprazole) are taken into consideration. Initially, these compounds are optimized at M062X/6-31+G(d) in water using Gaussian software [6,7]. No imaginary frequency is obtained from calculation results. The pdb file of studied inhibitors are obtained and re-minimized at OPLS3e method using LigPrep module of Maestro software [8-10]. As for the proteins, 3JS5 [11], 3IDO [11], 3ILY [11] are selected for molecular docking calculations. x, y,z coordinates of receptor binding domain of 3JS5, 3IDO and 3ILY were defined as (-17,17:19.7:-16.26), (67.01:68.15:9.39) and (-16.6:16.69:22.56), respectively. These proteins are related with tyrosine phosphate of Entamoeba histolytica.

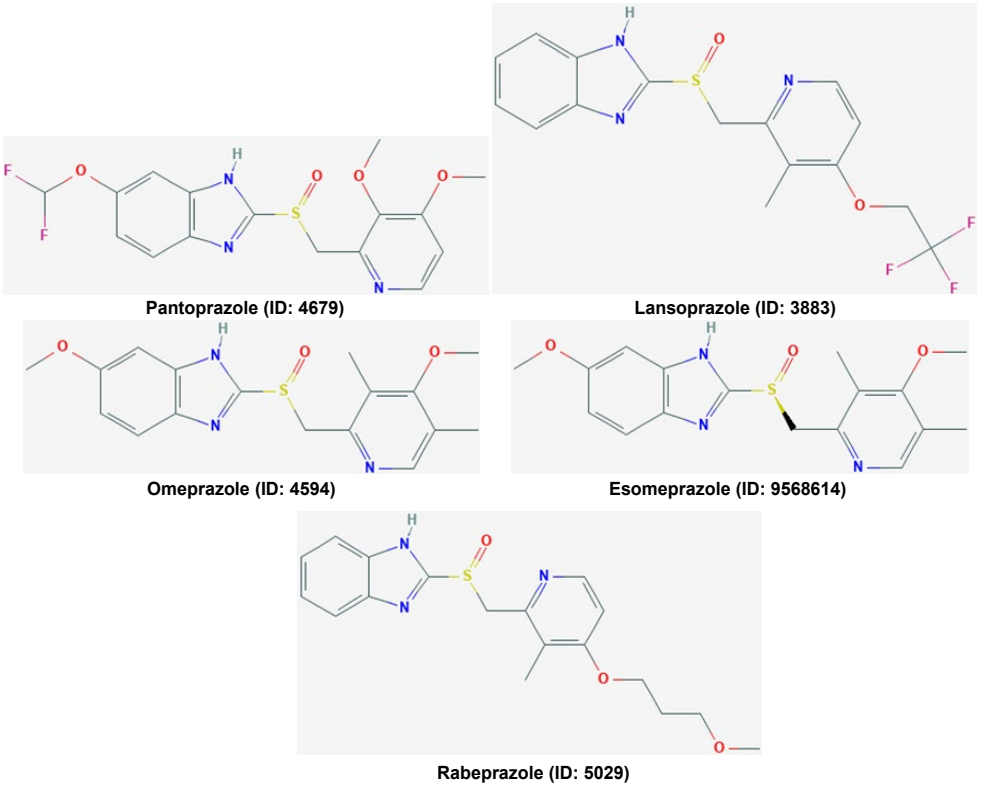

Fig. 1. The studied proton pump inhibitors

These proteins are prepared using Protein Prep module and receptor binding domain was defined by Receptor Grid Generation. Finally, molecular docking calculations were performed between PPIs and selected protein. In addition to these calculations, the molecular mechanics energies combined with the Poisson–Boltzmann and surface area continuum solvation (MM-PBSA) calculations were done for selected ligand-protein interactions. The binding energies are calculated in each 5 ns in

the range of 0 – 100 ns. In these calculations, Nanoscale Molecular Dynamics (NAMD) [12] and Visual Molecular Dynamics (VMD) [13] software programs were used. The Gibbs binding energy was calculated using Eq. (1).

$$\Delta G_{Binding} = G_{Complex} - (G_{Protein} + G_{Inhibitor} (1)$$

3. Results and discussion

3.1. Proton Pump Inhibitors

The related proton pump inhibitors which are pantoprazole, lansoprazole, omeprazole, esomeprazole and rabeprazole are taken into account for the optimization calculations. These inhibitors are optimized at M062X/6-31+G(d) in water. The structure of mentioned PPIs are represented in Fig. 1 with their PubChem ID.

There are a lot of heteroatoms in these inhibitors which are nitrogen, oxygen, sulphur and fluoride. Quantity of heteroatom are significant for the interaction between inhibitor and target protein. The heteroatom numbers of pantoprazole, lansoprazole, omeprazole, esomeprazole and rabeprazole are ten, nine, seven, seven and seven, respectively.

3.2. Tyrosine Phosphate of *Entamoeba histolytica*

Entamoeba histolytica is an anaerobic parasitic amoebozoan, part of the genus Entamoeba. Predominantly infecting humans and other primates causing amoebiasis, E. histolytica is estimated to infect about 35-50 million people worldwide. Some protein structures of this parasite are reported in literature which are calmodulin-like protein, calcium binding protein, coactosin, phosphoserine phosphatase, cysteine protease, tyrosine phosphatase, etc. Tyrosine phosphatase has significant role in the growing of *Entamoeba histolytica* [14]. One of the preventing of this parasite is the inhibiting of this protein. The protein structure and electrostatic potential map of receptor binding domain of it are represented in Fig. 2.

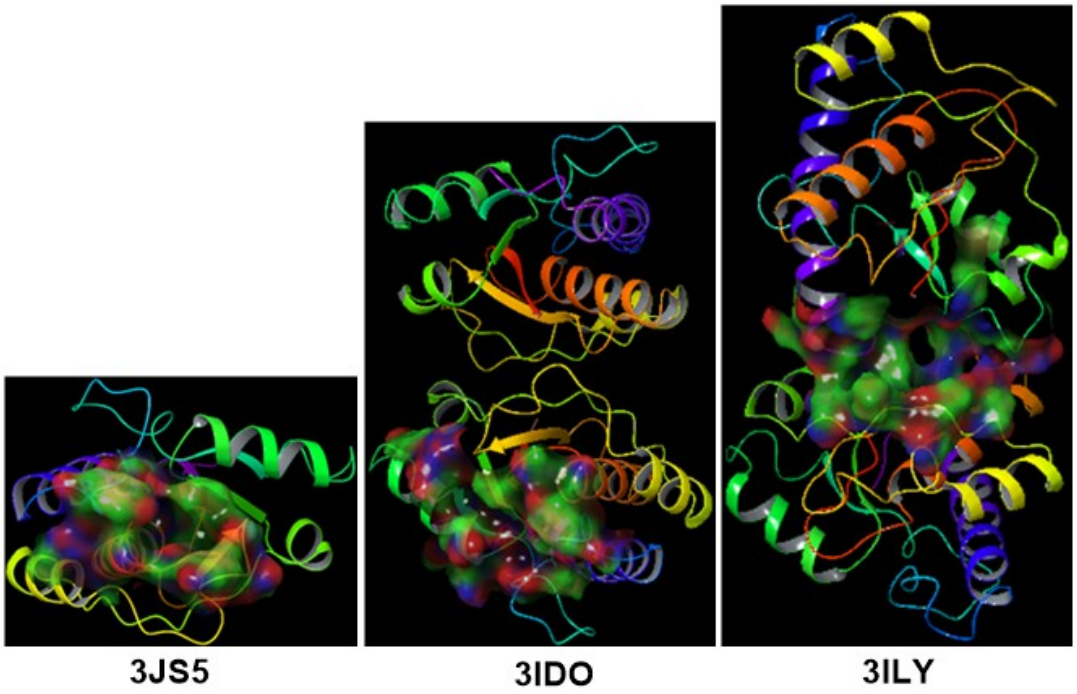

Fig. 2. Protein structure of tyrosine phosphatase of *Entamoeba histolytica* and electrostatic potential map.

According to Fig. 2, there are different color regions on the map which are mainly red, green and blue. Red color implies the most electron density region while blue shows the poorest electron region. As for the green, it implies neutral regions. Inhibitors are mainly interacted with these regions. The interaction structure varies according to the charge distribution on the molecule.

3.3. Molecular Docking Analyses

Biological activity of chemicals can be foreseen by molecular docking analyses by using computational

techniques. Molecular docking calculations are performed between studied PPIs and target proteins. The interaction between the related inhibitors and receptor binding domain of target proteins are examined in detail. The docking results which are docking score (DS), ligand efficiency (LE), van der Walls energy (E_{vdw}), coulomb energy (E_{coul}) and interaction energy (E_{Int}) are given in Table 1.

According to Table 1, the whole studied PPIs are not active against tyrosine phosphatase of Entamoeba histolytica. Especially, esomeprazole and rabeprazole are found as inactive. As for the others, pantoprazole and lansoprazole are found as the best two one at first sight. The docking score Pantoprazole is inhibited the 3JS5 and 3IDO while lansoprazole is inhibited the 3IDO and 3ILY. The docking score for 3ILY is bad for the docking results. Therefore, this protein is reckoned without for further analyses. For other results, it can be seen that pantoprazole is the best PPI in docking calculations. The docking structure of pantoprazole with 3JS5 and 3IDO are represented in Fig. 3. Additionally, the interaction structures of these dockings are represented in Fig. 4.

Table 1. Molecular docking results of sage herb against SARS-CoV-2

Compound	DS[a]	LE[a]	E_{vdw}[a]	E_{coul}[a]	E_{Int}[a]
For 3JS5					
Pantoprazole[b]	-3.83	-0.15	-29.77	-10.51	-40.28
Pantoprazole[c]	-3.65	-0.14	-28.28	-5.90	-34.18
Pantoprazole[c]	-2.72	-0.10	-28.99	-5.25	-34.24
For 3IDO					
Pantoprazole[b]	-3.36	-0.13	-27.69	-5.37	-33.06
Omeprazole[b]	-1.66	-0.07	-20.62	-7.71	-28.33
Lansoprazole[b]	-3.15	-0.13	-21.09	-10.52	-31.60
Lansoprazole[c]	-2.72	-0.11	-27.18	-6.08	-33.27
For 3ILY					
Lansoprazole[b]	-0.67	-0.03	-32.85	-4.19	-37.05
Lansoprazole[c]	-0.54	-0.02	-28.43	-7.89	-36.32

[a] in kcal/mol; [b] Orginal Pose; [c] Possible Pose

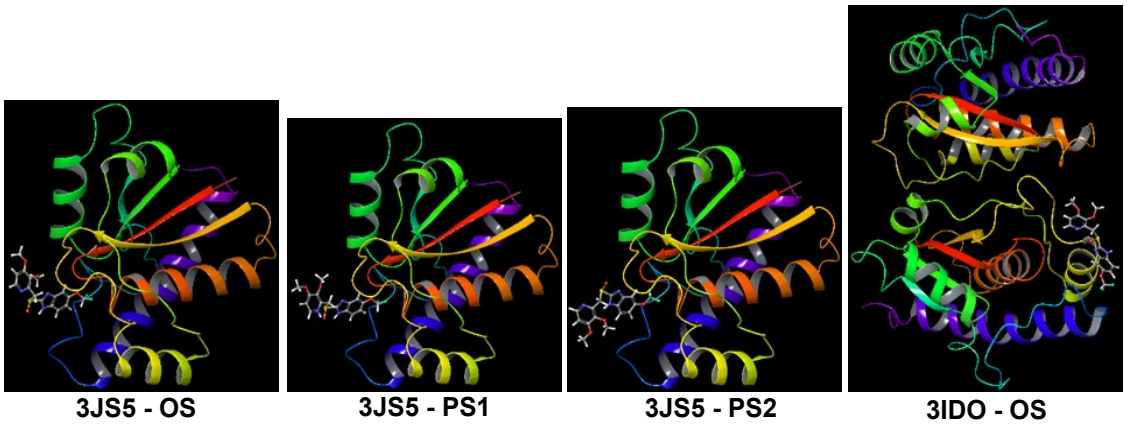

Fig. 3. Docking structure of pantoprazole.

According to Fig. 4, the interaction types between pantoprazole and target protein can be seen easily. The dominant interaction types are determined as solvent exposure, hydrophobic, polar, charged (negative), charged (positive), glycine, pi-pi stacking and pi-cation interactions. Additionally, the amino acids that interact the most with pantoprazole are seen as cysteine and tyrosine.

3.4. MM-PBSA Calculations

Determining of the interaction stability between inhibitor and receptor binding domain of target

protein is so significant in further analyses. For this purpose, poisson–boltzmann and surface area continuum solvation (MM-PBSA) calculations are performed using VMD and NAMD software. The interaction energy is calculated in each 1 ns in the range of 0 – 100 ns. The calculated results are given in Table 2 and the binding energy in each 1 ns are represented in Fig. 5.

According to Table 2 and Fig. 5, the interaction is seemed as wavy. Sometime the binding energy is positive while it is negative in the other time. But it can be said that the negative values are dominant for the interaction. Especially, the whole energy after the 88 ns is negative. This implies that there is strong interaction between protein and ligand. Therefore, pantoprazole can be used the inhibiting of the tyrosine phosphatase of Entamoeba histolytica.

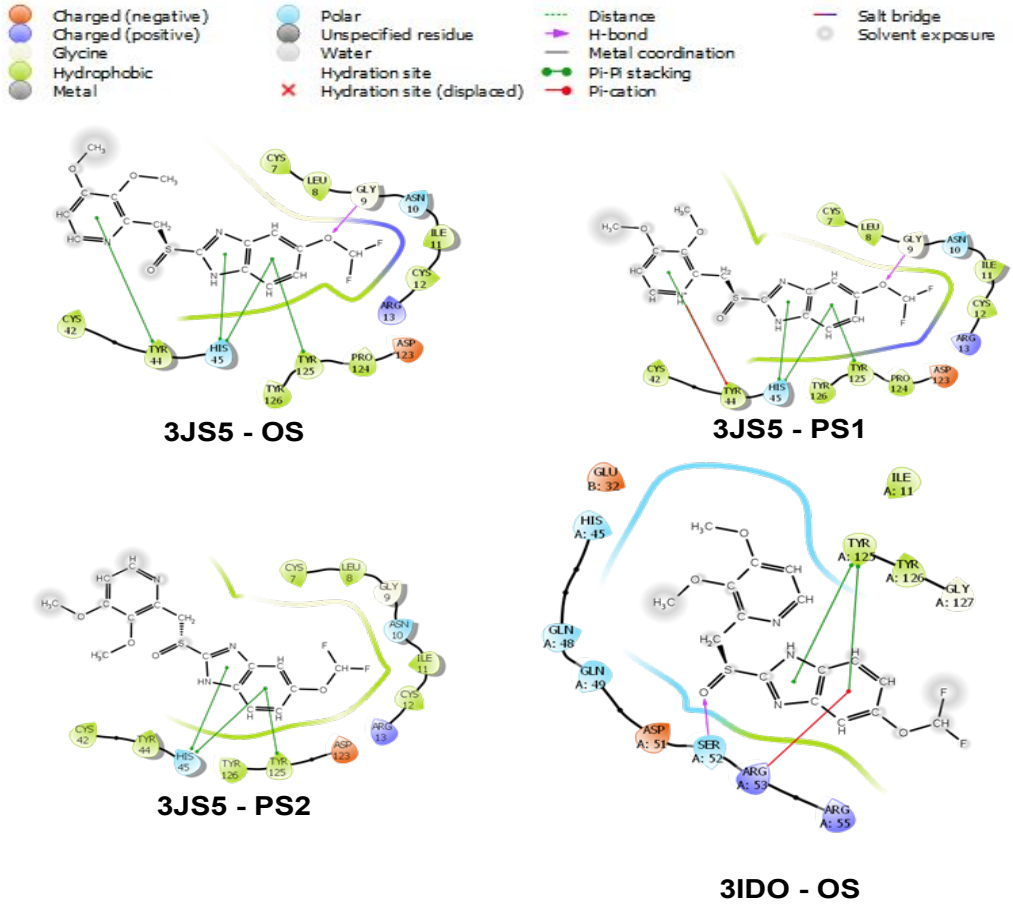

Fig. 4. Interaction structure of pantoprazole.

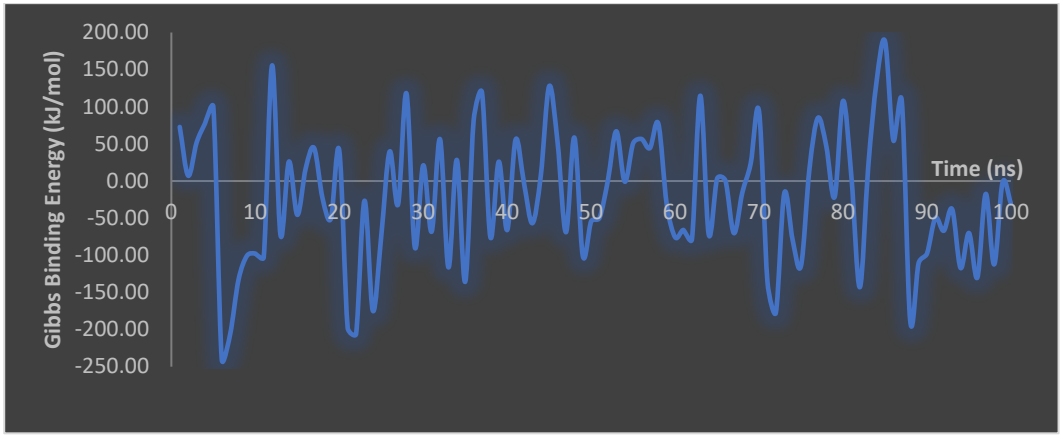

Fig. 5. The binding energy distribution of 3JS5-pantoprazole structure.

Table 2. The binding energy (kJ/mol) in each one nanosecond for 3JS5 – pantoprazole complex structure

Time	Energy	Time	Energy	Time	Energy	Time	Energy
1	72.79	26	39.75	51	-49.45	76	16.28
2	7.20	27	-31.47	52	1.20	77	84.94
3	51.49	28	118.03	53	67.06	78	47.31
4	77.30	29	-89.40	54	-0.88	79	-21.46
5	99.46	30	21.12	55	50.71	80	107.83
6	-239.56	31	-68.20	56	56.58	81	4.94
7	-206.51	32	56.31	57	44.35	82	-143.03
8	-132.87	33	-116.21	58	77.17	83	19.07
9	-100.73	34	28.43	59	-31.33	84	134.43
10	-97.67	35	-135.58	60	-75.68	85	188.09
11	-102.81	36	81.15	61	-65.92	86	54.55
12	155.66	37	119.01	62	-78.73	87	107.69
13	-72.74	38	-75.64	63	114.93	88	-188.04
14	26.16	39	26.10	64	-71.75	89	-110.73
15	-45.54	40	-65.99	65	3.13	90	-97.69
16	17.01	41	55.67	66	0.19	91	-49.84
17	44.45	42	-2.72	67	-70.14	92	-66.98
18	-22.89	43	-56.80	68	-15.76	93	-38.04
19	-50.58	44	7.08	69	23.56	94	-117.08
20	40.92	45	127.70	70	93.32	95	-69.98
21	-197.65	46	52.09	71	-135.98	96	-129.97
22	-206.16	47	-68.95	72	-177.28	97	-17.34
23	-26.70	48	58.51	73	-16.60	98	-112.33
24	-174.73	49	-100.53	74	-79.54	99	-2.02
25	-74.00	50	-54.35	75	-113.82	100	-30.46

4. Conclusion

Five proton pump inhibitors are taken into account for this study which are pantoprazole, lansoprazole, omeprazole, esomeprazole and rabeprazole. The structures of these compounds are taken from PubChem website. Tyrosine phosphatase of Entamoeba histolytica is selected as target protein due to the fact that inhibiting of the tyrosine phosphatase is the one of the preventing mechanism of Entamoeba histolytica. Three protein structures, 3JS5, 3IDO and 3ILY are selected for the docking analyses. The site maps of them are calculated and docking calculations are performed. According to docking results, pantoprazole is found as active against the target proteins. Finally, molecular dynamic calculation is performed between pantoprazole and 3JS5. Effective binding is found after 88 ns. As a result, it is found that pantoprazole can be effective in the inhibiting of Entamoeba histolytica.

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