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Inhibitory potentials of ivermectin, nafamostat, and camostat on spike protein and some nonstructural proteins of SARS-CoV-2: Virtual screening approach

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Abstract: The search for potential oral drugs either through synthetic routes or by drug repurposing for combating the dreaded covid-19 virus is still ongoing. The coronavirus spike glycoprotein and several other non-structural proteins play crucial roles in the replication and transmission of this virus. Recent research have identified ivermectin, nafamostat, and camostat as promising drug inhibitors of SARS-CoV-2 target proteins. The broad-spectrum inhibitory action of ivermectin, nafamostat, and camostat on the spike glycoprotein and some non-structural proteins of this virus was studied in silico. The spike glycoprotein, nsp3, nsp5, nsp9, nsp10, nsp13, and nsp16 were selected for this study and were downloaded from the protein data bank. Flexible docking procedure implemented in Auto Dock Vina module was deployed for the docking procedure of the drugs with the protein receptors. Although ivermectin had the best inhibitory action on the viral spike protein and nsp10, nafamostat was identified as the compound with the best broad-spectrum activity on this virus, having the highest binding affinity values of – 9.4kcal/mol, – 7.9 Kcal/mol, – 6.1 Kcal/mol, – 8.0 Kcal/mol, and – 8.7 Kcal/mol for nsp3, nsp5, nsp9, nsp13, and nsp16 respectively. This drug, in combination with ivermectin could therefore be explored further as potential compounds that could be modified to curb the menace of the covid-19 pandemic.

Keywords: Camostat; Ivermectin; Nafamostat; Nonstructural protein; Spike protein; Virtual screening.

INTRODUCTION

Nearly a century after the Spanish flu, the coronavirus disease 2019 (COVID-19) is a pandemic currently being faced by the global community[1]. The current pandemic is because of a novel beta Covid, SARS-CoV-2, systematically having a place with the coronaviridae family, known to cause respiratory diseases in people[2]. SARS-CoV-2 is a wrapped, single-stranded positive-sense RNA infection. The viral RNA genome contains 29,903 nucleotide bases and has ten

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open reading frames (ORF). The ORF1ab encodes for the enormous replicase polyprotein PP1ab, which is separated by papain-like protease (PLpro) and 3-chymotrypsin-like protease (3CLpro) to generate nonstructural proteins (nsps) 1–16, required for the replication of the virus. The primary proteins S, N, E, M, and supplementary proteins are encoded by ORF2-10 [2]. The S protein, anchored on the virus envelope, serves to attach coronavirus receptors and internalization[3]. This protein plays a crucial role in receptor recognition as well as the cell membrane fusion process. As soon as the virus interacts with the host cell, an extensive structural reorganization of the S protein occurs. This activity allows the virus to fuse with the host cell membrane. Polysaccharide molecules coat the spikes to camouflage them, thereby dodging surveillance of the host immune system during entrance [4].

Several non-structural proteins contribute to the replication and transcription of coronaviruses. Nsp3 is a multi-domain protein produced by coronaviruses. It is the largest of the non-structural proteins. It plays many roles in the viral life cycle, acting as a framework of protein that interacts with itself and binds to other viral nsps or host proteins [5] .Generally, nsp3 is crucial in coronaviruses for the formation of replication transcription complexes (RTC) assembly on the host cell membrane, where replication and transcription of the viral genome take occur [6]. Nsp5, often referred to as 3C-like protease, plays a crucial role in synthesizing viral proteins and generates many nonstructural viral proteins through its protease activity. Nsp5 plays a vital role in the coronavirus life cycle, making it a desirable target for producing antiviral drugs [7,8]. Nsp9 is an essential non-structural protein that links coronavirus replication to RNA. Several ways of nsp9 dimerization improve their binding affinity to nucleic acid[9]. Nsp10 is also a significant replication regulator with 148 amino acids and two zinc finger domains for enzymatic interactions. It could interact with nsp14 and nsp16 [10,11]. Nsp13 is one of the most conserved ancestral proteins in nido-viruses, making it an essential drug discovery target [12]. This protein can unwind double-stranded DNA and RNA through hydrolysis of deoxyribonucleotide triphosphates (dNTPs) and ribonucleotide triphosphates. This activity can be facilitated by nsp12 [13]. All the non-structural proteins, nsp16 is crucial in the viral replication cycle because it is important for coronavirus immune evasion [14]. Nsp16 being a 2'-O-methyltransferase, forms part of the replication transcription complex [15]. This protein particularly promotes the transfer of a methyl group from its S-adenosylmethionine cofactor to the 2-hydroxyl of ribose sugar of viral Mrna [16]. This activity improves translation efficiency and camouflages the mRNA so that intracellular pathogen recognition receptors do not recognize it. Essentially, the inhibition or knocking out of 2'-O-mTase activity severely reduces viral replication and infectivity of coronaviruses [17]. Therefore, developing inhibitors of nsp16 is a potential therapeutic approach.

Numerous studies related to identifying effective therapeutics for SARS-CoV-2 have been reported [18-20]. In our previous study [21], using in silico techniques, we evaluated the efficiency of eleven drugs, including chloroquine, hydroxychloroquine, lopinavir, ritonavir, nafamostat, camostat, famotidine, umifenovir, nitazoxanide, ivermectin, and fluvoxamine, in blocking the interactions between human ACE2 and coronavirus spike glycoprotein. Lopinavir, ritonavir, and nafamostat showed good binding affinity on ACE2, while ivermectin, nafamostat, and camostat had the best binding affinity on the coronavirus spike glycoprotein. In this study, the binding affinities of ivermectin, nafamostat, and camostaton the spike and some non-structural proteins of theSARS-CoV-2 were investigated in silico to identify the compound with the largest broad-spectrum inhibitory activity on this virus.

MATERIAL AND METHOD

Protein selection and preparation

Three dimensional (3D) X-ray crystallographic structure of SARS-CoV-2 spike protein, non-structural proteins 3, 5, 9, 10, 13, and 16 weresourced from the protein data bank (PDB) through protein-plus webserver of Hamburg University, Germany. These selected proteins were then prepared for *in silico* docking and minimization implemented via the appropriate tools in Cresset Flare© software, version 4.0 (https://www.cresset-group.com/flare/). The minimization was implemented by choosing the General Amber Force Field (GAFF) option, with a gradient cutoff of 0.200 Kcal/mol/A, and iteration was set to 2000 iterations [22].

Selection and preparations of drugs

Three dimensional (3D) structures of camostat, nafamostat, and ivermectin were recovered from an online chemical curation server called PubChem in simple document format (SDF). Open babel in Python Prescription (version 0.8) was deployed for the optimization of our selected ligands. This process converts ligands into the most stable structures energetically by choosing Universal Force Field (UFF) option.

Computational docking procedure

Flexible docking procedure implemented in the Auto Dock Vina module in Python Prescription suite [23] was deployed for the docking procedure of the drugs with the protein receptors. Target site specific to each protein receptor was adjusted through the grid box with parameters provided in Table 1, containing the dimensions and the binding regions of each protein. The binding affinity with the protein-drug complex was retrieved at the end of the docking run.

RESULTS AND DISCUSSION

The binding affinity of ivermectin, nafamostat, and camostat on the spike glycoprotein and some other non-structural proteins of SARS-CoV-2 are shown in Table 2 and the interactions of the drugs with the amino acids at the binding site of the proteins are given in Table 3.

Table 2. Binding affinity of the selected drugs on some SARS-CoV-2 proteins

Drugs	ΔG (Kcal/mol)						
	Spike	Nsp3	Nsp5	Nsp9	Nsp10	Nsp13	Nsp16
Ivermectin	**-8.4**	**-**6.4	**-**6.9	**-**3.7	**-8.0**	**-**4.1	**-**5.7
Nafamostat	**-**7.8	**-9.4**	**-7.9**	**-6.1**	**-**7.7	**-8.0**	**-8.7**
Camostat	**-**7.2	**-**8.3	**-**6.7	**-**5.4	**-**7.0	**-**7.3	**-**7.6

The antiviral agent camostat is a serine protease inhibitor that attacks SARS-CoV and SARS-CoV-2. Clinically, it is used to treat pancreatitis and reflux oesophagitis. It fights and reduces viral infection by blocking virus-membrane fusion. Studies show thatSARS-CoV-2 utilizes the human transmembrane protease serine 2, TMPRSS2, to enter the human cell, cleave and activate the spike protein [31,32]. This shows that the drug attacks and prevents virus-cell membrane fusion, thereby inhibiting viral replication.

Nafamostat approved for the treatment of acute pancreatitis is being studied as a drug that can block the viral entry of the new coronavirus, SARS-CoV-2. According to recent studies on SARS-CoV-2 cell entry on ACE2 and TMPRSS2, nafamostat can very well inhibit the membrane fusion of the virus's envelope with host cell surface membranes [32]. Results show that it efficiently blocked SARS-CoV-2 infection of human lungs. It has also been reported to block the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection in vitro [33]

Ivermectin is widely used as a broad-spectrum antiparasitic drug with known efficacy of antiviral properties. It is commonly used to treat several tropical diseases that include onchocerciasis, helminthiases, and scabies. It is also used

to control malaria transmission as it is appreciably tolerated and used. Reports from studies suggest that ivermectin inhibits key intracellular transport proteins hijacked by viruses that infect by attacking the host's antiviral response. A study by Chaccour [34] reported that patients treated with a single 400 mcg/kg dose of ivermectin for mild COVID-19 showed a tendency to lower viral load and cough within 72h. They suggested that there could be a down-regulation of the ACE-2 receptor and viral entry into the cells of the respiratory epithelium and olfactory bulb. It could also result from inhibition of the activation of pro-inflammatory pathways in the olfactory epithelium. It has also been shown to inhibit the replication of SARS-CoV-2 in cell cultures [35].

The binding affinity of nafamostat was significantly greater than those of camostat and ivermectin on all the non-structural proteins apart from nsp10 that showed the best binding with ivermectin. This observation indicated that nafamostat would give the broadest spectrum of inhibitory action on these SARS-CoV-2 non-structural proteins but may however not be as efficient as ivermectin in preventing the replication of the virus. Ivermectin had the best inhibitory action on the spike glycoprotein and nsp10, which showed that it could prevent the penetration of the viral spike protein into the host and prevent the virus's replication.

A greater number of hydrogen bond interactions were found in the binding of nafamostat and camostat with the amino acid residues at the active sites of the proteins than what was observed with their interaction with ivermectin. This indicated that the drug-protein complexes formed by these two drugs would be more stable than those formed by ivermectin [36]. All the drugs interacted with Arg403 in the spike glycoprotein, showing that this amino acid is essential in inhibiting the action of this protein. Also, nafamostat and camostat interacted with Tyr495 and Thr500 in the spike glycoprotein, suggesting that their mechanism of action are similar.

All the drugs studied interacted with Pro248 and Tyr268 at the active site of nsp3. Nafamostat and camostat showed very similar mode of action at this site by their interaction with Gly163, Asp164, Arg166, Pro248, Tyr268, Gln269, and Glu167. Different amino acids interacted with the drugs at nsp5, indicating that their mechanisms of action at this protein site were not related.At nsp9 and nsp16, the mode of action of ivermectin was different from the other two drugs. Nafamostat and camostatinteracted withAla8, Leu9, Gln11, and Met101 at nsp9, and also with the amino acids Asp130, Gly71, Leu100, and Phe149 at nsp16,suggesting a similar mode of action by these two drugs at these sites. All the drugs interacted with ILE55 and VAL116 at nsp10, with nafamostat and camostat having a closer relationship in their mode of action by having additional interactions with Asp91 and Thr111. At nsp13, similarity in the interaction of all the drugs was observed at Lys288 and Asp374. The remaining interactions by all the drugs occurred at different amino acids in the protein.

CONCLUSION

The potentials of camostat, nafamostat, and ivermectin to inhibit the spike glycoprotein, nsp3, nsp5, nsp9, nsp10, nsp13, and nsp16 of SARS-CoV-2 were studied in silico. Nafamostat showed good binding affinity on all target proteins. However, ivermectin was better at binding with the spike glycoprotein and nsp10 than this drug. The mechanism of action of nafamostat and camostat on the studied proteins were very similar but varied markedly with ivermectin. The good binding affinity demonstrated by nafamostat at nsp3, nsp5, nsp9, nsp13, and nsp16 showed that it could influence multi-target interactions of the five proteins of the virus and hence curtail the infection. The potentials of nafamostat and ivermectin in SARS-CoV-2 prevention could therefore be explored for the possible production of a single compound that can inhibit spike glycoprotein and human ACE2 binding,

and interfere with the replication and transcription of coronaviruses in Homo sapiens when the infection has already occurred.

AUTHORS' CONTRIBUTIONS

CED: Conceptualization, Data curation, Supervision, Methodology, Software HIU: Conceptualization, Supervision, Methodology, Data curation, Software. IAD: Visualization, Investigation. UEE: Visualization, Investigation. LCN: Original draft preparation, Writing- Reviewing and Editing. CEE: Original draft preparation, Writing- Reviewing and Editing.

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DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals. The authors declare that they have no competing interests.

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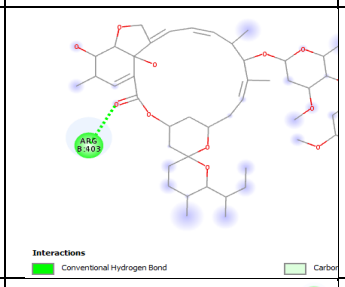
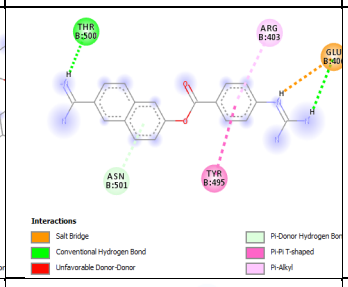
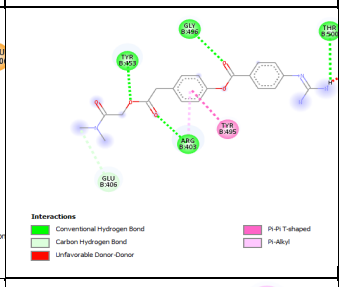
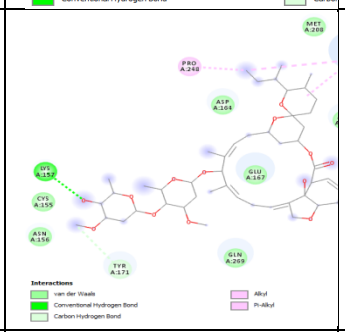
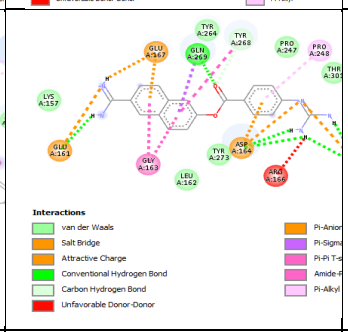
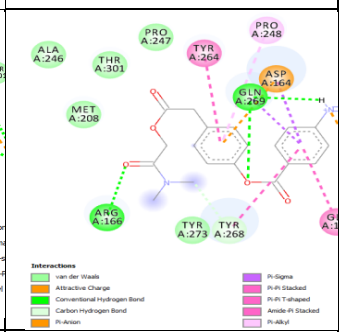
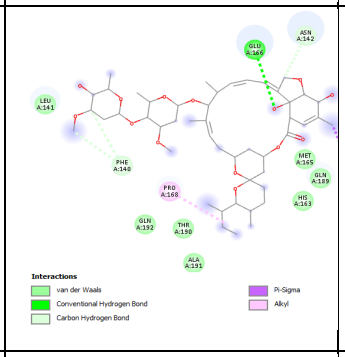
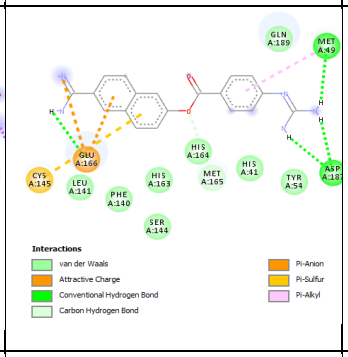
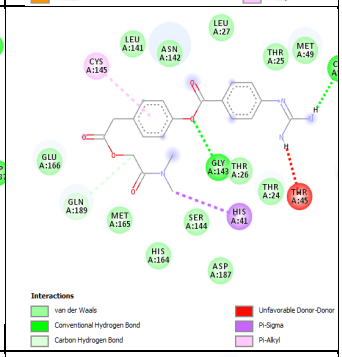
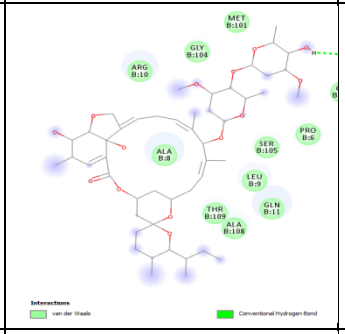
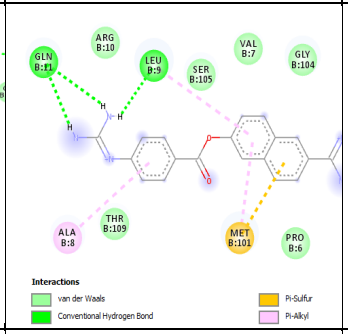
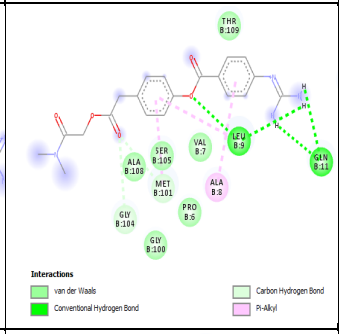
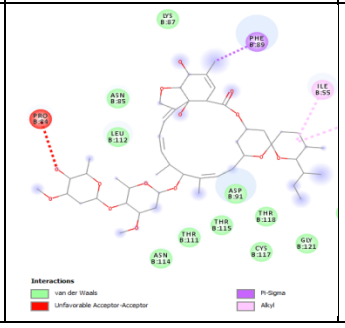
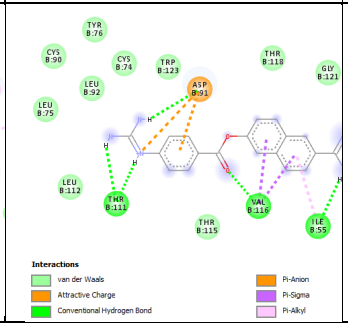
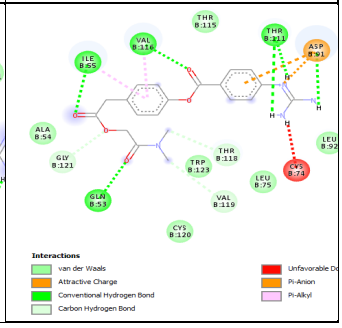
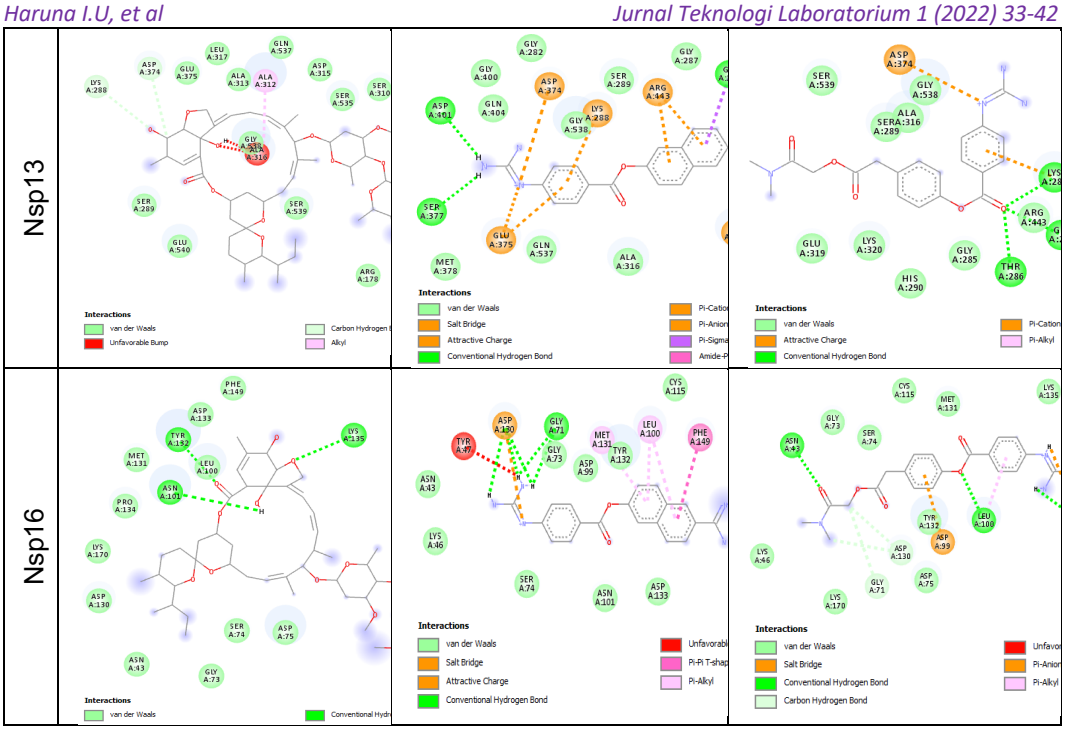
Table 1: Grid box parameters and amino acids in the binding site of our selected protein receptors

S/N	Target Proteins	Center grid box (XYZ), Å	Dimension (XYZ), Å	Active site amino acid residues
1.	Nsp3 (PDB ID: 7CMD)	-22.808 × -16.359 × -19.925	33.668 × 29.582 × 31.718	Gly286, Trp106, Gly271, His73, Cys111, His272, Asp286,Arg140 and Asn109 (Gao et al, 2020)
2.	Nsp5 (PDB ID: 6LU7)	-14.85 × 14.923 × 69.59	25.02 × 27.98 × 30.87	Thr25, Cys44, Thr26, His41, Met49, Tyr54, Phe140, Leu141, Gly143, Cys145, Asn142, His163, His164, Met165, Ser144, Glu166, Pro168, His172, Val186, Asp187, Arg188, Gln189, Phe185, Thr190 and Gln192 (Jin et al., 2020).
3.	Nsp9 (PDB ID: 6M71)	38.221 × -15.213 × 14.62	16.848 × 25.00 × 19.496	Leu9, Ser105, Val7, Pro6, Tyr31, Leu106, Ala8, Met101, Leu103 and Ala107 (Littler et al., 2020)
4.	Nsp10 (PDB ID: 6YZ1)	67.062 × -19.238 × 9.243	28.835 × 37.509 × 35.771	Val42, Leu45, Lys93, Thr106, Ala107, asn40, Thr49, Cys120, Cys128, Cys130, Cys117, Cys74, Cys77, Cys90 and His83 (Rosas-Lemus *et al*.,2020b).
5.	Nsp13 (PDB ID: 6XEZ)	-13.877 × 14.581 × -74.112	20.448 × 20.567 × 25.581	Lys288, Ser289, Asp374, Glu375, Gln404 and Arg567 (Chen et al., 2020).
6.	Nsp 16 (PDB ID: 6YZ1)	83.813 × 16.651 × 25.451	20.977 × 26.335 × 20.158	Tyr47, Asn43, His69, Asp99, Asn101, Asp114, Asp130 and Lys170(Krafcikova et al., 2020)
7.	Spike protein (PDB ID: 6LZG)	-37.386 × 31.021 × 12.733	22.603 × 43.431 × 41.774	Trp353, Arg355, Lys417, Gly446, Tyr449, Tyr453, Ala475, Gln484, Phe486, Thr478, Tyr489, Gly496, Gln498, Thr500 and Gly502 (Wang et al., 2020)

Table 3. Drug interactions with amino acids at the active sites of the SARS-CoV-2 proteins

Protein	Protein-Ligand interactions		
	Ivermectin	Nafamostat	Camostat
Spike			
Nsp3			
Nsp5			
Nsp9			
Nsp10			


Nsp13
Nsp16
Interactions
van der Waals
Unfavorable Bump
Carbon Hydrogen
Alkyl
Salt Bridge
Attractive Charge
Conventional Hydrogen Bond
Pi-Cation
Pi-Alkyl
Unfavorable
Pi-Pi T-shap
Carbon Hydrogen Bond