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The Interaction Study with Meropenem and Phenylalanine

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Abstract- Meropenem has its spectrum of antibiotic activity and it is similar to that of imipenem. Reaction mechanism of Meropenem treat a wide variety of infections and phenylalanine was studied.by using semi-empirical- Parameterization Method 6 (PM6). The stepwise mechanism was considered. The energetic requirements of reaction Meropenem with Phenylalanine for the stepwise were determined. This study can be a model to understand the mechanism due to importance of the peptide bond which can make conformational restir cition on molecule in protein chemistry.

Keywords- *Meropenem, Phenylalanine, Semi-Empirical, PM6.*

I. INTRODUCTION

Meropenem belonging to the subgroup of carbapenem is - broad-spectrum antibiotic and have excellent activity against many pathogens associated with complicated skin and soft tissue infections (cSSTIs). Sepsis is used in nosocomial infections, meningitis infection. It was reported that meropene should be considered for treatment of cSSTIs in higher-risk patients where Pseudomonas aeruginosa is a suspected or documented pathogen [1]. Meropenem was introduced in 1995 and its spectrum of antibiotic activity is similar to that of imipenem, however, it has got more activity Gram-negative aerobes and its stability to DHP-I.1 more than that of imipenem [2-4]. Which was introduced in 1995, has a spectrum of antibiotic activity broadly similar to that of imipenem, but is more active against Gram-negative aerobes, active against imipenemresistant P. aeruginosa, and is more stable to DHP-I.1.

Fazlul Hug [5] reported that both MER and UK-1 have a large HOMO-LUMO energy gap means that it is kinetically inert, and also reported that they have not got abound in electron deficient regions, so they could not readily react with nucleobases in DNA.

The UV, FT-IR and Raman spectra of meropenem were reported during a solid-state stability study according to the density-functional theory (DFT/B3LYP method) with a 6-31G (d,p) basis set. They found that the charge density and HOMO and LUMO was localized on the β-lactam and pyrrolidine 4:5 bicyclic fused rings and the carboxylic and carbonyl groups and In meropenem, the most pronounced are the negative regions, localized on the carboxylic and carbonyl groups, that indicate possible sites for nucleophilic activity [6].

Helfand et al. [7].reported that meropenem rapidly diffused into β-lactamase crystals to form an acyl enzyme species. Homoda et al. [8] developed a new sensitive spectrophotometric method to determine meropenem antibiotic and it was applied to determine meropenem in its pharmaceutical formulations with average recovery 101.7 for the Bip method for meropenem.

The main objective of the present study to understanding the interaction phenyl alanine with meropenem, and to describe energetic condition energy of activation and energy of reaction of formation peptide bonds and an electronic structure (orders of the broken off and formed bonds). The reaction mechanism of phenyl alanine (1) with meropenem (2) have been studied as stepwise mechanism by means of PM6-semi-empirical method which was parameterized for biochemical systems [9].

II. COMPUTATIONAL METHODOLOGY

The reaction mechanism for carboxyl group of meropenem with amino group of phenylalanine and carboxyl group of phenylalanine were performed with the theoretical method of PM6 with full geometry optimization for reactants, intermediates, and final products. Frequency calculations were carried out to see the imaginary frequency which is characteristic of an ordinary TS being first-order saddle point.

The intrinsic reaction coordinate (IRC) calculation was used to follow the reaction path with the GAUSSIAN 09W program [10].

III. RESULT AND DISCUSSION

Peptide bond between the amino acids can be formed ion-molecule reaction which the protonated amino acids react with its neutral form and finally by releasing the water molecule, a peptide bond is formed or molecule based reaction the two neutral amino acids react together and form a peptide bond between them by releasing a water molecule [11, 12]. In this study, we have performed a theoretical investigation to understand the reaction mechanism of between the phenyl alanine and meropenem in the gas phase using molecule based

reaction approach. We investigated the reaction mechanism between for peptide bond format the phenyl alanine and meropenem ion by stepwise mechanism. Three steps transition states named as TS1, TS2 and TS3 are involved.

In the first step pre-stage nucleophilic attack of the nitrogen atom of the phenyl alanine to the carboxylic carbon atom of the meropenem molecule. This reactant state structure drives it to an intermediate structure In1 via a transition state structure The bond lenghts were given in Table 1 for the intermediates,transition states and product.

TABLE I. BOND LENGTHS FOR THE MOLECULES BEING REACTION PARTICIPANTS IN THE REACTION PHEN-N-54)

	1+2	TS1	In1	TS2	In2	TS3	Product
C23-N52	3.110	2.464	1.770	1.610	1.466	1.473	1.390
C23-O4	1.372	1.375	1.417	1.427	1.415	1.269	1.232
C23-O5	1.214	1.215	1.242	1.312	1.430	1.681	3.640
O4-H45	0.995	0.994	0.983	0.980	0.993	1.019	1.797
O5-H74	3.898	3.134	2.496	1.490	0.988	1.712	1.017
O5-H54	4.228	3.585	3.245	2.761	2.586	2.258	4.853
C23-C15	1.473	1.481	1.513	1.501	1.513	1.514	1.482
C15-N7	1.453	1.455	1.456	1.450	1.447	1.438	1.450
C15-C14	1.359	1.357	1.352	1.354	1.350	1.352	1.356
N52- C53	1.480	1.485	1.512	1.499	1.484	1.486	1.477
C53-C56	1.541	1.540	1.538	1.540	1.542	1.530	1.534
C53-C57	1.535	1.533	1.529	1.530	1.530	1.542	1.540
N52- H54	1.018	1.019	1.033	1.299	1.036	1.033	1.032
N52- H74	1.023	1.025	1.040	1.040	3.180	3.071	3.180

TS1. C23-N52 bond length is 2.464 Å for TS1 where C-N bond is formed partially and 1.770 Å for In1 where C-N bond is formed completely. In the second step of reaction, the hydrogen atom of amine group shifts to oxygen atom of carboxylic group via transition state TS2. C23-O5 bond length is 1.242 Å for In1, 1.312 Å for TS1 and 1.430 for In2. In the third step of reaction, the hydrogen atom of hydroxyl group shifts to another hydroxyl group via transition state TS3 and water molecule is released at the end of the reaction, peptide bond is formed.

The Mulliken charges of phenylalanin (1) and meropenem (2) molecules and their transition states, intermediates of the reactions and final product were given in Table 2.

As it seen from the Table 2 the most positive carbon atom is C23 atom (0.685) of the phenylalanine molecule.

The meropenem molecule includes the most negative nitrogen atom N52 (-0.513) also. So the first effect of the reaction will occur on this atom. The C23 and N52 atoms distance is measured as 2.464 at first transition state TS1. The positive charge of C23 carbonyl carbon atom and negative charge on O5 atom increased in this stage. The negative charge on N52 atom decreased because of the positive charge of C23 atom. The positive charge on H54 and H74 atoms increased also in this stage. The energy value is calculated as 34.76 kcal/mol at this transition state form (Table 3).

TABLE II. THE MULLIKEN CHARGES OF THE REACTION INTERMEDIATES, TRANSITION STATES AND PRODUCT

	1phenyl +2merop	TS1	IN1	TS2	IN2	TS3	Product
C23	0.685	0.780	0.776	0.718	0.671	0.698	0.633
O4	-0.554	-0.539	-0.601	-0.605	-0.576	-0.677	-0.569
O5	-0.491	-0.597	-0.691	-0.726	-0.611	-0.621	-
H45	0.359	0.346	0.341	0.332	0.359	0.378	-
C15	-0.196	-0.177	-0.182	-0.169	-0.060	0.104	-0.125
N52	-0.513	-0.500	-0.366	-0.458	-0.521	-0.566	-0.504
H54	0.223	0.234	0.262	0.412	0.351	0.453	-
H74	0.224	0.249	0.253	0.274	0.262	0.378	0.313
C53	-0.018	-0.028	-0.060	-0.041	0.001	0.012	0.015
C56	-0.320	-0.338	-0.337	-0.328	-0.363	-0.367	-0.339
C57	0.554	0.568	0.576	0.586	0.620	0.614	0.588

TABLE III. THE FREE ENERGY AND NEGATIVE FREQ.VALUES OF THE STUDIED MOLECULES

	Free energy (kcal/mol)	Negative freq.(cm^{-1})
1+2	29.09	-
TS1	34.76	-104.07
IN1	35.30	-
TS2	50.73	-1150.24
IN2	26.71	-
TS3	55.94	-791.67
Product	17.53	-

The HOMO and LUMO orbitals of meropenem mainly collected on carbon and nitrogen atoms of β-lactam ring (Fig. 1). The LUMO orbital extended to carboxcylic acid group also. The phenylalanine HOMO orbital mainly collected on all molecule atoms except carbonyl group. The NH2 group has no effect on LUMO orbital.

So we can say that HOMO of phenylalanin interacts with LUMO of merophenem from the visualization result. After bonding of the C23 and N52 atoms first intermediate (In1) occurred and bond length is 1.76998 A. The charge on N52 atom is -0.366 at In1.The energy value is 35.30 kcal/mol for intermediate In1. This value is very near to TS1 energy value.

The transition state TS2 shows the moving of H54 atom to O5 oxygen atom. The energy increase to 50.73 kcal/mol. As it seen from the Table 2 the H54 atoms positive charge increased to 0.412. The N52 and O5 charges are more negative than In1 at this stage. This stage finishes with bonding of H54 and O5 atoms to forming In2 intermediate.

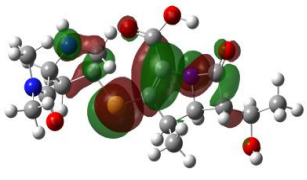
In2 intermediate includes two OH group on C23 atom. The energy decrease to 26.71 kcal/mol. The charges of H54 and H74 atoms are 0.351 and 0.262 respectively. O5 charge is -

0.611 and this charge is more negative than phenylalanine form (-0.491).

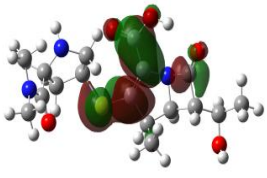
The TS3 is dehydration stage. The most biggest free energy is observed in this stage as 55.93 kcal/mol .The C23-O5 bond and C23-O4 bonds are 1.68070 and 1.26914 A respectively. C23-O5 bond is longer and charge on C23 is more positive than before. After dehydration process the reaction ends with occurring of product. The product energy is calculated as 17.53 kcal/mol.

Whether the optimized structures are at local minima (noimaginary frequency) or at transition states (one imaginary frequency) is checked by doing harmonic vibrational calculations with the same level of theory. TS1 geometry of the reactant (1+2) and intermediate state (IN1), TS2 geometry of the intermediate state (IN1), and TS3 geometry of the intermediate state (IN2), and product (3) were obtained by QST3 (Quadratic synchronous transit-guided quasi-Newton approach) method. Transition state (TS). Imaginary frequencies for TS1 and TS2 and TS3 in the reaction mechanism of mentioned molecules are -104 cm-1 and -1150 cm-1 and 792 cm-1, respectively. The intrinsic reaction coordinate [13] calculations (IRC) which is defined as the steepest descent path starting from the transition state, initially going in the direction of negative curvature in the Hessian are also performed for all transition states to find the right minima. (Fig. 2)

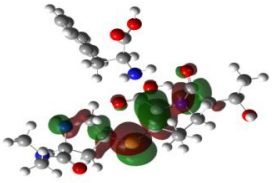
In order to enhance the effect of antibiotics used in infections such as meningitis and sepsis is necessary to increase the dose employed .Today the combining of the antibiotics is necessary to against the growing resistance to antibiotics in bacteria. In this case, it can cause many side effects due to antibiotics such as nefrotiksik first. The studies have been done to prevent the use of high doses which cause of interaction of different molecules with antibiotic. This study has especially been studied the reaction mechanisn between meropenem and phenylalanine which is the particularly one of the "Active mediated Transport System" in the blood brain in chemical media to show the applicability for in vitro.



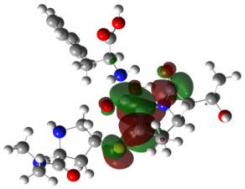
meropenem-HOMO



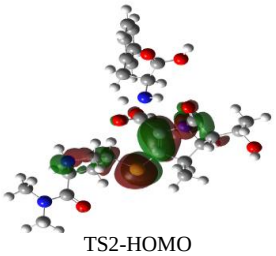
meropenem-LUMO



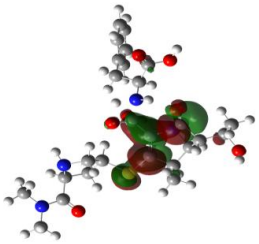
TS1-HOMO



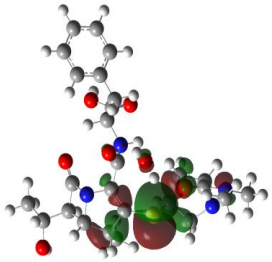
TS1-LUMO



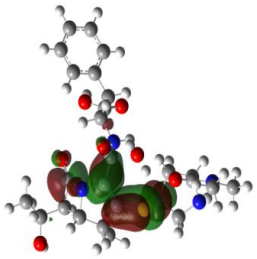
TS2-HOMO



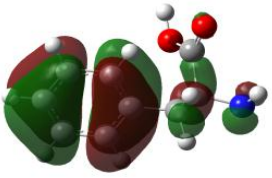
TS2-LUMO



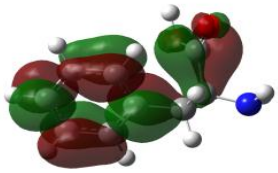
TS3-HOMO



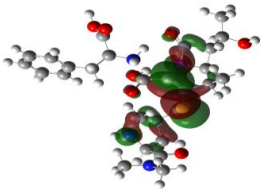
TS3-LUMO



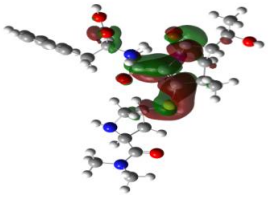
phenylalanin-HOMO



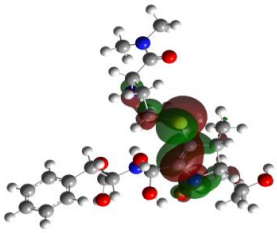
phenylalanin-LUMO



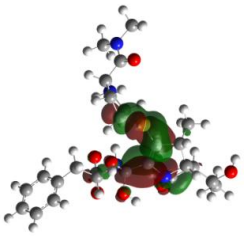
IN1-HOMO



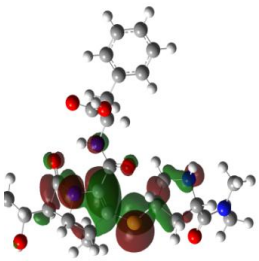
IN1-LUMO



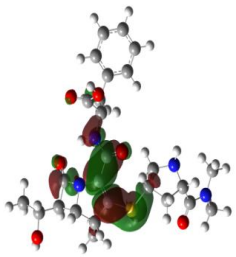
IN2-HOMO



IN2-LUMO

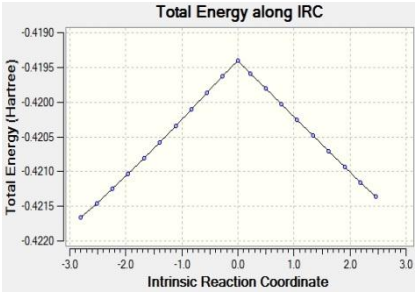


PRODUCT-HOMO

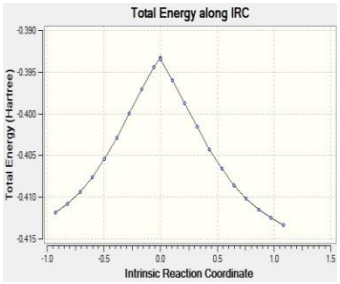


PRODUCT-LUMO

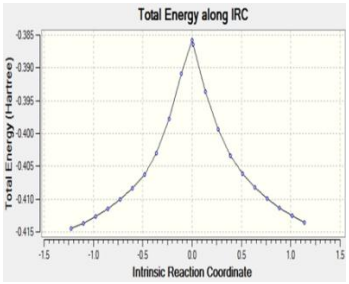
Figure 1. The HOMO and LUMO visualization of the s tudied molecules



TS1



TS2



TS2

Figure 2. IRC calculation graphics for the transition states

IV. CONCLUSION

Mechanisms between meropenem and phenylalanine were considered in this study. Stepwise mechanism proceeded through three transition states. The reaction mechanism for carboxyl group of meropenem with amino group of phenylalanine was explored by using semi-empirical-PM6 method. Because of the importance of the peptide bond which can make conformational restriction on molecule in protein chemistry, this study can be a model to understand the mechanism.

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