Investigation of the Interaction of Warfarin with Arginine and Thyroxine with Tyrosine.

Elham M. A. AL- Rufaie, Huda N. AL- Ania

and Abd AL. Kareem Khaleel.
Department of Chemistry, College of Science, University of Baghdad.
Jadiriya, Baghdad, Iraq.

E- mail: hudanajim2005@yahoo.com

Keywords: HAS: Human serum alboumin, (ΔHf): heat of formation, (HOMO): highest occupied molecular orbital, (LUMO): lowest unoccupied molecular orbital .

(Received : , Accepted :)

Abstract.

Moleculer complxes of drugs (Warfarin and Thyroxine) with amino acids (Arginine and Tyrosine) were studied theoretically using PM3 Semi-empirical calculations of molecular orbital model and IR- techniques. The studied systems was: Warfarin with Arginine and Thyroxine with Tyrosine , The optimized geometry of the complexes showed interaction between drugs and Amino Acid molecules, the bending energies for these interaction varies from (- 4.194 to – 0.529) Kcal.mol^{-1}. Which attributed to the formation of hydrogen bonding, and confirm experimentally by the IR- technique.

Keywords: HAS: Human serum alboumin, (ΔHf): heat of formation, (HOMO): highest occupied molecular orbital, (LUMO): lowest unoccupied molecular orbital .

إستقصاء تآثر الوافرين مع الارجنين والثيروكسين مع التيروسين.

الهام مجيد الرفيعي وهدى نجم الدين عبد اللطيف وعبد الكريم خليل

قسم الكيمياء، كلية العلوم ، جامعة بغداد، الجادرية ، العراق ، بغداد

الخلاصة:

استعملت طريقة حسابات الاوربتالات الجزيئية (PM3) شبه التجريبية لدراسة المعقدات المتكونة بين الدواء والحامض الاميني وقد تم دراسة نظامين منها هما (الوافرين مع الارجنين) و(الثيروكسين مع التيروسين) .الشكل الهندسي المثالي للمعقدات اعطى تاثرا بين جزيئتي الدواء والحامض الاميني بقوة تتراوح بين (4.194 - الى 0.529 -)كيلوسعرة للمول والذي اعزى الى تكوين اصرة هيدروجينية بين الجزيئتين واثبتت تجريبيا باستخدام تقنية الاشعة تحت الحمراء.

Introduction

Amino acids are the building blocks of proteins, contains both amine and carboxyl functional groups, combines in a condensation reaction forming peptides, polypeptides, and proteins[1]. Twenty standard amino acids are used by cells in protein biosynthesis, these are specified by the general genetic code[2].

Arginine (Arg.) is one of the 20 most common natural amino acids, the geometry, the charge distribution and ability to form multiple H-bonds make Arginine ideal for binding negatively charged groups[3]. Tyrosine (Tyr.) is anon- essential amino acid and it is found in large quantities in Casine, it has aspecial role by virtue of the phenol functionality[4]. Human serum alboumin (HAS) is the most abundant protein in plasma, which plays amajor role in the drug-binding process. For many compounds, HAS provides adepot so they will be available in quantities well beyond their solubility in plasma. Morever, HAS abundance (concentration of 45 mg. ml^{-1} in the serum of human adults) makes it an important determinant of the pharmacokinetic behavior of many drugs[5,6]. In other cases, HAS holds some ligands in astrained orientation, allowing their metabolic modification, and renders potential toxins harmless by transporting them to disposal sites. The amino acids sequence of HAS shows the occurance of three homologeous domains, each domain is known to consist of two separate sub- domans, connected by random coil,the flexability of the HAS structure allows it to adopt readily to ligands and that its three –domain design provides avarity of binding sites[7].

Warfarin, an anticoagulant drug[8], are considered as stereotypical ligands for sudlow; site II and sudlow; site I, respectively[9,10]. Thyroxine, or 3, 3`,5,5` tetraiodothyronine (T1) is ahormone produced by the thyroid gland, during the transport of (T4) to the peripheral tissues, it is complexed to several serum proteins (globuline , trans thyretin and albumin), together, these proteins bind more than 99% of circulating T4 [11,12] . In this research we use theoretical method to study the binding mode of thyroxine with Tyrosine and Warfarin with Arginine.

Computational and Experimental methods:

The molecular structures of the studied drugs (Wafarin and Thyroxine) and amino acids (Arginine and Tyrosine) were optimized , using PM3 Semi-empirical method as implemented in the program package Hyperchem version 7.25.

The geometry of lowest heat of formation (ΔH_f) was determined and considered as equilibrium conformation (lowest energy structure). Calculating the highest occupied molecular orbital (HOMO) energy and lowest unoccupied molecular orbital (LUMO) energy to identify the binding sites in each molecule. FT-IR. measurements were performed on ashimadzu- Infrared spectrophotometer, for each drug and amino acid alone and Amino acid-drug mixture(1:1) for the studied systems. HAS, Warfarin and Thyroxine (Fig: 1) were from sigma, and the amino acid; Arginine and Tyrosine were from, Biochemical BDH-chemicals Ltd poole, England.

Results and discussion.

The minimum energy for molecular structures of (Warfarin, Thyroxine, Tyrosine and Arginine) were calculated by using PM3 semi-emperical methods as implemented in the program package Hyper chem. Version 7.25 [13], that accomplished by drawing first building of the conformational geometry of the molecules then minimize its energy (minimization) to obtaine the equilibrium conformation(the optimized geometry) that have the lowest energy and lowest heat of formation [14]. The more stable geometries of them (optimized geometry) were shown in (figure :2) and the systematic numbering for these molecules as in (IUPAC) international system [15].

Heat of formation (ΔH_f) ,total energy , ionization potential, and dipole moments for the more stable geometry of the drugs and amino acids were calculated from PM3 semi-emperical method, table-1 showes these physical properties.

Drug amino acid interaction :

The electron density distribution and the electrostatic potential energy for the drugs and amino acid molecules ,were calculated using PM3 semi-emperical method, each one alone to recognize the location of the (HOMO) and (LUMO) molecular orbital to identify the probable interaction position between the drugs and amino acids , due to their importance to all chemical properties of the molecules. After knowing the reactive location in each molecule we start to build up molecular complexes according to it, and calculate the binding energy between each pair of the studied systems for the most probable position, as follows:

$$E_{binding} = E_{complex} - (E_A + E_D)$$

$E_{complex}$ = Heat of formation for the complex .

E_A , E_D = Heat of formation for the drug (D) and amino acid (A).

Arginine - Wafarin interaction:

The values of the binding energy for the optimized geometry of these three complexes which illustrated in table -2 making clear that the most probable intraction is the intraction between O_{15} in Warfarin molecule and H_{23} in Arginine molecule owing to its highest possessive of the binding energy of these three position, this interaction H_{23}..... O_{15} is due to the formation of hydrogen bond which is within the rang of hydrogen bond strength [16,17].

Figur -4: Shows the position of (HOMO) and (LUMO) moleculer orbitals for the two molecules (Arginine and Warfarin) , there may be three probable interaction position between these two molecules , one position for Warfarin molecule at oxygen No.15 (LUMO) and three position for Arginine molecule (hydrogen-9, 23, 25) (HOMO) .

Table-2 : illustrate each one of these position and its totale energy, heat of formation and the binding energy in ($Kcal.mol^{-1}$).

Figure-5 : Showes the optimized geometry of the W_{O15}-A_{H23} Complex calculated by PM3 semi-emperical method using Hyperchem. Program version 7.25.

Tyrosine and Thyroxine interaction:

The position of (HOMO) and (LUMO) orbitals for the two molecules (Tyrosine and thyroxine) were illustrated in figure-6, there was one probable intraction position between them, Oxygen No-28 which belong to Thyroxine molecule and hydrogen No-21 on Tyrosine molecule. The total energy, heat of formation and the binding energy in Kcal.mol-1. Were shown in table-3 for this complex that may be formed between these two molecules and in the expected position. The most probable interaction between Thyroxine and Tyrosin is in aposition of oxygen-28 in Tyrosin and hydrogen -21 on Thyroxine with abinding energy of - 0.529 Kcal.mol-1.

Infra- red spectroscopy:

Spectroscopic measurements for the interaction of Warfarin and Thyroxine (drugs) with Arginine and Tyrosine (A.A.) were donefor each one of the drugs and amino acids alone and also for the mixtur of Warfarin - Arginine and Thyroxine – Tyrosin in a ratio of 1:1. The spectrum of the mixtur of Thyroxine – Tyrosin shows ashift in the band that relate to the carboxyl group of Thyroxine by ($80cm^{-1}$) and a shift in the band that relate to the amino group of Tyrosin by ($20cm^{-1}$), this shift also recognized in the spectrum of the mixtur of Warfarin – Arginine in the band that relate to –NH- of Arginine and that of C=O of Warfarin . The observed shifts confirm the formation of hydrogen bond in this position as expected by our theoretical calculations.

Conclusion:

The interaction between Warfarin – Arginine and Thyroxine – Tyrosin lead to the formation of hydrogen bond were investigated by computational and spectroscopic techniques. The heat of formation was used as aparameter to evaluate the binding energy of the interacted molecules which were confined by the shift in the absorption band of the groups involved in the formation of the hydrogen bond.

References:

1- Grag Walsh, ″Protein Biochemistry and Biotechnology″ John Wiley &sons LTD., pp. 25-50 (2002).
2- L .Whitmore, B .Wallace, Eur, Biophys. J., vol 33(3), pp. 233-237 (2004).
3- P. J. Reeds, J. Nutr., volum 123, pp. (15435- 15485) ,(1993).
4- J.Vivion ,T. ,Biophys. J. , vol. 80 (5), PP. 2093- 2109, (2001)

5- Peters, T. Jr.ed, All about albomin ″ Biochemistry genetics and medical application.Academic press, San Diegoad London, (1996).
6- RD .Salakhov, LR. Stefan, Ktopma,G. Multiph Computer- automated structure evaluation model of the plasma Protein binding affinity of adiverse drugs. Perspectines in Drug Discovery and Design, 19 : 133-155,(2000).
7- M.Dockal, D.Corter and F. Ruker ″ The three recombinant domains of human serum albumin. Structural characterizatia and ligand binding properties.″ J. Bial. Chem. 274, 29303-29310,((1999).
8- E.James and F.Reynolds, eds Martindale : The Extra pharmacopoeia, 31 st edn. The pharmaceutical press, London(1996).
9- G .Sudlow, D. J Birkett, and D. N. Wade, (The characterizatia of two specific drug binding sites on human serum albumin. Mol. Pharmacol. 11. 824 -832,(1975).
10- 1.Petitpas, A. A. Bhattacharya, S.Twine, M. East, & S. Curry, Crystal structure analysis of warfarin binding to human serum albumin: anatomy of drug site I. J. Bial. Chem. 276,22804- 28809, (2001).
11- Petitpas et,al : Structural Basis of Albumin.Thyroxine interactions and Familial Dysalbuminemic Hyperthyroxinemia. proc. Nat. Acad. Sci, USA., vol 100 pp. 6440 (2003).
12- R.T.Zoeller, K.M.Crofton ; Thyroid Hormone Action in Fetal Brain Development and potential for Disruption by Environmental chemicals. Nevro Toxicology , vol 21 pp. 953-946 (2000).
13- M. J. S. Dewar, E. G. Zoebisch, E.F. Healy , J.P.Stewart, J. Am. Chem. Soc. , 107, 3902, (1985).
14- E.Kensal ,Van Holde, W.Curtis ,Johnson, P.Ho. Shing ; " Principles of physical Biochemistry.″ Pearson prentice Hall, New Jersey, p. 146, second edition ,(2006).
15- IUPAC- IUB Commission on biochemical nomenclature, Biochim. Biophys. Acta, 107 ,5 (1965).
16- J. A. Ketelaar, J. Chem. Phys., Dissociation of H. bond energy, 46, 425- 8 (1949).
17- G. C. Pimentel, A.L. Mc Clellan, The hydrogen bond, W. H. Freeman and company, San Fracisco and London. P. 350 (1960).

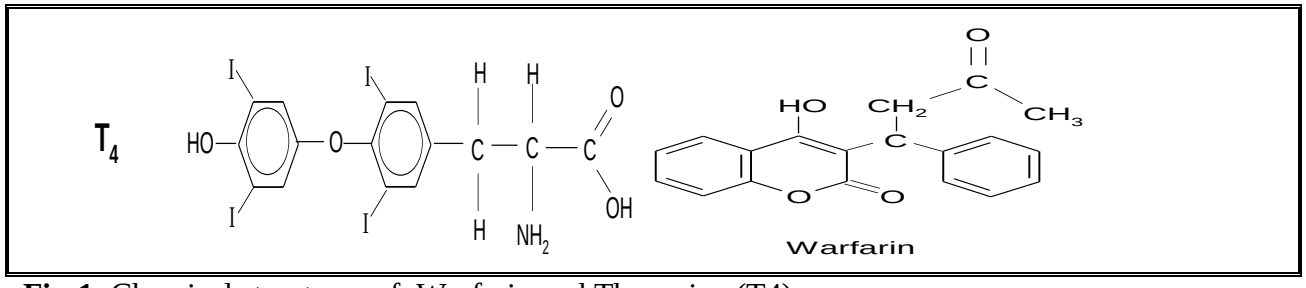
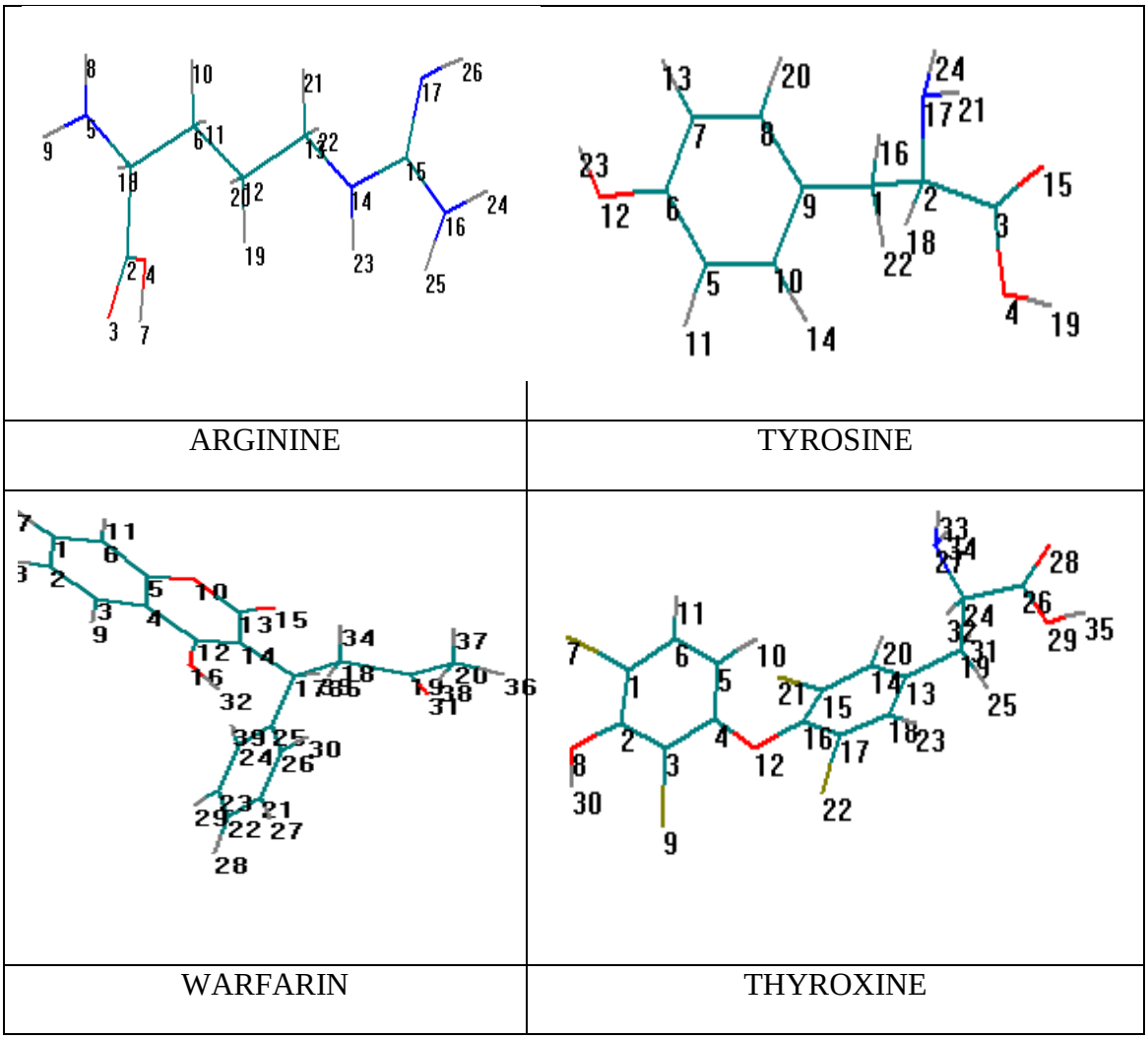


Fig-1: Chemical structures of Warfarin and Thyroxine (T4).



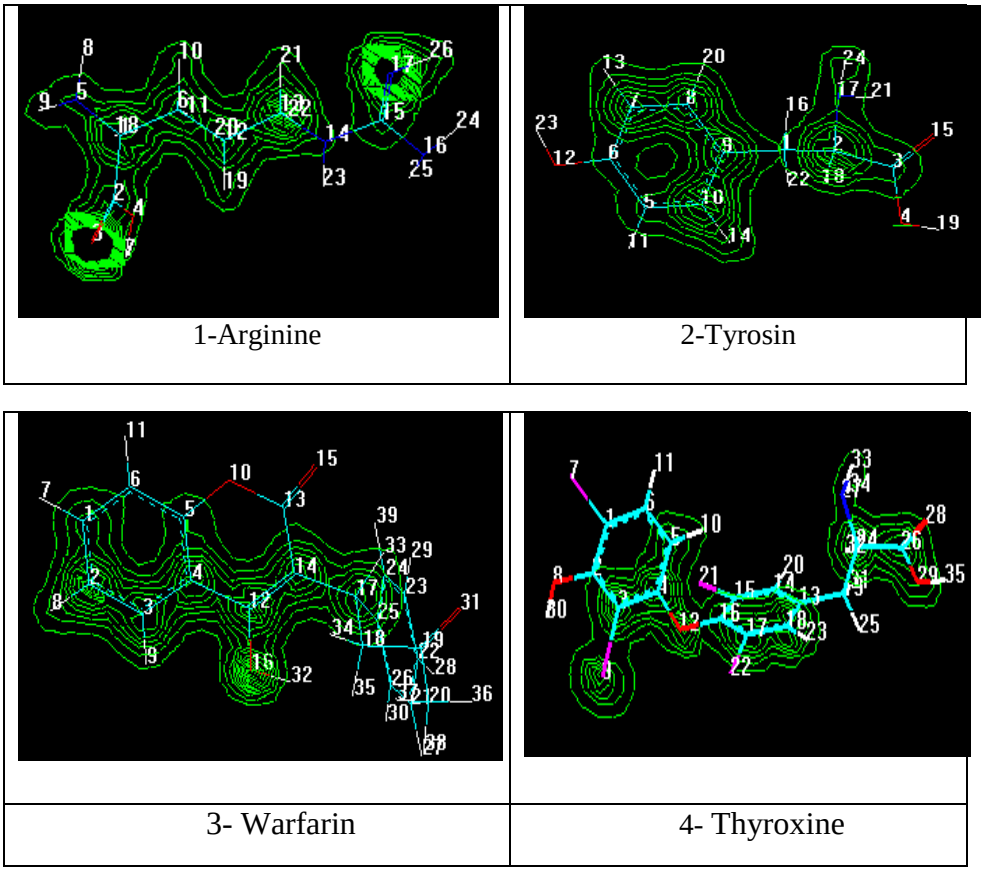
ARGININE | TYROSINE

WARFARIN | THYROXINE

Fig- 2: Optimized geometry of amino acids (1- Arginine, 2- Tyrosine) and the drugs (3- Warfarin, 4- Thyroxine T4) from PM3 semi-emperical calculations.

Table-1: some physical properties for the studied drugs and amino acids of the optimized geometry from PM3 semi-emperical calculations.

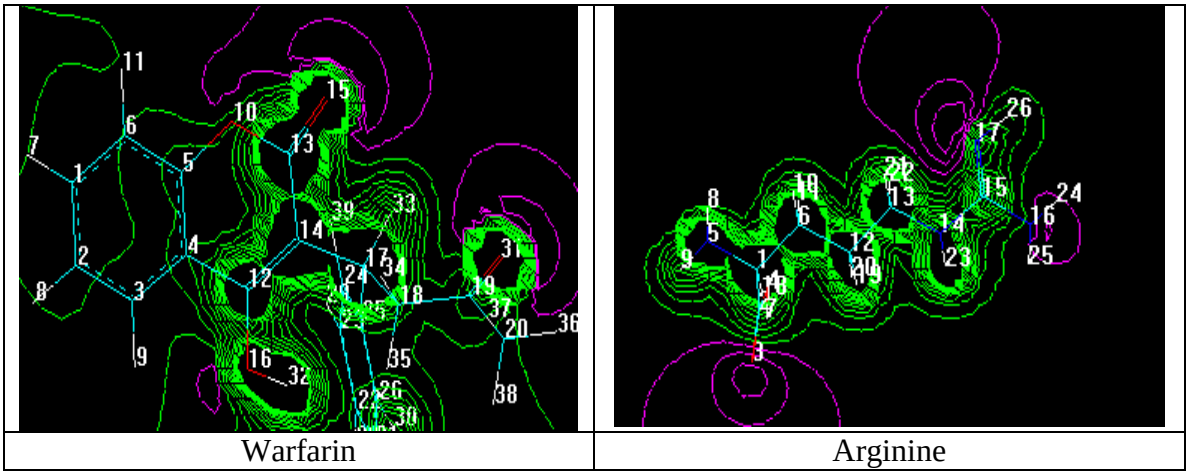
Molecule	**Total Energy Kcal .mol^{-1}**	**(ΔH_f) Heat of Formation Kcal.mol^{-1}**	**ionization potential(e.v)**	**Dipole moment (Debye)**
Arginine	**-49903.492**	**-82.508**	**9.318**	**2.375**
Tyrosine	**-52574.498**	**-117.489**	**9.092**	**0.535**
Warfarin	**-84672.137**	**-103.247**	**9.387**	**5.838**
Thyroxine	**-102307.061**	**-4.698**	**8.606**	**2.054**



1-Arginine | 2-Tyrosin

3- Warfarin | 4- Thyroxine

Figur -3: Electron density of Amino acid (1- Arginine, 2- Tyrosine) and the drugs (3- Warfarin, 4- Thyroxine T4) from PM3 semi-emperical calculations.



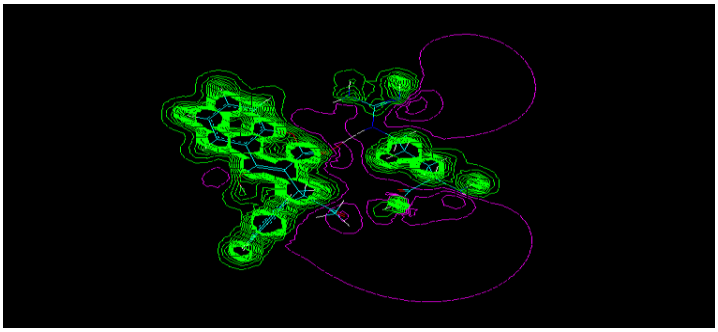
Warfarin | Arginine

Figur -4: Molecular orbitals (HOMO) and (LUMO) of the optimized configuration of Arginine and Warfarin by PM3 semi-emperical method.

Table-2 : Total energy , heat of formation and the binding energy for the expected complexes between Arginine (A) and Warfarin (W) from PM3 semi-emperical calculations.

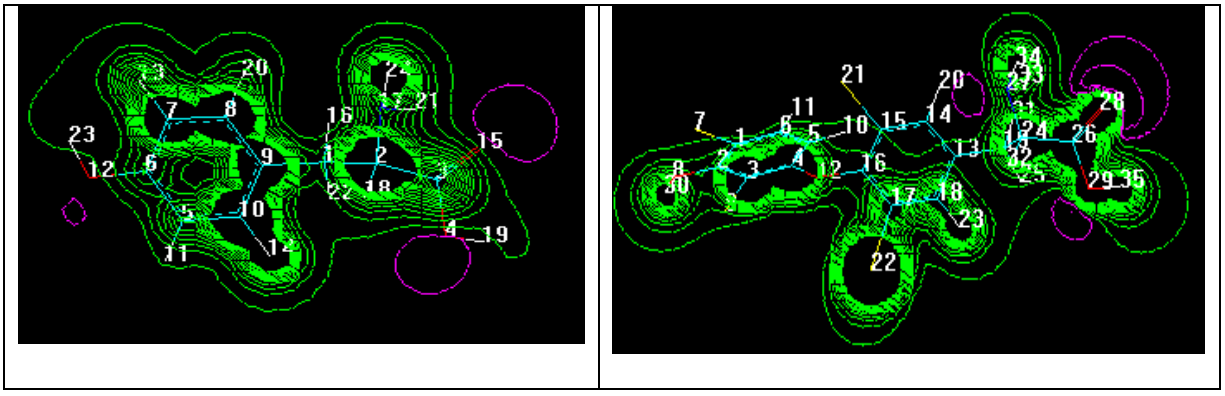
Arginine and Warfarin complex	**W_{O15}-A_{H9}**	**W_{O15}-A_{H23}**	**W_{O15}-A_{H25}**
Total energy (Kcal.mol^{-1})	**-134579.450**	**-134579.823**	**-134578.534**
Heat of formation ΔH°_{f} (Kcal.mol^{-1})	**-189.576**	**-189.949**	**-188.660**
Binding energy $\Delta\Delta H_f$ (Kcal/mol)	**-3.821**	**-4.194**	**-2.905**

$\Delta\Delta H_f$ = ΔH_f (complex) _ Σ_i ΔH_f (components: Arginine and Warfarin.).



Warfarine-O_{15} Arginine-H_{23}

Figure-5: The binding mode between Warfarin and Arginine in the position W_{O15}-A_{H23}.



(a) Tyrosin **(b) Thyroxine**

Figer -6: (HOMO) and (LUMO) Moleculer orbitals of the optimized configuration of :a- Tyrosin, b- Thyroxine from PM3 semi-emperical method.