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Computational study on the Metabolism of Antibacterial Prontisil (PROTO1) and Salfalazine(SASP4)

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Abstract

The ab- initio / HF of(6-31) (the basic sets parameters that make the molecule more stable) according to (Gaussian) program and density functional theory of polarization) and PM3 semiempericalmethod,showed that the net charge distributions for 4-aminobenzene sulfonamide (sulfanilamide)(SAM2) and Sulfa pyridine(SP) (the active drugs) were less than those of prodrugsProntisil (PROTO1) and Salfalazine (SASP4) which indicated the stabilities and easy of formation (or liberation) of these active drugs. In addition to that, the stabilities of these liberated drugs also proved by the steric energies which were less than those of the pro-drugs. The energy gaps between the HOMO and LUMO of the active drugs liberated in vivo(by metabolism) were very small which agreed with the previous two observations.

Keywords: 4-aminobenzene sulfonamide (sulifanilamed; Sulfapyridine); prodrugsProntisil ;Salfalazine; energy gap; HOMO and LOMO.

دراسة نظرية على الايض الحيوى للمضاد الحيوى(برونتوسيل(برونتو1) وسلفالازين(اس اى اس بى 4)

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الخلاصة

باستخدام ميكانيكية الكم (6-31 G)(d.p) HF\ abinitio ،(DFT) و(PM3)النظرية النصف عملية ونظرية دالة الكثافة الالكترونية عند مستوياتمختلفة من الطاقة لحساب طاقة اعلى اوربتال مشغول واوطئ اوربتال غير مشغول وحرارة التكوين للمركبات المتفاعلة والناتجة،وطاقة الحشد الفراغي للادوية التى ستتايض والناتجة من الايض الحيوى باستخدام الميكانيكية الجزيئية(MM2).باستخدام الهندسة الفراغية لإيجاد مركب في طاقة قريبة من الكمال او الفعالية. استطعنا ان نبرهن ان النتائج والحسابات تطابق ما هو معلن من فعالية المركبات وعدم فعاليتها وتبين ان صافي الشحنة على المركب 4-امينو بنزين سلفامايد(SAM2) وسلفابريدين (SP) تدل على ان الدواء الفعال الناتج من الايض الحيوي هو اقل طاقة وكثر استقرارا من ال (Prodrugs) وهو البرونتوسيل (PRONTO1) والسلفازين (SASP4) وقد تم تاكيد ذلك ايضا بحساب طاقة الحشد الفراغي .

الكلمات الدالة:4-امينوبنزين سلفونا ميد –(سلفانلايد—سلفابيريدين)—ماقبل الدواء—برونتيسيل—سلفالازين—فجوة الطاقة—طاقة اعلى اوربيتال مشغول –طاقة اوطا اوربيتيل فارغ).

Introduction

A pro-drug is a pharmacological substance administered in significantly less active form, and when administered, the pro-drug is metabolized enzymatic and/or chemical transformation in vivo into an active metabolite(a drug which can then exert the desired pharmacological effect), a process termed bio activation. The rationale behind the use of a pro-drug is generally for Absorption, Distribution, Metabolism, and Excretion (ADME) optimization. Pro-drugs are usually designed to improve oral bioavailability, with poor absorption from the gastrointestinal tract usually being the limiting factor, pro-drugs are used when drugs have unattractive physicochemical properties[1]. About 5–7% of drugs approved worldwide can be classified as pro-drugs, and the implementation of a prodrug approach in the early stages of drug discovery is a growing trend[2].Prod rugs can be classified into two major types, based on their cellular sites of bio activation into the final active drug form, with Type I being those that are bio activated intracellular and Type II being those that are bio activated extracellular, especially in digestive fluids or the systemic[1-2] (PABA).

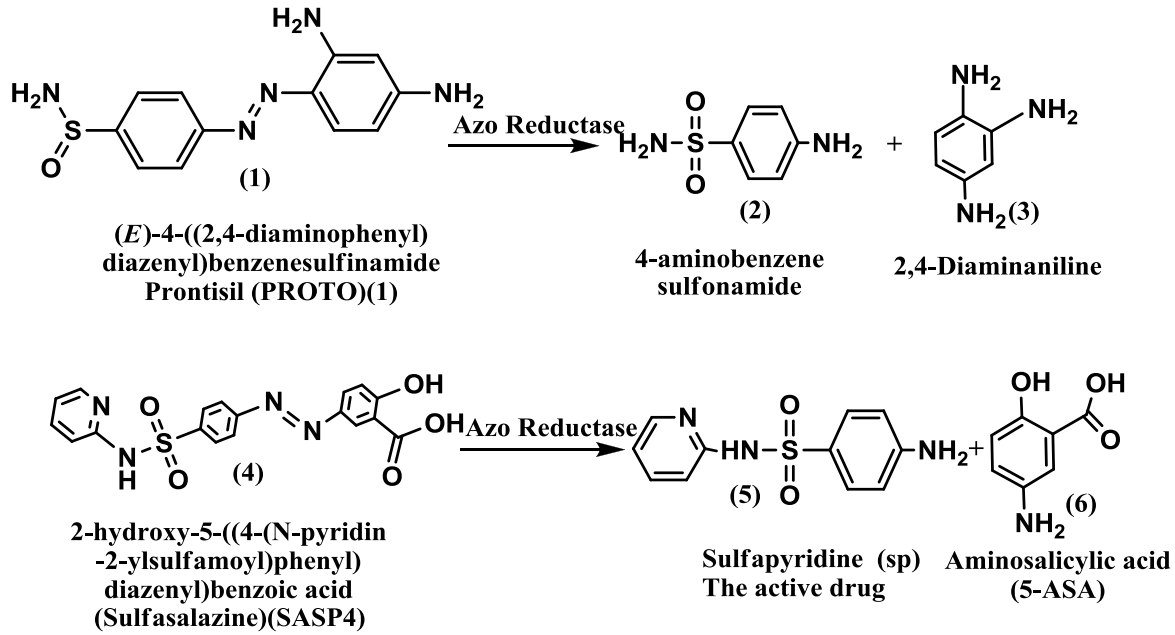
PABA is needed in enzymatic reactions that produce folic acid . These two prod rugs were metabolized in vivo to give the two active sulfonamide antibacterial agents Sulfanilamide (SAM 2) or Sulfapyridin (SP) . Chemically, these two molecules containing the sulfonamide functional group attached to an aniline. As sulfonamide antibiotics, they function by competitively inhibiting enzymatic reactions involving par-aminobenzoic acid which acts as a coenzyme in the synthesis of purine, pyrimidine and other amino acids[3].of bacterial azo-reduction. Prontosil is a lipid-soluble azo-dye, and was readily was administered orally to infected human patients, Scheme(1)[4].

Sulfasalazine (SASP4), is an anti- The high polarity of Prontosil (Ponto1) appeared to severely limit its absorption from the small intestine, and allowed it to reach the caecum which is the major site absorbed from the rat intestine.

However, antibiotic pretreatment, designed to suppress the intestinal flora, resulted in a decrease in the excretion of azo-reduction products after oral administration of Prontosil . This suggested that the intestinal flora is also a major site of azo-reduction of Prontosil.

Sulphanilamide (Sam2), the active antibacterial agent produced by intestinal bacterial metabolism of Prontosil in experimental animals, was released in a similar way when Prontosil inflammatory drug that is widely used in the treatment of diseases such as ulcerative colitis and Crohn's disease.

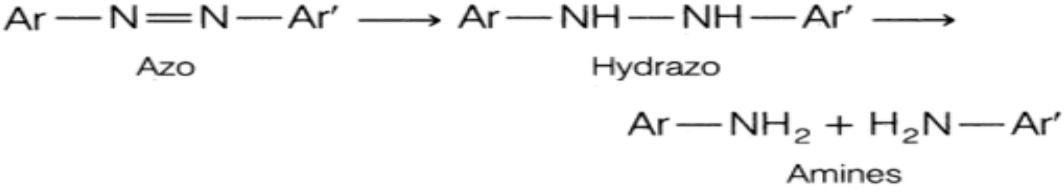
The diazobond in Sulfasalazine (SASP4) is cleaved in vivo to provide sulfa pyridine (SP) and 5-aminosalicylic acid (5SAS), Scheme (1)[5] .



Scheme (1)
Action of azo reductase on Prontisil (PROTO)(1) Sulfasalazine (SASP)(4)

The mechanism of this reaction is occurred in the terminal ileum and colon, bacterial flora release azoreductase that cleaves the azo bond (N=N) and releases the sulfa drug Sulphapyridine and 5-ASA[4].Scheme (2)Azo reduction, however, is believed to proceed via ahydrazo intermediate (-NH-NH-) that subsequently is cleavedreductively to yield the corresponding aromatic amines.

Bioreduction of nitro compounds is carried out by NADPH(reduced form f nicotinamide adenosine dinucleotide phosphatedependent) microsomal and soluble nitro reductases present in the liver [4].Scheme (2)



Scheme (2)
Mechanism of Azo compound cleavage by reductase enzyme

A multicomponent hepatic microsomal reductase system requiring NADPH appears to be responsible for azo reduction.

In addition, bacterial reductases present in the intestine can reduce nitro and azo compounds, especially those that are absorbed poorly or excreted mainly in the bile[4].

In vivo studies have indicated that the absolute bioavailability of orally administered Sulfasalazine (SASP4) is less than 15% for parent drug. Like SASP4, it is metabolized by micro flora present in the colon and cecum to active drug and 5-ASA. Of the two species, SP is relatively well absorbed from the intestine and highly metabolized, while 5-ASA is much less well absorbed[6] .

Material and Methods

The 3D Conformation of prontisil (PROTO1)andSalifaazine (SASP4) were showed in Figs (1 and 2) respectively.

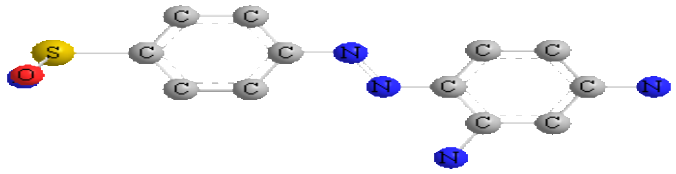


Figure 1: 3D conformation of Prontisil (PROTO1)

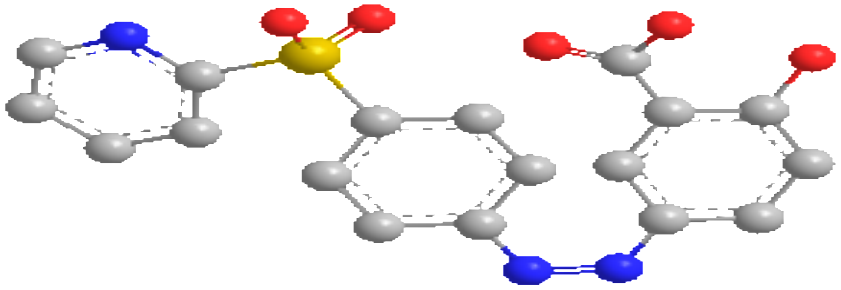
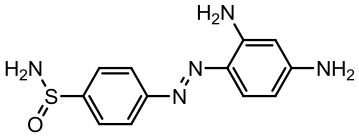
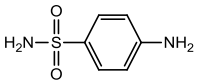
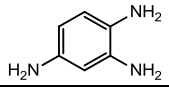


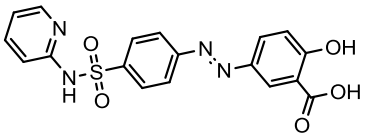
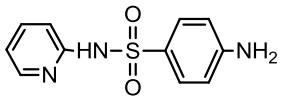
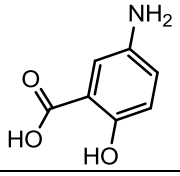
Figure 2: 3D conformation of Salfalazine(SASP4)

The theoretical method currently used was the semi-empirical PM3for calculation of the heat of formations. Molecular mechanics were used for calculation Steric Energy, Steric energies of pro drugs and active drugs produced from metabolism were minimized with the MM2 Force Field(Molecular mechanics method). The Physical properties of Prontisil (PROTO1) and Salfalazine (SASP4) and their metabolic Products were showed in Tables (1 and 2).and its metabolic Produ**g**

Table(1): Physical properties of Prontisil (PROTO1)

Compounds	HOMO (e.v)	LUMO (e.v)	H_f Kcal\mol	Steric E Kcal\ mole	Δ(L-H) Gap(e.v)
	-0.2649	-0.0659	121.702	67.436	0.3308
	-0.255	0.0342	-44.6348	14.4268	0.2208
	-0.338	0.0173	36.348	35.1314	0.3207

Table(2) : Physical properties of Salfalazine(SASP4)

Compounds	HOMO (e.v)	LUMO (e.v)	H_f Kcal\mol	Steric Kcal\ mole	Δ(L-H) Gap(e.v)
	-0.3396	-0.0349	-9.5537	-19.1066	0.3047
	-0.3173	-0.0126	-19.5537	-43.442	0.3047
	-0.3067	-0.0163	-16.9989	-20.66	0.2904

Density Functional Theory (DFT). The electronic structure study includes all-electrons within the Kohn-Sham implementation of the. Density Functional Theory (DFT). The level of theory used in this work corresponds to the non-local hybrid functional developed by Beck, Lee Yang-Parr (B3LYP), whereas the Kohn-Sham orbitals are represented by(6-31G) basis set and a triple-æ numerical with double polarized functions (d,p) plus one diffuse basis set estimation.Net charge distribution for conformations in Tables (3-4).

Results

Molecular Mechanics method determines the steric energy of conformations of a molecule as a measure of their relative stabilities. The Steric Energy is computed at the end of an MM2 Energy minimization.is a sum of bonded(stretch, bend, stretch-bend, and torsion,non-1,4 van vanderWaalsanddipole/dipol.1,4der Waals and

Parameters for the energy functions were standard once [7],the*ab initio* molecular orbital calculations, were carried out using the GAUSSIAN 98 program V(0.3) [8-9].Geometries for all structures were fully optimized by means of analytical energy gradients by Birney optimizer with no geometrical onstraints. The restricted Hartree-Fock calculations with the split-valence 6-31G basis set, which includes a set of d-type polarization functions on all non-hydrogen atoms, were used in these calculations [10].

HOMO and LUMO are acronyms for highest occupied molecular orbital and lowest unoccupied molecular orbital, respectively. The energy difference between the HOMO and LUMO is termed the LUMO- HOMO gap. HOMO and LUMO are referred to as frontier orbitals energy [12] .

Table 3: Net charge distribution for 4-minobenzene sulfonamide

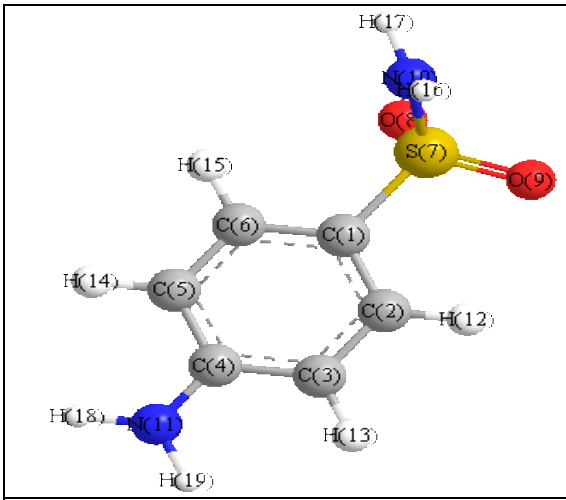
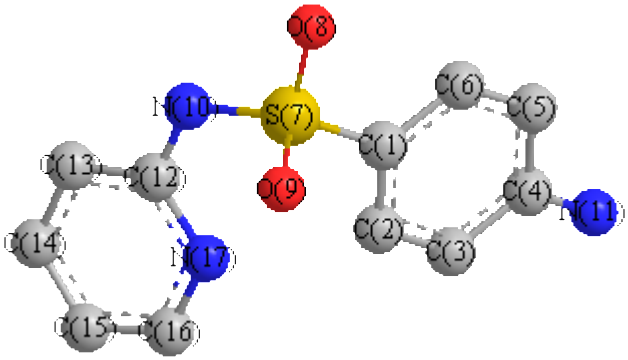
	AB initio/HF (6-31G)(d.p)	DFT/B3LYP (6-31G)(d.p)
C1	-0.356445	-0.202787
C2	-0.080221	-0.202787
C3	-0.191810	-0.124160
C4	0.301076	0.437506
C5	-0.187868	-0.123468
C6	-0.083504	-0.091554
S7	1.593523	1.125794
O8	-0.675796	1.125794
O9	-0.671278	-0.517829
N10	-0.671278	-0.724184
N11	-0.746465	-0.709283

Table 4: Net charge distribution for sulfa pyridine

	AB initio/HF (6-31G)(d.p)	DFT/B3LYP (6-31G)(d.p)
C1	-0.395478	-0.205083
C2	-0.075402	-0.096191
C3	-0.221301	-0.118898
C4	-0.221301	0.432402
C5	-0.216891	-0.118617
C6	-0.081044	-0.097025
S7	-0.081044	1.218975
O8	-0.081044	-0.541467
O9	-0.704726	0.526151
N10	-0.704726	-0.498380
N11	-0.831203	0.087018
C12	0.675072	-0.170459
N13	-0.592935	-0.166376
C14	0.112976	0.106681
C15	-0.297543	-0.707689
C16	-0.210371	-0.539330
C17	-0.059010	-0.695672

Discussion

Geometric and Electronic Structure of Prontisil (PROTO1)andSalfalazine(SASP4)and their active products. The efficiency of DFT/B3LYP method may be scrutinized by comparison with the results obtained by more elaborate calculation such as ab initio / HF. Present results concern products. Charge densities in table (3,4).

1-The results of ab initio calculations for structure optimization and conformational interconversion pathways of were shown in Tables(1-2). The ab- initio / HF of (the basic sets parameters that make the molecule more stable) according to (Gaussian) program and D.P(the density of polarization) showed that the net charge distributions for 4-aminobenzene sulfonamide (sulfanilamide)(SAM 2) and Sulfa pyridine (SP) (the active drugs) were less than those of prodrugs which indicate the stabilities and easy of formation or liberation of these active drugs.

2-In addition to that, the stabilities of these drugs also can proved by the steric energies which were less than those of theprodrugs [7] .

3-The energy gaps between the HOMO and LOMO of the active drugs liberated from the prodrugs by metabolism were very small which agreed with the previous two observations(energy gaps of the compounds directly proportional with stability of compounds)[13].

Conclusions

A reduced HOMO−LUMO gap, which is defined as the HOMO−LUMO energy separation of a molecule can be used as an index of kinetic stability for a variety of compounds. The reduced HOMO−LUMO gap < 1.00 indicates that the HOMO contributes to the decrease in the topological resonance energy. The active drugs liberated from the prodrugs by metabolism were kinetically very stable with very large reduced HOMO−LUMO gaps .

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