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A REVIEW ON COMPUTATIONAL STUDY IN THE PROTONATION OF BENZYLPENICILLIN TAUTOMERS BY AUSTIN MODEL-1(AM1) METHOD

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ABSTRACT

The optimized electronic structure of benzylpenicillin tautomers and its mono-protonated, di-protonated and anion in the gas phase by semi-empirical molecular orbital AM1 method have been reported. In this review, the protonation of benzylpenicillin tautomers in terms of geometry, conformation, the heats of formation (ΔH_f^o), dipole moment (μ), ionization potential (IP), full atomic charges and energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) have been discussed.

KEYWORDS: AM1, tautomerism, protonation, benzylpenicillin, induction effect, frontier molecular orbital.

1. INTRODUCTION

Penicillin derivatives have been studied extensively because of their broad anti-microbial spectra, more favourable absorption patterns and reduced undesirable side effects.[1] Penicillin G is a first antibiotic in chemotherapy of bacterial infections and isolated through biosynthetic methods.[2] Benzylpenicillin is active against gram-positive bacteria and is readily absorbed into the blood stream where it is partially bound to plasma proteins in different species as horse (54%), cow (49%) and human (65%).[3,4] The main cause of penicillin deterioration is the reactivity of the strained β-lactam ring and the nature of degradation products; these are influenced by the pH of the solution. In strongly acidic solutions (pH < 3), penicillin undergoes a complex series of reactions leading to a variety of inactive degradation products. Acid-catalysed degradation of penicillin in the stomach contributes strongly to the poor oral absorption.[5] It has a high order of selective toxicity to

micro-organisms which are pathogenic to human beings, and no obvious side effects in humans.[6] The stability of tautomers[7] and equilibrium constants in electrostatic reaction field for heterocyclic compounds in aqueous solution[8] was studied.

2. Importance of Computational studies

Quantum chemistry is the field in which solutions to the Schrodinger's equation ($H\Psi=E\Psi$) are used to predict the properties of molecules for solving chemical problems for calculation of various properties.[9] Austin Model-1 (AM1) is one of the semi-empirical methods based on the neglect of differential diatomic overlap integral approximation which uses experimental parameters and extensive simplification of the Schrodinger's equation to optimize molecules. AM1 Semi-empirical molecular orbital calculations were performed on the molecules shown in Scheme-1 to 5, using the MOPAC93 in WinMOPAC ver 5.13 program. Geometry calculations in the ground state (keywords: PRECISE, equivalent to GNORM=5.0, CHARGE, GEO-OK, and MMOK to correct the increase in the barrier to rotation of the amide linkage) were completely optimized until the formation of lowest energy conformation. In this context, the numbering of benzylpenicillin tautomers were shown in Figure -1 to 4. The initial molecular geometry was adopted as Pople's standard data[10], and subsequently optimized using an energy gradient method. The conformations were designated by Klyne-Prelog terms[11] using *s* = syn, *a* = anti, *p* = peri-planar (0 ± 30^0 & 180 ± 30^0) and all other angles *c* = clinal.

3. Importance of Austin Model-1 (AM1) method

Rajeev Sing et al reported[12] the optimization of structural parameters (bond lengths and bond angles) of S-2-picolyl-β-N-(2-acetylpyrrole) dithiocarbazate by semi-empirical AM1 and PM3 methods and compared with those of experimentally available x-ray diffraction data. They found calculated bond lengths are in good agreement with experimental values. For bond angles none of the methods produce excellent correlation but out of the two methods AM1 method gives slightly better results than PM3 methods (correlation coefficients, CC=0.506). The theoretical calculations of the molecules are performed in gaseous phase and the experimental results of molecules are recorded in solid phase. In spite of the differences, calculated geometric parameters represent a good approximation and they are the basis for calculating other parameters such as vibration frequencies and thermodynamic properties.

C.D. Gutsche[13] carried out computational study by AM1 semi-empirical method on isomer structures of four Calix[4] resorcinarenes functionalized with organic phosphorus groups, but the conformational and configurational structures were realized and optimized by Hyper Chem programmer. Because of the sterical-hindrances the "cone" conformation with all its six configurations couldn't be realized, so the heats of formation were calculated only for others four conformations, together with the adequate configurations. M. Tsintsadze et al[14] have studied the effect of ssolvents' on the formation ability of ligand to form metal complexes. M.J.S. Dewar et al[9a] have calculated the effect of solvent on the ability of formation of complexes of acetamide with metals and structural, energetic and electronic characteristics of dimethylacetamide in the state of gas and in solvents by means of the quantum-chemical semi-empirical AM1 method. The calculation results were indicated that reduction of heat of formation during transition from gas condition to solvent for the two molecules at an increase of dielectric permeability of the solvent is observable. It means that the stability of amides increases together with the polarity of solvents.

Mehdi Salihshihab[15] used semi-empirical calculations of AM1 method to characterize the self-assembly of carbazole, tetracyanoethylene, 2,3-dichloro-5,6-dicyano-p-benzoquinone, and 2,4,7-trinitro-9-fluorenone. Carbazole is an electron-rich system, while tetracyanoethylene, 2,3-dichloro-5,6-dicyano-p-benzoquinone, and 2,4,7-trinitro-9-fluorenone are electron-deficient systems. Since all of these molecules contain aromatic rings and π systems, their self-organization is mainly based on aromatic interactions and donor–acceptor interactions. The energetic and physical properties of these molecular systems, such as heat of formation, HOMO–LUMO gap, binding energy, dipole moment, and mean polarizability were calculated. Supratim Ray[16] used the AM1 semi empirical levels to calculate the Mullikan's charges and dipole moment of common atoms for twenty six 1, 3-diarylpyrazole derivatives is used as chemometric tool. The model indicates the importance of hydroxyl group at various position of the moiety. E.R. Charmorrol et al[17] reported the conformational energy surface at compound (Z)-13-hexadecen-11-ynyl acetate as well as the electronic properties of a few analogues at varying the torsion angles, using semi-empirical methods. The structural and electronic parameters as atomic charges and orbital energies were calculated. Total Electronic Charge Density maps were also determined for the pheromone molecule and their analogue derivatives. The results obtained at semi-empirical level of theory with AM1 Hamiltonian were related to the stereoelectronic requirements

necessary to produce the activity on biological receptor, by comparative electro antennogram responses.

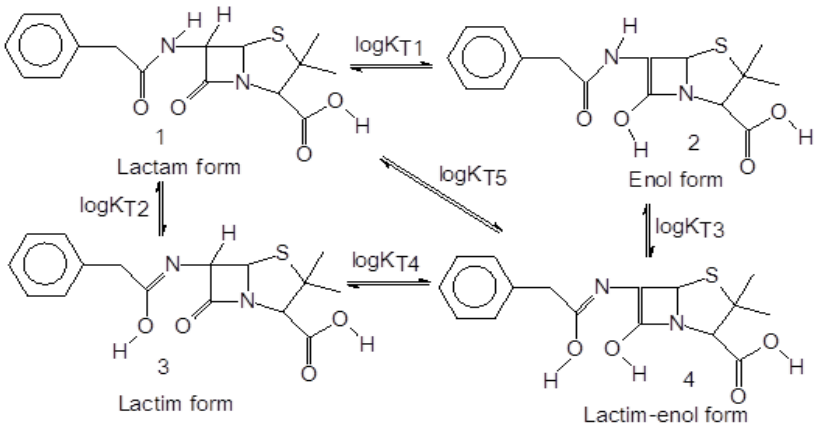
M. Bossa et al[18] has applied AM1 method satisfactorily in many hydrogen-bonded systems specially in pentachlorophenol and dihydroxybenzoquinone-amine systems. It is known however, that the quality of semi-empirical results depends on the nature of the investigated system and its chemical properties which are the targets of the study. For such reasons, these methods need to be extensively tested before employed with confidence. Therefore, they correlated the results of AM1 method with the FTIR spectral data of interesting hydrogen-bonded complexes between chloranilic acid and anilines. S.M. Janes et al[19] studied the influences of the chemical substitution on the proton transfer process and the strength of the hydrogen bond from the experimental values and correlated with the theoretical values calculated by using AM1 method for the quinones. Th.Zeegers-Huyskens[20] calculated the proton affinities of the substituted anilines by using AM1 approach and using it as a basicity scale in the system under investigation. Lemi Turker[21] reported AM1-type semi-empirical quantum chemical calculations to explain differences in herbicidal activity between certain phenoxyacetic acid derivatives. It was found that the proper orientation and shape of the both COOH group and the phenyl moiety mutually and individually affected the observed activities.

E.F. Sheka et al[22] has applied AM1 method successfully to study the chemical and physical properties of metal oxides and different reactions with participation of oxide catalysts. A.P. Marchand et al[23] reported an interesting theoretical study of the Diels-Alder reaction of polychlorinated cyclopentadiene to norbornadiene. They employed semi-empirical AM1methods to study the transition states and energy levels of this, at one time industrially important reaction. They also applied low level ab initio (HF/3-21) calculations on these AM1 optimized structures to study the transition states of the exo- and endo- approaches of the diene to the dienophile. Shruti Maheswari et al[24] studied energetic of the ground and excited state intra-molecular proton transfer in salicylic acid by using the semi-empirical method AM1 at the RHF level as well as with single and pair doubles excitation configuration interaction spanning eight frontier orbitals (PECI). The ab initio potential energy profile for intra-molecular proton transfer in the ground state reveals a single minimum corresponding to the primary form. In the first excited singlet state, however, there are two minima corresponding to the primary and tautomeric forms, separated by a barrier of-

6 kcal/mol, so that it is accounting for dual emission in salicylic acid. Electron density changes with electronic excitation and tautomerism indicate no zwitterion formation.

Peter et al[25] used the semi-empirical AM1 SCF-MO method to study the benzyne mechanism for aromatic nucleophilic substitution of various *m*-substituted chloro-benzenes would induce the formation of 2,3-arynes through their electron-withdrawing resonance or inductive effects. The geometry and electronic structure of the 2,3- and 3,4-arynes investigated here, confirm the generally accepted *o*-benzyne structure postulated for arynes. The sites of nucleophilic addition to arynes as predicted here are in fair agreement with expectation and experimental findings. H.Kara et al[26] applied Semi-empirical AM1 SCF-MO calculations to find the structure optimization and conformational inter-conversion pathways of a system containing a six-membered ring. The system has the two symmetrical energy-minimum conformations, chair and twist. The chair conformation has the most stable geometry. Some quantum parameters such as HOMO and LUMO energy, the chemical hardness and chemical potential are discussed.

4. Importance of Tautomeric equilibrium in benzylpenicillin[27]



Scheme - 1

Equilibrium is normally established in polar solvents, in order to investigate the stable tautomer of benzylpenicillin (1) which is confirmed by the calculated heats of formation with full geometry optimization. The tautomers can exist in *anti*- or *syn*-conformations as per Scheme-1. The AM1 calculated heat of formation, and the tautomeric equilibrium constants $\log K_T$ was calculated[28] according to the equation (1):

$$\log K_T = \frac{\Delta G_T}{2.303\ R\ T} = \frac{\delta \Delta H_f^{\circ}}{2.303\ R\ T} \quad \text{- - - (1)}$$

Where ΔG_T is the free energy of the tautomeric equilibrium, $\delta\Delta H_f^o$ is the difference in the calculated heats of formation of tautomeric species participating in this equilibrium. R is the gas constant and T is the absolute temperature. From this equation (1), it is observed that the tautomeric equilibrium is increased in the order of $\log K_{T3} < \log K_{T2} < \log K_{T4} < \log K_{T1} < \log K_{T5}$.

5. Proton affinity of nitrogen atoms in benzylpenicillin tautomers

The proton affinity (PA)[29] values for the different nitrogen atoms of benzylpenicillin tautomers were calculated by using the equation (2).

$$\mathbf{PA = \Delta H_f^o(H^+) + \Delta H_f^o(B) - \Delta H_f^o(BH^+) \ldots (2)}$$

Where PA is the proton affinity, $\Delta H_f^o(B)$ is the heat of formation for benzylpenicillin tautomers, $\Delta H_f^o(BH^+)$ is the heat of formation for the cation, and $\Delta H_f^o(H^+)$ is heat of formation for the proton (367.2kcal/mol). The stable conformation of the cations were calculated from AM1 method.

5.1. Electronic structure of benzylpenicillin (1) and its tautomers (2 to 4)

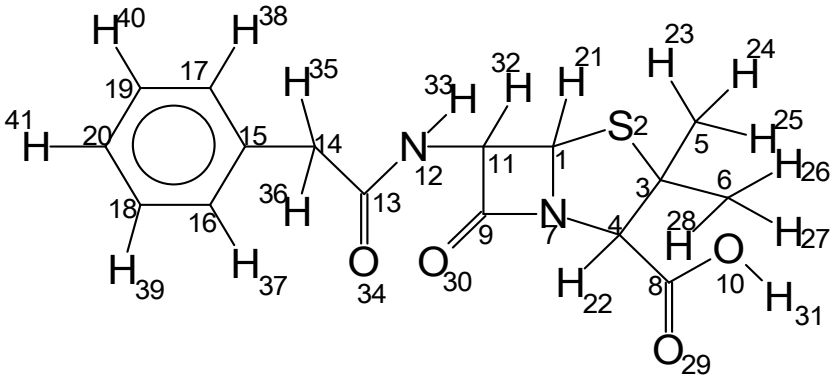


Figure - 1

The optimized electronic structure of benzylpenicillin (**1**) and its tautomers (**2** to **4**) are shown in Scheme-1. The calculated heats of formation (ΔH_f^o), ionization potential (IP), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero atoms of the molecules (**1** to **4**) are presented in Table-I. It is observed that the net charges on N_7- and N_{12}- atoms are -0.2584 and -0.3614 respectively in the case of benzylpenicillin (**1**) in the order of $N_7 < N_{12}$. The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **1** and **4** have more electron-donor character whereas other tautomers have electron-acceptor property. The promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed in the

case of molecules **1** and **4,** due to the presence of same sign and other molecules undergo antara-facial path way is allowed due to the opposite sign.[30]

Table I: Heat of formation (ΔH_f^o in kcal/mol), ionization potential (eV), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV), electron excitation energies ($\Delta E = E_{LUMO} - E_{HOMO}$) (in eV) and the atomic charges on hetero-atoms of benzyl penicillin(1) and its tautomers (2 to 4) from AM1 calculations.

Parameters	1	2	3	4
ΔH_f^o (kcal/mol)	-94.5278	-70.6933	-82.8829	-68.0049
Ionization potential (eV)	9.310	8.799	8.947	8.378
μ (Debye)	5.546	2.426	1.835	3.013
E_{HOMO} (eV)	-9.310	-8.799	-8.947	-8.379
E_{LUMO} (eV)	-0.062	+0.063	0.086	-0.204
Electron excitation energies (eV)	9.248	8.862	9.033	8.175
S_2	+0.0366	+0.0685	+0.0935	+0.0974
N_7	-0.2584	-0.1588	-0.2572	-0.1606
N_{12}	-0.3614	-0.2917	-0.2701	-0.1957
O_{10}	-0.3225	-0.3200	-0.3178	-0.3050
O_{29}	-0.3282	-0.3540	-0.3342	-0.3755
O_{30}	-0.2396	-0.2025	-0.2740	-0.2346
O_{34}	-0.3570	-0.3412	-0.2892	-0.2875

The dipole moment is increasing in the order of molecules **3** < **2** < **4** < **1**. The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[31] (μ_{ind}) of molecules can be estimated with respect to benzylpenicillin lactim form (**3**). It is found that the induction effect is increasing in the order of $\Delta\mu_{ind}$ (**2**) 0.591D < $\Delta\mu_{ind}$ (**4**) 1.178 D < $\Delta\mu_{ind}$ (**1**) 3.711 D. According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **4** < **2** < **3** < **1**. The stable tautomers of benzylpenicillin (1) can be assigned by comparison of its geometry and electronic structure as per Scheme-1. Three tautomeric forms of benzylpenicillin (**1**) are possible at chemical equilibrium under ordinary conditions. which are capable of inter-conversion at higher temperatures, often with the aid of catalyst. As per electron excitation energies (ΔE) (in eV), it is observed the reactivity is decreased in the order of **4** > **2** > **3** > **1**. The shifting of H_{32}-proton and H_{33}-proton of benzylpenicillin **(1)** to respective O_{30}-atom and O_{34}-atom are predicted for the formation of respective enol-form(**2**) and lactim-form(**3**). The simultaneous shifting of H_{32}-proton and H_{33}-proton of benzylpenicillin **(1)** to respective O_{30}-atom and O_{34}- atom is predicted for the formation of lactim-enol form **(4)** of benzylpenicillin. The AM1 calculated heat of formation, and the tautomeric equilibrium constants $\log K_T$ was calculated according to the equation (1) and the change of net charges were calculated and

incorporated in Table- II. The tautomeric equilibrium is increased in the order of $logK_{T3}$ < $logK_{T2}$ < $logK_{T4}$ < $logK_{T1}$ < $logK_{T5}$. At the time of tautomeric conversion of (**1**) to (**2**), (**1**) to **(4)** and (**3**) to (**4**) the net charges are increased at O_{29}- atom and decreased at all other hetero atoms. In the case of tautomeric conversion of (**1**) to (**3**) the net charge is increased at O_{29}-atom, O_{30}-atom and decreased at all other hetero-atoms, but the tautomeric conversion of (**2**) to (**4**), the net charge is increased at N_7-atom, O_{29}-atom, O_{30}-atom and decreased at all other hetero-atoms.

Table II: Tautomeric Equilibrium of Benzylpenicillin.

$logK_T$	Equilibrium	$logK_T$ - Values	Change of Net Charges on Hetero-atoms	
			Increasing	Decreasing
$logK_{T1}$	1 ↔ 2	17.47	O_{29}	S_2, N_7, N_{12}, O_{10}, O_{30}, O_{34}
$logK_{T2}$	1 ↔ 3	8.53	O_{29}, O_{30}	S_2, N_7, N_{12}, O_{10}, O_{34}
$logK_{T3}$	2 ↔ 4	1.97	N_7, O_{29}, O_{30}	S_2, N_{12}, O_{10}, O_{34}
$logK_{T4}$	3 ↔ 4	10.90	O_{29}	S_2, N_7, N_{12}, O_{10}, O_{30}, O_{34}
$logK_{T5}$	1 ↔ 4	19.44	O_{29}	S_2, N_7, N_{12}, O_{10}, O_{30}, O_{34}

From the Table-III, the benzylpenicillin (**1**) may undergo lactam-enol tautomerism and form benzylpenicillin-enol (**2**) with increasing bond length of O_{30}-C_9 (1.3468 Å) and decreasing bond length of C_{11}-C_9 (1.3757 Å) with the formation of H_{32}-O_{30} bond (0.9752Å). If the benzylpenicillin (**1**) may undergo lactam-lactim tautomerism and form benzylpenicillin-lactim (**3**) with increasing bond length of O_{34}-C_{13} (1.3798 Å) and decreasing bond length of C_{13}-N_{12} (1.2940 Å) with the formation of H_{33}-O_{34} bond (0.9709 Å). But the formation of lactim-enol tautomerism (**4**) from benzyl penicillin (**1**) with increasing bond lengths of O_{30}-C_9 (1.3486 Å) and O_{34}-C_{13} (1.3789 Å), decreasing bond lengths of C_{11}-C_9 (1.3831 Å) and C_{13}-N_{12} (1.3017 Å) with the formation of H_{32}-O_{30} bond (0.9751Å) and H_{33}-O_{34} bond (0.9690 Å).

Table III: Bond lengths of benzyl penicillin(1) and its tautomeric forms (2 to 4) from AM1 calculations.

Bond lengths (Å)	1	2	3	4
C_9-N_7	1.4512	1.4685	1.4342	1.4608
C_{11}-C_9	1.5689	1.3757	1.5659	1.3831
O_{30}-C_9	1.2176	1.3468	1.2225	1.3486
C_{13}-N_{12}	1.3873	1.3904	1.2940	1.3017
C_{14}-C_{13}	1.5176	1.5227	1.5192	1.5171
O_{34}-C_{13}	1.2452	1.2426	1.3798	1.3789
H_{32}-O_{30}	- -	0.9752	- -	0.9751
H_{33}-O_{34}	- -	- -	0.9709	0.9690

5.2. The conformations of benzylpenicillin (1) and its tautomers (2 to 4)

The spatial arrangement of atoms in tautomers are considered to study the conformations of benzylpenicillin (**1**), and its enol form (**2**), lactim form (3) and lactim-enol form (**4**) of benzylpenicillin with a view to investigate molecular deformations. Fully optimized AM1 calculations of dihedral angles of molecules (**1** to **4**) were incorporated Table-IV. As per Scheme-1, the H_{32}-proton shifting to O_{30}- atom in the benzylpenicillin **(1)** is predicted for the formation of enol-form(**2**). The conformations of ***–ac*** of $C_{13}N_{12}C_{11}C_9$, ***-ac*** of $O_{29}C_8C_4C_3$ and ***–sc*** of $O_{30}C_9N_7C_4$ are changed to conformation ***+sc***. Dihedral angle of $C_8C_4C_3S_2$, $O_{10}C_8C_4C_3$, $C_{15}C_{14}C_{13}N_{12}$, $H_{31}O_{10}C_8C_4$,$O_{34}C_{13}N_{12}C_{11}$ and $H_{33}N_{12}C_{11}C_9$ are changed to respectively from *–ac* to *+ap*, *+sc* to *–ac*, *-sp* to *–sc*, *+ap* to *–ap*, *+sp* to *–sp* and *+sc* to *-ac* conformations and all other conformations are moderately changed. After lactam-enol rearrangement, the enol form of benzylpenicillin (**2**) is formed with the *+sp* conformation in the case of dihedral angle of $H_{32}O_{30}C_9N_7$. If the H_{33}-proton shifting to O_{34}- atom in the benzylpenicillin **(1)** is predicted for the formation of lactim-form(**3**). The change of conformation from *–ac* of $C_8C_4C_3S_2$ and *+sc* of $O_{10}C_8C_4C_3$ are changed to *+ac* conformation. The conformation of –ac of $C_{13}N_{12}C_{11}C_9$, –sp of $C_{15}C_{14}C_{13}N_{12}$ and*–ac* of $O_{29}C_8C_4C_3$ are changed to –sc conformation. After lactam-lactim rearrangement, the lactim form of benzylpenicillin (**3**) is formed with the +sp conformation in the case of dihedral angle of $H_{33}O_{34}C_{13}N_{12}$.

Table IV: Dihedral angle (^{o}) of benzyl penicillin (1) and its tautomeric forms (2 to 4), from AM1 calculations.

Dihedral angle (^{o})	1		2		3		4	
	Angle	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**
$C_4C_3S_2C_1$	-18.79	*-sp*	-22.38	*-sp*	-12.39	*-sp*	-18.81	*-sp*
$C_8C_4C_3S_2$	-137.46	*-ac*	+162.73	*+ap*	+129.36	*+ac*	+157.03	*+ap*
$O_{10}C_8C_4C_3$	+88.68	*+sc*	-136.12	*-ac*	+98.40	*+ac*	+64.85	*+sc*
$C_{13}N_{12}C_{11}C_9$	-127.62	*-ac*	+59.03	*+sc*	-52.15	*-sc*	-12.94	*-sp*
$C_{14}C_{13}N_{12}C_{11}$	-177.09	*-ap*	-178.79	*-ap*	-176.35	*-ap*	-179.34	*-ap*
$C_{15}C_{14}C_{13}N_{12}$	-26.76	*-sp*	-39.91	*-sc*	-84.33	*-sc*	-76.38	*-sc*
$O_{29}C_8C_4C_3$	-91.56	*-ac*	+47.29	*+sc*	-81.92	*-sc*	-117.61	*-ac*
$O_{30}C_9N_7C_4$	-52.24	*-sc*	+67.28	*+sc*	-51.95	*-sc*	+68.72	*+sc*
$H_{31}O_{10}C_8C_4$	+178.59	*+ap*	-178.41	*-ap*	+179.04	*+ap*	+179.01	*+ap*
$H_{32}O_{30}C_9N_7$	- -	- -	+26.05	*+sp*	--	- -	-0.29	*-sp*
$H_{33}O_{34}C_{13}N_{12}$	- -	- -	- -	- -	+8.21	*+sp*	+4.59	*+sp*
$O_{34}C_{13}N_{12}C_{11}$	+2.00	*+sp*	-1.27	*-sp*	+1.40	*+sp*	-1.08	*-sp*
$H_{33}N_{12}C_{11}C_9$	+56.65	*+sc*	-126.67	*-ac*	- -	- -	- -	- -

* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - Signs[11]

The simultaneous shifting of H_{32}-proton and H_{33}-proton of benzylpenicillin **(1)** to respective O_{30}- atom and O_{34}- atom is predicted for the formation of lactim-enol form **(4)**. The conformations of *–ac* of $C_8C_4C_3S_2$ and $C_{13}N_{12}C_{11}C_9$ are changed to ***+ap*** and ***–sp*** conformations respectively to form stable conformation and rest of positions have moderate changes. Dihedral angle of $C_{15}C_{14}C_{13}N_{12}$ and $O_{34}C_{13}N_{12}C_{11}$ are changed respectively *-sp* to *–sc* and *+sp* to *-sp* conformation and all other conformations are moderately changed. It is observed that the shifting of H_{32}-proton and H_{33}-proton of benzylpenicillin **(1)** to respective O_{30}- atom and O_{34}- atom is predicted for the formation of lactim-enol form **(4)** with the formation of ***-sp*** and ***+sp*** conformations in the case of $H_{32}O_{30}C_9N_7$, and $H_{33}NO_{34}C_{13}N_{12}$ respectively.

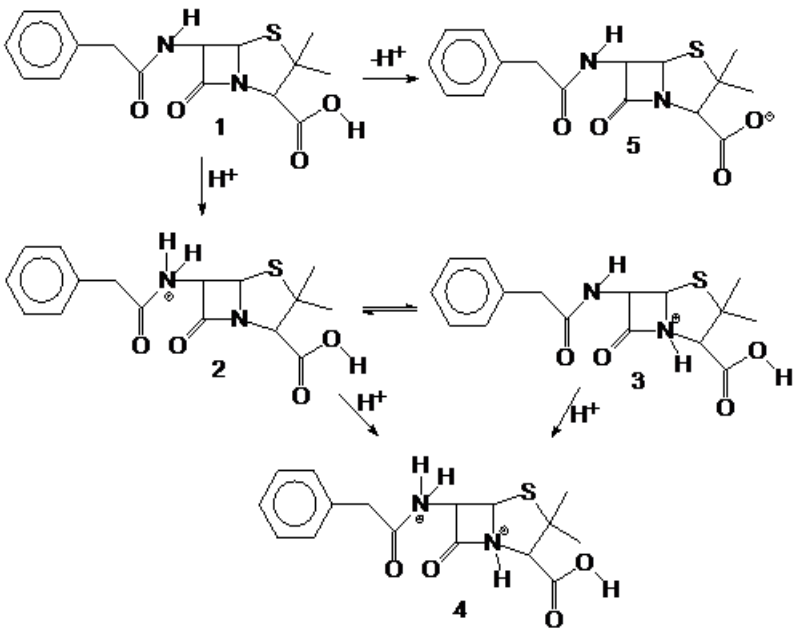
6.1.Computational study[32] on electronic structure of benzylpenicillin (1) and its mono-protonated (2&3), di-protonated (4) and anion (5)

The optimized electronic structure of benzylpenicillin (**1**) and its mono-protonated (**2** & **3**), di-protonated (**4**) and anion (**5**) are shown in Scheme-2. In this context, the numbering of benzylpenicillin is shown in Figure -1. The calculated heats of formation (ΔH_f^o), ionization potential (IP), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero atoms of the molecules (**1** to **5**) are presented in Table-V. It is observed that the net charges on N_7- and N_{12}- atoms are -0.2584 and -0.3614 respectively in the order of $N_7 < N_{12}$ in the case of benzylpenicillin (1).

Table V: Heat of formation (ΔH_f^o in kcal/mol), ionization potential (eV), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV), electron excitation energies ($\Delta E = E_{LUMO} - E_{HOMO}$) (in eV) and the atomic charges on S_2, N_7, N_{12}, O_{10}, O_{29}, O_{30} and O_{34} of benzyl penicillin(1) and its mono-protonated forms (2 & 3), di-protonated form (4), and anion (5) from AM1 calculation.

Parameters	1	2	3	4	5
ΔH_f^o (kcal/mol)	-94.5278	79.4018	67.6497	325.4611	-122.6205
Ionization potential (eV)	9.310	13.011	12.306	15.626	4.864
μ (Debye)	5.546	6.725	4.691	2.652	19.282
E_{HOMO} (eV)	-9.310	-13.011	-12.306	-15.626	-4.865
E_{LUMO} (eV)	-0.062	-4.486	-4.587	-8.726	+1.894
Electron excitation energies (eV)	9.248	8.525	7.719	6.900	6.759
S_2 (atomic charge)	+0.0366	+0.0296	+0.1982	+0.1828	-0.0839
N_7 (atomic charge)	-0.2584	-0.2188	-0.1291	-0.1002	-0.1923
N_{12} (atomic charge)	-0.3614	-0.1035	-0.3784	-0.1268	-0.3450
O_{10} (atomic charge)	-0.3225	-0.3064	-0.2730	-0.2600	-0.5572
O_{29} (atomic charge)	-0.3282	-0.3041	-0.3212	-0.3004	-0.5322
O_{30} (atomic charge)	-0.2396	-0.1893	-0.0914	-0.0253	-0.2888
O_{34} (atomic charge)	-0.3570	-0.1657	-0.3135	-0.1452	-0.3853

The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **1** and **5** have more electron-donor character whereas other molecules have electron-acceptor property. The promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed in the case of molecules 1 to 4, due to the presence of same sign.[30] The dipole moment of molecules is observed in the order of molecules **4** < **3** < **1** < **2** < **5**. Anion (**5**) shows higher dipole moment. The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[31] (μ_{ind}) of molecules can be estimated with respect to benzylpenicillin (**1**). It is found that the induction effect is increasing in the case of $\Delta\mu_{ind}$ (**2**) 1.179D and $\Delta\mu_{ind}$ (**5**) 13.736D and decreasing in the case of $\Delta\mu_{ind}$ (**3**) -0.855D and $\Delta\mu_{ind}$ (**4**) -2.894D. According to the heat of formation (ΔH_f^o) data, the stability of compounds have decreased in the order of **5** > **1** > **3** > **2** > **4**. It is predicted that the protonation would take place preferably at N_7-atom than N_{12}-atom in the case of benzylpenicillin (**1**), this is due to the increased bond length of C_9-N_7 (1.4512 Å) than C_{13}-N_{12} (1.3873Å). It is confirmed that the stability of mono-protonated benzylpenicillin **3** (ΔH_f^o, +67.6497Kcal/mol) is more stable than **2** (ΔH_f^o, +79.4018Kcal/mol).



Scheme - 2

The formation of di-protonated benzylpenicillin **(4),** from mono-protonated benzylpenicillins (**2** & **3**) is possible with the heat of formation (ΔH_f^o) of +325.4611Kcal/mol. The protonation site of benzylpenicillin **(1)** at N_7- atom is predicted to be the main basic centre of molecule.

However, negative atomic charges are also present on the other atoms of the molecule. The protonation at N_7-atom in the case of neutral benzylpenicillin (**1**) to mono-protonated form (**3**) is considered by increasing net atomic charges at N_{12}-atom and decreasing at N_7-, O_{10}-, O_{29}-, O_{30}-, and O_{34}- atoms. The protonation site of benzylpenicillin (**1**) at N_{12}- atom to mono-protonated form (**2**) is considered by decreasing net atomic charges at N_7-, N_{12}-, O_{10}-, O_{29}-, O_{30}- and O_{34}-atoms. In the case of di-protonated form (**4**), the negative atomic charges are decreased at all hetero atoms. Anion of benzylpenicillin (**5**) is formed by the removal of a proton on O_{10}-atom with increasing net charges at O_{10}-, O_{29}-, O_{30}- and O_{34}-, and decreasing at N_7- and N_{12}- atoms.

The cations formed by the protonation at N_7- or N_{12}- atoms of benzylpenicillin (**1**) can exist in *anti*- or *syn*-conformations, according to the position of N-atoms as shown in Scheme-2. The proton affinity (PA) values for the different nitrogen atoms of benzylpenicillin RH (**1**) were calculated by using the equation (2) and found to be 193.2704kcal/mol and 205.0225kcal/mol respectively in the case of mono-protonated benzylpenicillins (**2** and **3**). Di-protonated benzylpenicillin (**4**) was formed from either of mono-protonated benzylpenicillins (**2** and **3**) respectively with PA 121.1407 kcal/mol and 109.3886 kcal/mol. The proton affinity is in the order of N_7 (205.0225kcal/mol) > N_{12} (193.2704kcal/mol) and mono-protonated benzylpenicillin (**3**) appears to be more stable.

6.2.The conformations of benzylpenicillin (1) and its mono-protonated (2 and 3), di-protonated (4) and anion R^- (5)

The spatial arrangement of atoms in a molecule is considered to study the conformations of benzylpenicillin (**1**), and its mono-protonated forms (**2 & 3**), di-protonated form (**4**) and anion (**5**) with a view to investigate molecular deformations.

Table VI: Bond lengths of benzyl penicillin(1) and its mono-protonated forms (2 & 3), di-protonated form (4), and anion (5) from AM1 calculation.

Bond lengths (Å)	1	2	3	4	5
C_9-N_7	1.4512	1.5269	1.5764	1.5342	1.4286
C_{13}-N_{12}	1.3873	1.5205	1.4111	1.5637	1.2495
O_{30}-C_9	1.2176	1.2138	1.1966	1.1985	1.2230
O_{34}-C_{13}	1.2452	1.2190	1.2385	1.2122	1.2495
C_{14}-C_{13}	1.5176	1.5032	1.5113	1.5000	1.5221

From the Table-VI, Table-VII and Scheme-2, mono-protonated benzylpenicillin (**2**) is formed by the addition of proton at N_{12}-atom of benzylpenicillin (**1**), with increasing bond lengths at C_{13}-N_{12} and C_9-N_7 and decreasing bond lengths at O_{30}-C_9, O_{34}-C_{13} and C_{14}-C_{13}. The change of conformation from *–ac* of $C_8C_4C_3S_2$, $C_{13}N_{12}C_{11}C_9$ and $O_{29}C_8C_4C_3$, are changed to *+ac*, *-ap* and *–sc* conformation. Dihedral angle of $O_{10}C_8C_4C_3$ is changed *+sc* to *+ac* conformation. It is also observed that the protonation at N_{12}- atom is shown *–sc* conformation. If the mono-protonated benzylpenicillin (**3**) is formed by the addition of proton at N_7- atom of benzylpenicillin (**1**), with increasing bond lengths at C_{13}-N_{12} and C_9-N_7 and decreasing bond lengths at O_{30}-C_9, O_{34}-C_{13} and C_{14}-C_{13}. The change of dihedral angle of $C_8C_4C_3S_2$ is converted *-ac* to *+ac* conformation and all other conformations are unaltered. It is observed that the protonation at N_7-atom is shown *+ac* conformation.

Table VII: Dihedral angle (o) of benzyl penicillin (1) and its mono-protonated forms (2 & 3), di-protonated form (4), and anion (5) from AM1 calculation.

Dihedral angle (^{o})	1		2		3		4		5	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-18.79	*-sp*	-14.43	*-sp*	-21.51	*-sp*	-23.59	*-sp*	-9.67	*-sp*
$C_8C_4C_3S_2$	-137.46	*-ac*	+129.36	*+ac*	+148.64	*+ac*	+147.77	*+ac*	+129.88	*+ac*
$O_{10}C_8C_4C_3$	+88.68	*+sc*	+98.09	*+ac*	+71.79	*+sc*	+70.30	*+sc*	+95.22	*+ac*
$C_{13}N_{12}C_{11}C_9$	-127.62	*-ac*	-155.17	*-ap*	-111.86	*-ac*	-106.04	*-ac*	-124.35	*-ac*
$C_{14}C_{13}N_{12}C_{11}$	-177.09	*-ap*	-178.85	*-ap*	-178.35	*-ap*	+179.63	*+ap*	-176.13	*-ap*
$C_{15}C_{14}C_{13}N_{12}$	-26.76	*-sp*	-18.59	*-sp*	-25.19	*-sp*	+0.83	*+sp*	-41.53	*-sc*
$O_{29}C_8C_4C_3$	-91.56	*-ac*	-82.03	*-sc*	-109.57	*-ac*	-111.21	*-ac*	-83.19	*-sc*
$O_{30}C_9N_7C_4$	-52.24	*-sc*	-52.56	*-sc*	-68.31	*-sc*	-67.12	*-sc*	-54.89	*-sc*
$H_{31}O_{10}C_8C_4$	+178.59	*+ap*	+178.69	*+ap*	+179.08	*+ap*	+178.54	*+ap*	- -	- -
$H_{33}N_{12}C_{11}C_9$	+56.64	*+sc*	+83.29	*+sc*	+71.74	*+sc*	+133.42	*+ac*	+62.19	*+sc*
$O_{34}C_{13}N_{12}C_{11}$	+2.00	*+sp*	+0.49	*+sp*	+1.00	*+sp*	-0.28	*-sp*	+2.62	*+sp*
$HN_{12}C_{11}C_9$	- -	- -	-33.45	*-sc*	- -	- -	+16.80	*+sp*	- -	- -
$HN_7C_4C_3$	- -	- -	- -	- -	+110.98	*+ac*	+112.87	*+ac*	- -	- -

* Conformational analyses using prefixes a = anti, s = syn, p = peri-planar, c = clinal, and + & -signs.[11]

In the case of formation of di-protonated benzylpenicillin (**4**), it is found that the dihedral angle of $C_8C_4C_3S_2$, $C_{14}C_{13}N_{12}C_{11}$, $C_{15}C_{14}C_{13}N_{12}$, $H_{33}N_{12}C_{11}C_9$, and $O_{34}C_{13}N_{12}C_{11}$ are changed conformation, *-ac* to *+ac*, *-ap* to *+ap*, *-sp* to *+sp*, *+sc* to *+ac* and *+sp* to *–sp* conformations respectively. It is also investigated that the protonation at N_7- atom and N_{12}-atom are shown respectively *+ac* and *+sp* conformations to form stable di-protonated benzylpenicillin (**4**). It can be concluded that the anion (**5**) is formed with the removal of a proton on O_{10}- atom of benzylpenicillin (**1**), and the change of conformation from *-ac* of $C_8C_4C_3S_2$ and $O_{29}C_8C_4C_3$ are changed to *+ac* and *–sc* conformations respectively. Dihedral angle of $O_{10}C_8C_4C_3$, and

$C_{15}C_{14}C_{13}N_{12}$ are changed the conformations from *+sc to +ac* and *-sp* to *-sc* respectively to form stable anion R^- (**5**) and rest of positions have moderate changes.

7.1.Computational study[33] on Electronic structure of benzylpenicillin (1) and its lactim tautomer (2) mono-protonated (3 & 4), di-protonated (5) and anion (6)

The optimized electronic structure of benzylpenicillin (**1**) and its lactim tautomer (**2**) mono-protonated (**3** & **4**), di-protonated (**5**) and anion (**6**) are shown in Scheme- 3. In this context, the numbering of lactim from of benzylpenicillin (**2)** is shown in Figure - 2. The calculated heats of formation (ΔH_f^o), ionization potential (IP), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero atoms of the molecules (**1** to **6**) are presented in Table-VIII. It is observed that the net charges on N_7- and N_{12}- atoms are -0.2572 and -0.2701 respectively in the case of lactim tautomer of benzylpenicillin (**2**). The sequence of protonation for nitrogen atoms of lactim tautomer of benzylpenicillin (**2**) is increasing in the order of $N_7 < N_{12}$.

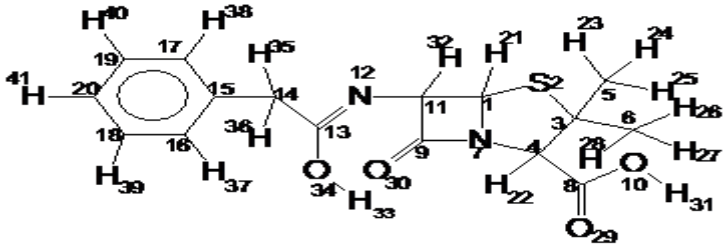


Figure - 2

The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **1** and **5** have more electron-donor character whereas other molecules have electron-acceptor property. In the case of HOMO, the electron density is more at N_{12}- atoms for all molecules. The promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed in the case of molecules **1, 3, 4**, and **5**, due to the presence of same sign and other molecules undergo antara-facial path way us allowed due to the opposite sign.[30] The dipole moment of molecules depends on the nature of the atoms and bonds comprising the molecules and on their arrangement. The dipole moment is increasing in the order of molecules **2** < **1** < **4** < **3** < **5** < **6**. Anion (**6**) shows higher dipole moment. The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[31] (μ_{ind}) of molecules can be estimated with respect to benzylpenicillin lactim form (**2**). It is found that the

induction effect is increasing in the order of $\Delta\mu_{ind}$ (**1**) 3.711D < $\Delta\mu_{ind}$ (**4**) 4.125D < $\Delta\mu_{ind}$ (**3**) 4.472D < $\Delta\mu_{ind}$ (**5**) 4.761D < $\Delta\mu_{ind}$ (**5**) 16.887D. According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **5** < **4** < **3** < **2** < **1** < **6**. It is predicted that the protonation would take place preferably at N_{12}-atom than N_7-atom in the case of lactim form of benzylpenicillin (**2**). It is confirmed that the stability of mono-protonated lactim form of benzylpenicillin **3** (ΔH_f^o, +63.6902Kcal/mol) is more stable than **4** (ΔH_f^o, +80.0409Kcal/mol). The formation of lactim form of di-protonated benzylpenicillin **(5),** from mono-protonated lactim form of benzylpenicillins (**3** & **4**) is possible with the heat of formation (ΔH_f^o) of +305.4984Kcal/mol. The protonation site of lactim form of benzylpenicillin **(2)** at N_{12}- atom is predicted to be the main basic centre of molecule. However, negative atomic charges are also present on the other atoms of the molecule. The protonation at N_{12}-atom in the case of lactim form of benzylpenicillin (**2**) to mono-protonated form (**3**) is considered by decreasing net atomic charges at N_7-, N_{12}-, O_{10}-, O_{29}-, O_{30}-, and O_{34}- atoms. The protonation site of lactim form of benzylpenicillin (**2**) at N_{12}- atom to mono-protonated form (**4**) is considered by decreasing net atomic charges at N_7-, O_{10}-, O_{29}-, O_{30}- and O_{34}-atoms and increasing at N_{12}-atom. In the case of di-protonated form (**5**), the negative atomic charges are decreased at all hetero atoms except at N_{12}-atom. Anion of lactim form of benzylpenicillin (**6**) is formed by the removal of a proton on O_{10}-atom with increasing net charges at O_{10}-, O_{29}-, O_{30}- and O_{34}-, and decreasing at N_7- and N_{12}- atoms.

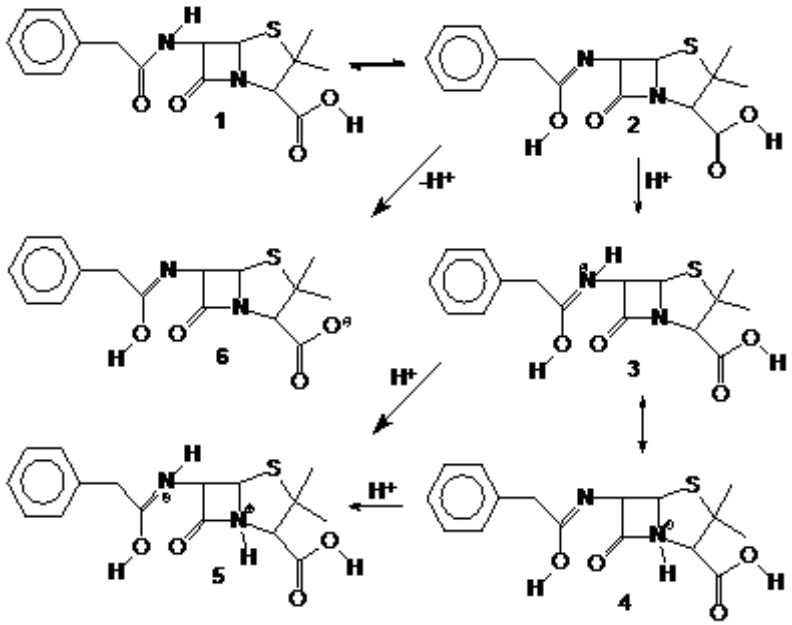
Table VIII: Heat of formation (ΔH_f^o in kcal/mol), ionization potential (eV), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV), electron excitation energies (ΔE) (in eV) and the atomic charges on S_2, N_7, N_{12}, O_{10}, O_{29}, O_{30} and O_{34} of benzyl penicillin(1) and its lactim form(2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculation.

Parameters	1	2	3	4	5	6
ΔH_f^o (kcal/mol)	-94.5278	-82.8829	63.6902	80.0409	305.4984	-109.8311
Ionization potential (eV)	9.310	8.947	12.577	11.927	15.383	4.816
μ (Debye)	5.546	1.835	6.307	5.960	6.596	18.722
E_{HOMO} (eV)	-9.310	-8.947	-12.577	-11.927	-15.383	-4.816
E_{LUMO} (eV)	-0.062	+0.086	-5.056	-4.487	-8.529	+2.137
Electron excitation energies ($\Delta E = E_{LUMO} - E_{HOMO}$) (eV)	9.248	9.033	7.521	7.440	6.854	6.953
S_2 (atomic charge)	+0.0366	+0.0935	+0.0614	+0.2260	+0.2100	-0.0244
N_7 (atomic charge)	-0.2584	-0.2572	-0.2260	-0.1207	-0.0997	-0.1919
N_{12} (atomic charge)	-0.3614	-0.2701	-0.2293	-0.3283	-0.2881	-0.2324
O_{10} (atomic charge)	-0.3225	-0.3178	-0.3042	-0.2710	-0.2605	-0.5574
O_{29} (atomic charge)	-0.3282	-0.3342	-0.3103	-0.3263	-0.3047	-0.5349
O_{30} (atomic charge)	-0.2396	-0.2740	-0.2367	-0.0924	-0.0438	-0.3337
O_{34} (atomic charge)	-0.3570	-0.2892	-0.1883	-0.2528	-0.1551	-0.3206

The cations formed by the protonation at N_7- or N_{12}- atoms of lactim form of benzylpenicillin (**2**) can exist in *anti*- or *syn*-conformations, according to the position of N-atoms as shown in Scheme- 3. The proton affinity (PA) values for the different nitrogen atoms of lactim form of benzylpenicillin RH (**2**) were calculated by using the equation (2) and found to be 220.6269 kcal/mol and 204.2762 kcal/mol respectively in the case of mono-protonated benzylpenicillins (**3** and **4**). Di-protonated form (**5**) was formed from either of mono-protonated benzylpenicillins (**3** and **4**) respectively with PA 125.3918kcal/mol and 141.7425 kcal/mol. The proton affinity is in the order of N_{12} (220.6269kcal/mol) > N_7 (204.2762kcal/mol) and mono-protonated benzylpenicillin (**3**) appears to be more stable. All cations are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium. As per electron excitation energies (ΔE) (in eV), it is observed the reactivity is decreased in the order of **5** > **6** > **4** > **3** > **2** > **1**. It is confirmed that benzylpenicillin (**1**) is more stable than its lactim-form (**2**).

7.2.The conformations of benzylpenicillin-lactim form (2) and its mono-protonated (3 & 4), di-protonated (5) and anion (6)

The spatial arrangement of atoms in a molecule is considered to study the conformations of benzylpenicillin (**1**), and its lactim form of benzylpenicillin (**2**), mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) with a view to investigate *anti*- or *syn*- conformation, according to the position of atoms. In this context, the change in energy content of the protonation may depend on the changes in the parameters of dihedral angles.



Scheme - 3

From the Table-IX, Table-X and as per Scheme- 3, mono-protonated lactim form of benzylpenicillin (**3**) is formed by the addition of proton at N_{12}-atom of lactim tautomer of benzylpenicillin (**2**), with increasing bond lengths at C_{13}-N_{12} and decreasing bond lengths at C_9-N_7, O_{30}-C_9, O_{34}-C_{13} and C_{14}-C_{13}.

Table IX: Bond lengths of benzyl penicillin(1) and its lactim form (2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculation.

Bond lengths (Å)	1	2	3	4	5	6
C_9-N_7	1.4512	1.4341	1.4174	1.5650	1.5354	1.4133
O_{30}-C_9	1.2176	1.2225	1.2184	1.1974	1.1989	1.2296
C_{13}-N_{12}	1.3873	1.2940	1.3342	1.3039	1.3493	1.2902
C_{14}-C_{13}	1.5176	1.5192	1.5141	1.5212	1.5164	1.5176
O_{34}-C_{13}	1.2452	1.3798	1.3422	1.3713	1.3347	1.3866
O_{34}-H_{33}	- -	0.9708	0.9834	0.9686	0.9786	0.9712

The change of conformation from –*sc* of $C_{15}C_{14}C_{13}N_{12}$ is changed to +*sp* conformation and all other conformations are moderately changed. It is observed that the protonation at N_{12}-atom is shown +*ac* conformation. If the mono-protonated lactim form of benzylpenicillin (**4**) is formed by the addition of proton at N_7- atom of lactim tautomer of benzylpenicillin (**2**), with increasing bond lengths at C_{13}-N_{12}, C_{14}-C_{13} and C_9-N_7 and decreasing bond lengths at O_{30}-C_9, and O_{34}-C_{13}. The change of dihedral angle of $O_{10}C_8C_4C_3$, $C_{13}N_{12}C_{11}C_9$, $C_{14}C_{13}N_{12}C_{11}$, $O_{29}C_8C_4C_3$, $O_{30}C_9N_7C_4$, $H_{31}O_{10}C_8C_4$, and $O_{34}C_{13}N_{12}C_{11}$ are converted from +*ac* to +*sc*, -*sc* to –*ap*, -*ap* to +*ap*, -*sc* to –*ac*, -*sc* to –*sp*, +*ap* to –*ap*, and +*sp* to -*sp* conformations and all other conformations are unaltered. It is observed that the protonation at N_7-atom is shown +*ac* conformation. In the case of formation of di-protonated lactim form of benzylpenicillin (**5**), it is found that the dihedral angle of $O_{10}C_8C_4C_3$, $C_{13}N_{12}C_{11}C_9$, $C_{15}C_{14}C_{13}N_{12}$ and $O_{29}C_8C_4C_3$ are changed conformation, +*ac* to +*sc*, -sc to -ac, -*sc* to +*sp*, and -*sc* to -*ac* conformations respectively. It is also investigated that the protonation at N_7- atom and N_{12}-atom are shown respectively +*ac* and +*sc* conformations to form stable di-protonated lactim form of benzylpenicillin (**5**).

Table X: Dihedral angle (o) of benzyl penicillin (1) and its lactim form (2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculation.

Dihedral angle (^{o})	1		2		3		4		5		6	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-18.79	*-sp*	-12.39	*-sp*	-10.26	*-sp*	-7.18	*-sp*	-20.48	*-sp*	-12.36	*-sp*
$C_8C_4C_3S_2$	-137.46	*-ac*	+129.36	*+ac*	+123.42	*+ac*	+140.53	*+ac*	+145.17	*+ac*	+129.85	*+ap*
$O_{10}C_8C_4C_3$	+88.68	*+sc*	+98.40	*+ac*	+101.16	*+ac*	+73.43	*+sc*	+69.53	*+sc*	+95.24	*+ap*
$C_{13}N_{12}C_{11}C_9$	-127.62	*-ac*	-52.15	*-sc*	-63.79	*-sc*	-152.14	*-ap*	-101.58	*-ac*	-51.21	*-sc*
$C_{14}C_{13}N_{12}C_{11}$	-177.09	*-ap*	-176.35	*-ap*	-176.64	*-ap*	+177.21	*+ap*	-175.99	*-ap*	-176.74	*-ap*
$C_{15}C_{14}C_{13}N_{12}$	-26.76	*-sp*	-84.33	*-sc*	+20.44	*+sp*	-73.26	*-sc*	+12.01	*+sp*	-95.39	*-ac*
$O_{29}C_8C_4C_3$	-91.56	*-ac*	-81.92	*-sc*	-79.38	*-sc*	-107.89	*-ac*	-112.24	*-ac*	-83.52	*-sc*
$O_{30}C_9N_7C_4$	-52.24	*-sc*	-51.94	*-sc*	-51.74	*-sc*	-77.30	*-sp*	-67.23	*-sc*	-51.25	*-sc*
$H_{31}O_{10}C_8C_4$	+178.59	*+ap*	+179.04	*+ap*	+179.38	*+ap*	-179.96	*-ap*	+178.80	*+ap*	- -	- -
$H_{33}O_{34}C_{13}N_{12}$	- -	- -	+8.21	*+sp*	+7.93	*+sp*	+4.24	*+sp*	+0.42	*+sp*	+11.41	*+sp*
$O_{34}C_{13}N_{12}C_{11}$	+2.00	*+sp*	+1.40	*+sp*	+2.79	*+sp*	-4.05	*-sp*	+4.99	*+sp*	+0.98	*+sp*
$HN_{12}C_{11}C_9$	- -	- -	- -	- -	+115.77	*+ac*	- -	- -	+84.98	*+sc*	- -	- -
$HN_7C_4C_3$	- -	- -	- -	- -	- -	- -	+108.62	*+ac*	+114.31	*+ac*	- -	- -

* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs[11].

It can be concluded that the anion (**6**) is formed with the removal of a proton on O_{10}- atom of lactim tautomer of benzylpenicillin (**2**), and the change of conformation from *+ac* of $C_8C_4C_3S_2$ and $O_{10}C_8C_4C_3$ are changed to *+ap* conformation. Dihedral angle of $C_{15}C_{14}C_{13}N_{12}$ is changed the conformation from *-sc* to *–ac* to form stable anion (**6**) and rest of positions have moderate changes.

8.1. Computational study[34] on electronic structure of benzylpenicillin (1) and its enol tautomer (2) mono-protonated (3 & 4), di-protonated (5) and anion (6)

The optimized electronic structure of benzylpenicillin (**1**) and its enol tautomer (**2**) mono-protonated (**3** & **4**), di-protonated (**5**) and anion (**6**) are shown in Scheme- 4. In this context, the numbering of enol form of benzylpenicillin (**2**) is shown in Figure - 3. The heat of formation (ΔH_f^o), ionization potential (IP), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero atoms of the molecules (**1** to **6**) were calculated and presented in Table-XI. It is observed that the net charges on N_7- and N_{12}- atoms are -0.1588 and -0.2917 respectively in the case of benzylpenicillin-enol tautomer (**2**) and in the order of sequence of protonation $N_7 < N_{12}$.

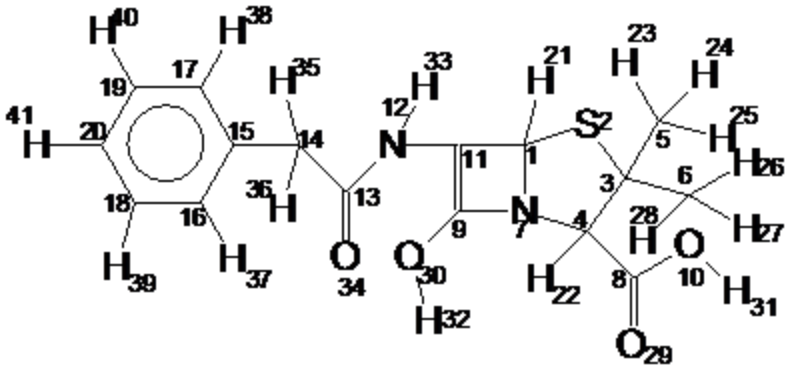


Figure - 3

The promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed in the case of molecules **1, 3, 4**, and **5**, due to the presence of same sign and other molecules undergo antara-facial path way us allowed due to the opposite sign.[30] The dipole moments of molecules are increasing in the order of molecules **2** < **1** < **4** < **3** < **5** < **6**. The electronegative hetero-atoms are observed to cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[31] (μ_{ind}) of molecules can be estimated with respect to benzylpenicillin enol form (**2**). It is found that the induction effect is increasing in the order of $\Delta\mu_{ind}$ (**1**) 3.12 D < $\Delta\mu_{ind}$ (**4**) 3.827 D < $\Delta\mu_{ind}$ (**3**) 3.954 D < $\Delta\mu_{ind}$ (**5**) 4.306 D < $\Delta\mu_{ind}$ (**5**) 14.204 D. According to the heat of formation (ΔH_f^o) data, it is observed that the stability of compounds have increased in the order of **5** < **3** < **4** < **2** < **1** < **6**. It is examined the protonation at N_{12}-atom or N_7-atom in the case of enol form of benzylpenicillin (**2**). It is estimated that the stability of mono-protonated enol form of benzylpenicillin **4** (ΔH_f^o, +83.1997 Kcal/mol) is more stable than **3** (ΔH_f^o, +93.7366 Kcal/mol). The enol form of di-protonated benzylpenicillin **(5)** is possible (with the heat of formation (ΔH_f^o) of +336.9712 Kcal/mol**)** from mono-protonated enol form of benzylpenicillins (**3** & **4**).

Table XI: Heat of formation (ΔH_f^o in kcal/mol), ionization potential (eV), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV), electron excitation energies (ΔE) (in eV) and the atomic charges on hetero-atoms of benzyl penicillin(1) and its enol form(2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculations.

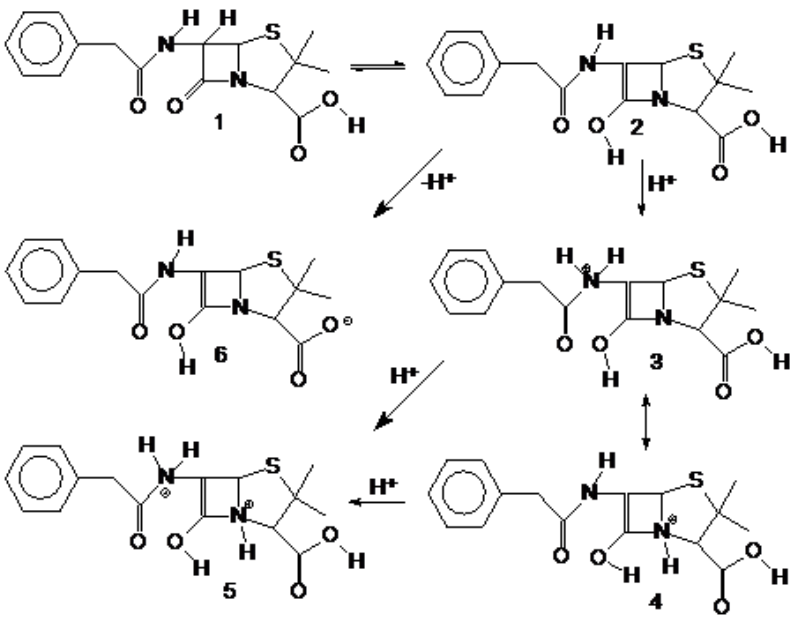
Parameters	1	2	3	4	5	6
ΔH_f^o (kcal/mol)	-94.5278	-70.6933	93.7366	83.1997	336.9712	-108.7441
Ionization potential (eV)	9.310	8.799	12.100	12.282	15.322	5.357
μ (Debye)	5.546	2.426	6.380	6.253	6.732	16.630
E_{HOMO} (eV)	-9.310	-8.799	-12.100	-12.282	-15.322	-5.357
E_{LUMO} (eV)	-0.062	+0.063	-3.983	-4.332	-8.454	+1.988
Electron excitation energies ($\Delta E = E_{LUMO} - E_{HOMO}$) (eV)	9.248	8.862	8.117	7.950	6.868	7.345
S_2 (atomic charge)	+0.0366	+0.0685	+0.1268	+0.2041	+0.2945	-0.0627
N_7 (atomic charge)	-0.2584	-0.1588	-0.1504	-0.0060	-0.0428	-0.1616
N_{12} (atomic charge)	-0.3614	-0.2917	+0.0283	-0.2851	-0.0324	-0.2570
O_{10} (atomic charge)	-0.3225	-0.3200	-0.3590	-0.3189	-0.3803	-0.5815
O_{29} (atomic charge)	-0.3282	-0.3540	-0.2949	-0.2744	-0.2139	-0.5366
O_{30} (atomic charge)	-0.2396	-0.2025	-0.1817	-0.1676	-0.1443	-0.2423
O_{34} (atomic charge)	-0.3570	-0.3412	-0.1575	-0.3381	-0.1606	-0.3668

The protonation of benzylpenicillin-enol form **(2)** at N_{12}- atom, negative atomic charges are changed on the other atoms of the molecule. The protonation at N_{12}-atom is spotted to generate mono-protonated form (**3**) by decreasing net atomic charges at N_7-, N_{12}-, O_{29}-, O_{30}-, and O_{34}- atoms and increasing at O_{10}- atom. It is detected that the protonation at N7- atom of enol form of benzylpenicillin (**2**) to generate mono-protonated form (**4**) with decreasing net atomic charges at N_7-, N_{12}-, O_{10}-, O_{29}-, O_{30}- and O_{34}-atoms. It is inspected that the di-protonated form (**5**) is perceived with decreasing the negative atomic charges at all hetero atoms except at O_{10}-atom. It is also examined that the removal of a proton from O_{10}-atom of enol form of benzylpenicillin(**2**) for the formation of Anion(6); then the net charges are increasing at N_7-, O_{10}-, O_{29}-, O_{30}- and O_{34}- and decreasing at N_{12}- atom. In order to investigate the equilibrium, the exact protonation centres of benzylpenicillin- enol form (**2**) is determined from the proton affinities (PA) for the different nitrogen atoms of the molecule have been calculated by means of AM1 method. The proton affinity (PA) values for the different nitrogen atoms of benzylpenicillin- enol form RH (**2**) were calculated by using the equation (2) and found to be 202.7701 kcal/mol and 213.307 kcal/mol respectively in the case of mono-protonated benzylpenicillins (**3** and **4**). Di-protonated form (**5**) was formed from

either of mono-protonated benzylpenicillins (**3** and **4**) respectively with PA 123.9654 kcal/mol and 113.4285 kcal/mol.

It is predicted that the proton affinity is in the order of N_{12} (202.7701 kcal/mol) < N_7 (213.307 kcal/mol) and mono-protonated benzylpenicillin (**4**) appears to be more stable. As per electron excitation energies (ΔE) (in eV), it is observed the reactivity is decreased in the order of **5** > **6** > **4** > **3** > **2** > **1**. It is confirmed that benzylpenicillin (**1**) is more stable than its enol-form (**2**).

8.2. The conformations of benzylpenicillin-enol form (2) and its mono-protonated (3 & 4), di-protonated (5) and anion (6)



Scheme - 4

The spatial arrangement of atoms in a molecule is investigated to study the conformations of benzylpenicillin (**1**), and its enol form of benzylpenicillin (**2**), mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) with a view to detect *anti*- or *syn*- conformation, according to the position of atoms. In this context, the changes in the parameters of bond lengths (Table-XII) dihedral angles (Table- XIII) of molecules (**1** to **6**) were observed and discussed. As per Scheme- 4, mono-protonated enol form of benzylpenicillin (**3**) is formed by the addition of proton at N_{12}-atom of enol tautomer of benzylpenicillin (**2**), with increasing bond lengths at C_{13}-N_{12}, H_{32}-O_{30} and C_{11}-C_9 and decreasing bond lengths at C_9-N_7, O_{30}-C_9, O_{34}-C_{13} and C_{14}-C_{13}. It is examined that the conformations of $C_{13}N_{12}C_{11}C_9$, $C_{14}C_{13}N_{12}C_{11}$, $C_{15}C_{14}C_{13}N_{12}$, $H_{31}O_{10}C_8C_4$, $H_{32}O_{30}C_9N_7$, and $H_{33}N_{12}C_{11}C_9$ are changed respectively from +*sc*

to +*sc*, -*ap* to +*ap*, -*sc* to +*sp*, -*ap* to +*sp*, +*sp* to –*sp*, and –*ac* to +*sc* conformations and all other conformations are moderately changed. It is observed that the protonation at N_{12}-atom in the case of $HN_{12}C_{11}C_9$ is shown +*ap* conformation. If the mono-protonated benzylpenicillin enol (**4**) is formed by the addition of proton at N_7- atom of benzylpenicillin enol tautomer (**2**), with increasing bond lengths at C_{13}-N_{12}, H_{32}-O_{30} and C_9-N_7 and decreasing bond lengths at C_{14}-C_{13} O_{30}-C_9, and O_{34}-C_{13}. It is examined that the dihedral angle of $O_{10}C_8C_4C_3$, $C_{13}N_{12}C_{11}C_9$, $C_{15}C_{14}C_{13}N_{12}$, $O_{29}C_8C_4C_3$, $H_{32}O_{30}C_9N_7$, and $H_{33}N_{12}C_{11}C_9$ are converted from -*ac* to -*ap*, +*sc* to +*sp*, -*sc* to +*sp*, +*sc* to +*sp*, +*sp to* +*ac*, and -*ac* to –*ap* conformations respectively and all other conformations are unaltered. It is observed that the protonation at N_7-atom is shown -*ap* conformation.

Table XII: Bond lengths of benzyl penicillin(1) and its enol form (2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculations.

Bond lengths (Å)	1	2	3	4	5	6
C_9-N_7	1.4512	1.4685	1.4550	1.5086	1.5036	1.4559
C_{11}-C_9	1.5689	1.3757	1.3852	1.3741	1.3731	1.3839
O_{30}-C_9	1.2176	1.3468	1.3295	1.3375	1.3220	1.3358
C_{13}-N_{12}	1.3873	1.3904	1.5265	1.4135	1.5568	1.3778
C_{14}-C_{13}	1.5176	1.5227	1.5054	1.5109	1.5011	1.5317
O_{34}-C_{13}	1.2452	1.2426	1.2181	1.2418	1.2135	1.2458
H_{32}-O_{30}	- -	0.9752	0.9813	0.9839	0.9866	0.9869

Table XIII: Dihedral angle (o) of benzyl penicillin (1) and its enol form (2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculations.

Dihedral angle (°)	1		2		3		4		5		6	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-18.79	-*sp*	-22.38	-*sp*	-20.29	-*sp*	-28.83	-*sp*	-26.12	-*sp*	-17.04	-*sp*
$C_8C_4C_3S_2$	-137.46	-*ac*	+162.73	+*ap*	+161.15	+*ap*	+164.58	+*ap*	+160.49	+*ap*	+158.36	+*ap*
$O_{10}C_8C_4C_3$	+88.68	+*sc*	-136.12	-*ac*	-106.94	-*ac*	-168.87	-*ap*	-146.07	-*ac*	-108.88	-*ac*
$C_{13}N_{12}C_{11}C_9$	-127.62	-*ac*	+59.03	+*sc*	-65.99	-*sc*	+5.53	+*sp*	-64.59	-*sc*	+75.48	+*sc*
$C_{14}C_{13}N_{12}C_{11}$	-177.09	-*ap*	-178.79	-*ap*	+178.55	+*ap*	-175.76	-*ap*	-179.29	-*ap*	-174.49	-*ap*
$C_{15}C_{14}C_{13}N_{12}$	-26.76	-*sp*	-39.91	-*sc*	+13.53	+*sp*	+14.84	+*sp*	-0.71	-*sp*	-54.87	-*sc*
$O_{29}C_8C_4C_3$	-91.56	-*ac*	+47.29	+*sc*	+72.79	+*sc*	+16.77	+*sp*	+38.79	+*sc*	+71.00	+*sc*
$O_{30}C_9N_7C_4$	-52.24	-*sc*	+67.28	+*sc*	+73.76	+*sc*	+60.61	+*sc*	+72.49	+*sc*	+70.42	+*sc*
$H_{31}O_{10}C_8C_4$	+178.59	+*ap*	-178.41	-*ap*	+178.29	+*sp*	-178.84	-*ap*	-171.32	-*ap*	- -	- -
$H_{32}O_{30}C_9N_7$	- -	- -	+26.05	+*sp*	-7.89	-*sp*	+123.88	+*ac*	-2.12	-*sp*	-22.80	-*sp*
$O_{34}C_{13}N_{12}C_{11}$	+2.00	+*sp*	-1.27	-*sp*	-0.59	-*sp*	+6.29	+*sp*	+0.99	+*sp*	+3.57	+*sp*
$H_{33}N_{12}C_{11}C_9$	+56.65	+*sc*	-126.67	-*ac*	+56.53	+*sc*	-168.98	-*ap*	+56.83	+*sc*	-103.92	-*ac*
$HN_7C_4C_3$	- -	- -	- -	- -	- -	- -	-155.64	-*ap*	-146.04	+*ac*	- -	- -
$HN_{12}C_{11}C_9$	- -	- -	- -	- -	+173.27	+*ap*	- -	- -	+173.60	+*ap*	- -	- -

*Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs.[11]

In the case of formation of di-protonated benzylpenicillin-enol (**5**), it is found that the dihedral angle of $C_{13}N_{12}C_{11}C_9$, $H_{32}O_{30}C_9N_7$, $O_{34}C_{13}N_{12}C_{11}$ and $H_{33}N_{12}C_{11}C_9$ are changed conformation, +*sc* to -*sc*, +*sp* to -*sp*, -*sp* to +*sp* and -*ac* to +s*c* conformations respectively. It is also investigated that the protonation at N_7- atom and N_{12}-atom are shown respectively -*ac* and +*ap* conformations to form stable di-protonated benzylpenicillin enol (**5**). It can be concluded that the anion (**6**) is formed with the removal of a proton on O_{10}- atom of benzylpenicillin enol tautomer (**2**), and the change of conformation from +*sp* of $H_{32}O_{30}C_9N_7$ and –sp of $O_{34}C_{13}N_{12}C_{11}$ are changed to -*sp* and +*sp* conformations respectively to form stable anion (**6**) and rest of positions have moderate changes.

9.1. Computational study[35] on electronic structure of benzylpenicillin (1) and its lactim-enol tautomer (2) mono-protonated (3 & 4), di-protonated (5) and anion (6)

The optimized electronic structure of benzylpenicillin (**1**) and its lactim-enol tautomer (**2**) mono-protonated (**3** & **4**), di-protonated (**5**) and anion (**6**) are shown in Scheme- 5. The calculated heats of formation (ΔH_f^o), ionization potential (IP), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero atoms of the molecules (**1** to **6**) are presented in Table-XIV. It is observed that the net charges on N_7- and N_{13}- atoms are -0.1606 and -0.1957 respectively in the case of benzylpenicillin lactim-enol tautomer (**2**) in the order of the sequence of protonation at $N_7 < N_{13}$.

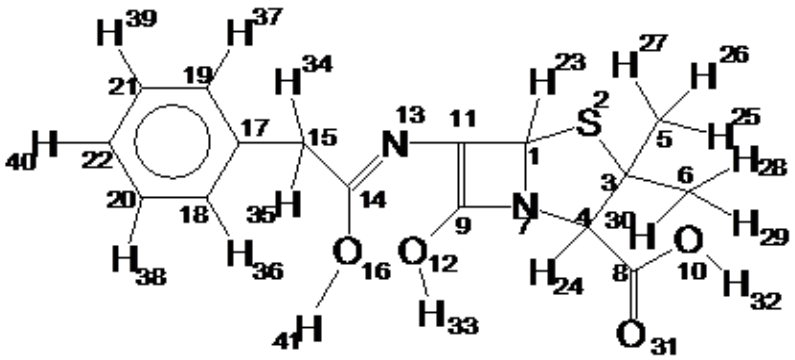


Figure - 4

It is investigated that the promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed in the case of molecules **1** to **5**. This is due to the presence of same sign of HOMO and LUMO energy levels. Anion (**6**) may undergo antara-facial path way. This is due to the opposite sign[30] of HOMO and LUMO energy levels. The dipole moment of molecules is increasing in the order of molecules **3** < **2**

< **5** < **1** < **4** < **6**. It is observed that the anion (**6**) shows higher dipole moment. The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[31] (μ_{ind}) of molecules is estimated with respect to benzylpenicillin lactim-enol form (**2**). It is found that the induction effect is increasing in the order of $\Delta\mu_{ind}$ (**3**) -0.604 D < $\Delta\mu_{ind}$ (**5**) 1.824 D < $\Delta\mu_{ind}$ (**1**) 2.538 D < $\Delta\mu_{ind}$ (**4**) 4.880 D < $\Delta\mu_{ind}$ (**6**) 10.855 D.

Table XIV: Heat of formation (ΔH_f^o in kcal/mol), ionization potential (eV), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV), electron excitation energies ($\Delta E = E_{LUMO} - E_{HOMO}$) (in eV) and the atomic charges on hetero-atoms of benzyl penicillin(1) and its lactim-enol form(2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculations.

Parameters	1	2	3	4	5	6
ΔH_f^o (kcal/mol)	-94.5278	-68.0049	+73.3233	+89.2562	+316.2056	-106.9222
Ionization potential (eV)	9.310	8.378	12.138	11.865	14.799	5.116
μ (Debye)	5.546	3.013	2.409	7.893	4.837	13.868
E_{HOMO} (eV)	-9.310	-8.379	-12.138	-11.865	-14.800	-5.116
E_{LUMO} (eV)	-0.062	-0.204	-4.760	-4.603	-8.728	+2.300
Electron excitation energies (eV)	9.248	8.175	7.378	7.262	6.072	7.416
S_2 (atomic charge)	+0.0366	+0.0974	+0.1277	+0.2640	+0.3047	-0.0263
N_7 (atomic charge)	-0.2584	-0.1606	-0.1438	-0.0208	-0.0381	-0.1626
N_{13} (atomic charge)	-0.3614	-0.1957	-0.0993	-0.2617	-0.2023	-0.1507
O_{10} (atomic charge)	-0.3225	-0.3050	-0.2782	-0.2781	-0.2492	-0.5831
O_{12} (atomic charge)	-0.2396	-0.2346	-0.2300	-0.1917	-0.2096	-0.2673
O_{16} (atomic charge)	-0.3570	-0.2875	-0.1884	-0.2395	-0.1464	-0.3103
O_{31} (atomic charge)	-0.3282	-0.3755	-0.3874	-0.3438	-0.3678	-0.5303

According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **5** < **4** < **3** < **2** < **1** < **6**. It can be predicted that the anion (**6**) is more stable and di-protonated form (**5**) is less stable. It is detected that the protonation may take place preferably at N_{13}-atom than N_7-atom in the case of lactim-enol form of benzylpenicillin (**2**). It is also confirmed that the stability of mono-protonated enol form of benzylpenicillin **4** (ΔH_f^o, +89.2562 Kcal/mol) is less stable than **3** (ΔH_f^o, +73.3233 Kcal/mol). The lactim-enol form of di-protonated benzylpenicillin **(5)** is possible (with the heat of formation (ΔH_f^o) of +316.2056 Kcal/mol**)** from mono-protonated lactim-enol form of benzylpenicillins (**3** & **4**). At the time of protonation at N_{13}-atom or N_7-atom, are also changed the net atomic charges on other atoms. The protonation at N_{13}-atom in the case of lactim-enol form of benzylpenicillin (**2**) to mono-protonated form (**3**) is considered by decreasing net atomic charges at N_7-, N_{13}-, O_{10}-,

O_{12}-, and O_{16}- atoms and increased at O_{31}- atom. The protonation site of lactim-enol form of benzylpenicillin (**2**) at N_7- atom to mono-protonated form (**4**) is considered by decreasing net atomic charges at N_7-, O_{10}-, O_{12}-, O_{16}- and O_{31}-atoms and increasing at N_{13}-atom. In the case of di-protonated form (**5**), it is observed that the negative atomic charges are decreased at all hetero atoms except at N_{13}-atom. It is investigated that the anion of lactim-enol form of benzylpenicillin (**6**) is formed by the removal of a proton on O_{10}-atom with increasing net charges at N_7-, O_{10}-, O_{12}-, O_{16}- and O_{31}-, and decreasing at N_{13}- atom. In accordance with the negative charge distribution on N-atoms, the proton affinity (PA) values for lactim-enol form of benzylpenicillin RH (**2**) were calculated by using the equation (2) and found to be 225.8718 kcal/mol and 209.9389 kcal/mol respectively in the case of mono-protonated benzylpenicillins (**3** and **4**). It is also inspected that the di-protonated form (**5**) was formed from either of mono-protonated benzylpenicillins (**3** and **4**) respectively with PA 124.3177 kcal/mol and 140.2506 kcal/mol. The proton affinity is viewed in the order of N_{13} (225.8718 kcal/mol) > N_7 (209.9389 kcal/mol) and mono-protonated benzylpenicillin lactim-enol form (**4**) appears to be more stable. As per electron excitation energies (ΔE) (in eV), it is observed the reactivity is increased in the order of **1** < **2** < **6** < **3** < **4** < **5**. It is confirmed that benzylpenicillin (**1**) is more stable than its lactim-enol form (**2**).

Table XV: Bond lengths of benzyl penicillin(1) and its lactim-enol form(2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculations.

Bond lengths (Å)	1	2	3	4	5	6
C_9-N_7	1.4512	1.4608	1.4534	1.5088	1.5089	1.4486
C_{11}-C_9	1.5689	1.3831	1.3866	1.3789	1.3709	1.3922
O_{12}-C_9	1.2176	1.3486	1.3779	1.3378	1.3312	1.3377
H_{33}-O_{12}	- -	0.9751	0.9839	0.9827	0.9907	0.9888
C_{14}-N_{13}	1.3873	1.3017	1.3337	1.3200	1.3564	1.2984
N_{13}-C_{11}	1.4092	1.3601	1.3778	1.3446	1.3790	1.3586
O_{16}-C_{14}	1.2452	1.3789	1.3432	1.3589	1.3283	1.3873
C_{15}-C_{14}	1.5177	1.5171	1.5202	1.5206	1.5272	1.5141
H_{41}-O_{16}	- -	0.9690	0.9786	0.9746	0.9828	0.9689
O_{10}-C_8	1.3651	1.3617	1.3517	1.3523	1.3419	1.2573
O_{31}-C_8	1.2294	1.2351	1.2366	1.2316	1.2341	1.2645
H_{32}-O_{10}	0.9716	0.9731	0.9839	0.9776	0.9824	- -
H-N_{13}	- -	- -	1.0137	- -	1.0135	- -
H-N_7	- -	- -	- -	1.0197	1.0241	- -

From Table-XV, it is confirmed that benzylpenicillin (**1**) would undergo lactim-enol tautomerism of benzylpenicillin (**2**) with increasing bond lengths of O_{12}-C_9 (1.3486 Å), O_{16}-C_{14} (1.3789 Å) and decreasing bond lengths of C_{11}-C_9 (1.3831 Å), C_{14}-N_{13} (1.3017 Å), with

the formation of H_{13}-O_{12} (0.9751 Å) and H_{41}-O_{16} (0.9690 Å) bonds. It is observed the change of conformation from *–ac* of $C_8C_4C_3S_2$, $C_{14}N_{13}C_{11}C_9$ and $N_{13}C_{11}C_9N_7$, are changed respectively to *+ap*, *-sp* and *-ap* conformations. It is noticed that the dihedral angle of $O_{16}C_{14}N_{13}C_{11}$ and $C_{17}C_{15}C_{14}N_{13}$ are changed respectively *+sp* to *–sp* and *-sp* to *-sc* conformations. It is detected that the lactim-enol form of benzylpenicillin (**2**) is formed with ***–sp*** and ***+sp*** conformations respectively in the case of dihedral angle of $H_{33}O_{12}C_9N_7$ and $H_{41}O_{16}C_{14}N_{13}$.

9.2. The Computational study[35] on conformations of lactim-enol form of benzylpenicillin (2) and its mono-protonated (3 & 4), di-protonated (5) and anion (6)

The spatial arrangement of atoms in a molecule is examined to study the conformations of benzylpenicillin (**1**), and its lactim-enol form of benzylpenicillin (**2**), mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) with a view to investigate *anti*- or *syn*-conformation. In this context, the change in energy content of the protonation may depend on the changes in the parameters of dihedral angles (Table- XVI) of molecules (**1** to **6**).

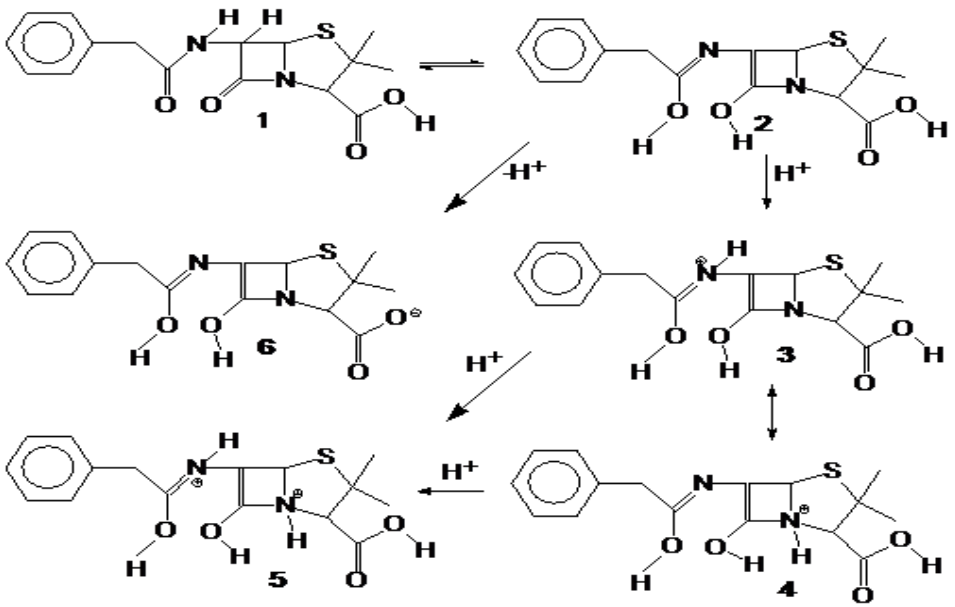
Table XVI: Dihedral angle (°) of benzyl penicillin (1) and its lactim-enol form (2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculations.

Dihedral angle (°)	1		2		3		4		5		6	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-18.79	***-sp***	-18.81	***-sp***	-17.94	***-sp***	-24.18	***-sp***	-28.40	***-sp***	-18.51	***-sp***
$C_8C_4C_3S_2$	-137.46	***-ac***	+157.03	***+ap***	+155.52	***+ap***	+154.13	***+ap***	+158.47	***+ap***	+159.19	***+ap***
$O_{10}C_8C_4C_3$	+88.68	***+sc***	+64.85	***+sc***	+68.44	***+sc***	+76.94	***+sc***	+73.46	***+sc***	-105.15	***-ac***
$H_{32}O_{10}C_8C_4$	+178.60	***+ap***	+179.00	***+ap***	+178.75	***+ap***	-179.42	***-ap***	+178.94	***+ap***	- -	-
$O_{31}C_8C_4C_3$	-91.56	***-ac***	-117.61	***-ac***	-122.64	***-ac***	-103.27	***-ac***	-107.48	***-ac***	+74.41	***+sc***
$N_{13}C_{11}C_9N_7$	-125.24	***-ac***	-168.66	***-ap***	-175.38	***-ap***	+177.65	***+ap***	+174.99	***+ap***	-168.53	***-ap***
$H_{33}O_{12}C_9N_7$	- -	- -	-0.29	***-sp***	-13.09	***-sp***	-88.18	***-sc***	-66.22	***-sc***	-24.24	***-sp***
$C_{14}N_{13}C_{11}C_9$	-127.63	***-ac***	-12.94	***-sp***	+5.38	***+sp***	+12.03	***+sp***	+29.61	***+sp***	-12.56	***-sp***
$O_{16}C_{14}N_{13}C_{11}$	+2.00	***+sp***	-1.08	***-sp***	+0.18	***+sp***	+0.42	***+sp***	-2.74	***-sp***	-0.29	***-sp***
$H_{41}O_{16}C_{14}N_{13}$	- -	- -	+4.59	***+sp***	+3.11	***+sp***	+6.93	***+sp***	+5.91	***+sp***	+2.78	***+sp***
$C_{15}C_{14}N_{13}C_{11}$	-177.09	***-ap***	-179.34	***-ap***	-117.64	***-ac***	-179.02	***-ap***	+178.52	***+ap***	-178.61	***-ap***
$C_{17}C_{15}C_{14}N_{13}$	-26.76	***-sp***	-76.38	***-sc***	-49.37	***-sc***	-90.70	***-ac***	-100.29	***-ac***	-76.68	***-sc***
$HN_7C_4C_3$	- -	- -	- -	- -	- -	- -	-144.25	***-ac***	-147.93	***-ac***	- -	- -
$HN_{13}C_{11}C_9$	- -	- -	- -	- -	-176.22	***-ap***	- -	- -	-153.81	***-ap***	- -	- -

* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs[11].

As per Scheme- 5, mono-protonated lactim-enol form of benzylpenicillin (**3**) is formed by the addition of proton at N_{13}-atom of benzylpenicillin lactim-enol tautomer (**2**). At this time, it is perceived that the increasing bond lengths at O_{12}-C_9, H_{33}-O_{12}, C_{14}-N_{13}, N_{13}-C_{11}, H_{41}-O_{16}, H_{32}-O_{10} and decreasing bond lengths at O_{16}-C_{14}, O_{10}-C_8. The conformations of $C_{14}N_{13}C_{11}C_9$, and

$O_{16}C_{14}N_{13}C_{11}$ are changed from -*sp* to +*sp*, the conformation of $C_{15}C_{14}N_{13}C_{11}$ is changed from -*ap* to -*ac* and all other conformations are moderately changed. It is observed that the protonation at N_{13}-atom in the case of $HN_{13}C_{11}C_9$ is shown -*ap* conformation. It is examined that the mono-protonated benzylpenicillin lactim-enol (**4**) is formed by the addition of proton at N_7- atom of benzylpenicillin lactim-enol tautomer (**2**). In this context, it is detected that the increasing bond lengths at C_{14}-N_{13}, H_{33}-O_{12}, H_{41}-O_{16}, C_9-N_7 and decreasing bond lengths at C_{11}-C_9, O_{12}-C_9, N_{13}-C_{11}, O_{16}-C_{14}, O_{10}-C_8. The conformations of $C_{14}N_{13}C_{11}C_9$, and $O_{16}C_{14}N_{13}C_{11}$ are changed from -*sp* to +*sp*, the conformations of $H_{32}O_{10}C_8C_4$, $N_{13}C_{11}C_9N_7$, $H_{33}O_{12}C_9N_7$, and $C_{17}C_{15}C_{14}N_{13}$ are changed from +*ap* to –*ap*, -*ap* to +*ap*, -*sp* to -*sc* and –*sc* to –*ac* respectively and all other conformations are moderately changed. It is inspected that the protonation at N_7-atom in the case of $HN_7C_4C_3$ is shown –*ac* conformation. The formation of di-protonated benzylpenicillin lactim-enol (**5**) is observed by the addition of protons at N_7- and N_{13}-atoms of benzylpenicillin lactim-enol tautomer (**2**). It is evidenced with increasing bond lengths at C_{14}-N_{13}, H_{33}-O_{12}, H_{41}-O_{16}, C_9-N_7, N_{13}-C_{11}, C_{15}-C_{14}, H_{32}-O_{10} and decreasing bond lengths at C_{11}-C_9, O_{12}-C_9, O_{16}-C_{14}, O_{10}-C_8. It is scrutinized that the dihedral angle of $N_{13}C_{11}C_9N_7$, $H_{33}O_{12}C_9N_7$, $C_{14}N_{13}C_{11}C_9$, $C_{15}C_{14}N_{13}C_{11}$ and $C_{17}C_{15}C_{14}N_{13}$ are changed conformation, -*ap* to -*ap*, -*sp* to -*sc*, -*sp* to +*sp*, -*ap* to +*ap* and -*sc* to -*ac* conformations respectively and all other conformations are unaltered.



Scheme - 5

It is also investigated that the protonation at N_7- atom and N_{13}-atom are shown respectively -*ac* and -*ap* conformations to form stable di-protonated lactim-enol benzylpenicillin (**5**). It can

be concluded that the anion (**6**) is formed with the removal of a proton on O_{10}- atom of benzylpenicillin lactim-enol tautomer (**2**). At this movement, it is perceived the increasing bond lengths at C_{11}-C_9, H_{33}-O_{12}, O_{16}-C_{14}, O_{31}-C_8 and decreasing bond lengths at C_9-N_7, O_{12}-C_9, O_{10}-C_8. It is also observed that the conformations of $O_{10}C_8C_4C_3$ and $O_{31}C_8C_4C_3$ are changed from +*sc* to –*ac* and –*ac* to +*sc* respectively to form stable anion (**6**) and rest of positions have moderate changes.

This review is the continuation of previous reviews on tutomerism[36] and protonation studies.[37]

10. CONCLUSIONS

Benzylpenicillin is one of the most widely used antibiotics in animals and humans, and readily absorbed into the blood stream with partially bound to plasma proteins. The main cause of penicillin deterioration is the reactivity of the strained β-lactam ring and the nature of a complex series of reactions leading to a variety of inactive degradation products. Austin Model-1 (AM1) is one of the semi-empirical method, which includes experimental parameters and extensive simplification of the Schrodinger's equation (HΨ=EΨ) to optimize molecules in gas phase. The various properties of molecules are to be predicted like electronic properties, conformational changes, stability, reactivity, pharmacological action and exact position of protonation for solving chemical problems.

1. The spatial arrangement of atoms in the conformations of benzylpenicillin (**1**), and its enol form (**2**), lactim form (**3)** and lactim-enol form (**4**) are existed in *anti*- or *syn*-conformations. The tautomeric equilibrium is increased in the order of $\log K_{T3} < \log K_{T2} < \log K_{T4} < \log K_{T1} < \log K_{T5}$, at the time of tautomeric conversion of **2** ↔ **4, 1** ↔ **3, 3** ↔ **4, 1** ↔ **2** and **1** ↔ **4** respectively. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **4 < 2 < 3 < 1**. Ionization potential (IP) is increased in the order of **4 < 2 < 3 < 1**. The dipole moment is increased in the order of molecules **3 < 2 < 4 < 1**. As per electron excitation energies (ΔE) (in eV), a large gap implies high stability and small gap implies low stability. The reactivity is decreased in the order of **4 > 2 > 3 > 1**.
2. Benzylpenicillin (**1**), and its mono-protonated cations (**2 & 3**), di-protonated cation (**4**) and anion (**5**) is existed in *anti*- or *syn*- conformation. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **4 < 2 < 3 < 1 < 5**. The ionization potential values are increased in the order of **5 < 1 < 3 < 2 < 4**. The dipole moment is

increased in the order of molecules **4** < **3** < **1** < **2** < **5**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **5** > **4** > **3** > **2** > **1**.

3. Benzylpenicillin (**1**) and its lactim tautomer (**2**) mono-protonated (**3** & **4**), di-protonated (**5**) and anion (**6**) are existed in *anti*- or *syn*- conformation. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **5** < **4** < **3** < **2** < **1** < **6**. The ionization potential values are decreased in the order of **5** > **3** > **4** > **1** > **2** > **6**. The dipole moment is increased in the order of **2** < **1** < **4** < **3** < **5** < **6**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **5** > **6** > **4** > **3** > **2** > **1**.
4. Benzylpenicillin (**1**) and its enol-form (**2**), mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) are existed in *anti*- or *syn*- conformation. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **5** < **3** < **4** < **2** < **1** < **6**. Ionization potential (IP) is increased in the order of **6** < **2** < **1** < **3** < **4** < **5**. The dipole moment is increased in the order of **2** < **1** < **4** < **3** < **5** < **6**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **5** > **6** > **4** > **3** > **2** > **1**.
5. Benzylpenicillin (**1**) and its lactim-enol form (**2**), mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) are formed in *anti*- or *syn*- conformation. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **5** < **4** < **3** < **2** < **1** < **6**. Ionization potential (IP) is increased in the order of **6** < **2** < **1** < **4** < **3** < **5**. The dipole moment is increased in the order of molecules **3** < **2** < **5** < **1** < **4** < **6**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **5** > **4** > **3** > **6** > **2** > **1**.

The utility of these theoretical predictions is essential for the penetration through the porin channels of cell membrane, biochemical mechanism to prevent cell wall synthesis and binding to plasma protein.

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