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

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ABSTRACT

The optimized electronic structure of phenethicillin tautomers and its mono-protonated, di-protonated and anion in the gas phase by semi-mpirical molecular orbital AM1 method have been reported. In this review, the protonation of phenethicillin 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, phenethicillin, induction effect, frontier molecular orbital.

1. INTRODUCTION

Phenethicillin (Broxil) is one of the penicillin derivatives and studied extensively due to their favourable absorption patterns and reduced undesirable side effects.[1] It is less active in-vitro against streptococci and pneumococci, but in-vivo this is partly offset by the higher blood levels in chemotherapy of bacterial infections.[2] Enzymatic splitting of natural penicillins and isolation of the important intermediate, 6-aminopenicillanic acid was led the preparation of several semi-synthetic penicillins.[3] Phenethicillin is widely used in the treatment of gonorrhoea and the chemotherapy of bacterial infections compared with other semi-synthetic penicillin derivatives.[4] Phenethicillin has been used most widely against gram-positive bacteria and readily absorbed into the blood stream where it is partially bound to plasma proteins in both animals and humans.[5] It has a high order of selective toxicity to micro-rganisms which are pathogenic to human beings without obvious side effects.[6] In this review, the study of phenethicillin tautomeres,[7] and its protonated forms and anion have

been optimized,[8] with a view to investigate its polarity and reactivity, which are an advantage for the penetration through the porin channels of cell membrane.[9] In this context, the numbering of phenethicillin **(1)** is shown in Figure - 1.

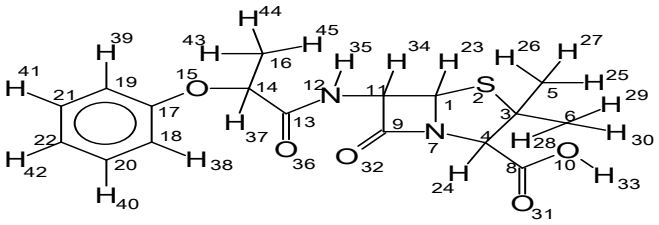


Figure - 1

2. Importance of computational method

Austin Model-1 (AM1) is one of the semi-empirical quantum calculations based on the neglect of differential diatomic overlap integral approximation,[10] which includes experimental parameters and extensive simplification of the Schrodinger's equation (HΨ=EΨ) to optimize molecules in gas phase usually considering an isolated molecule surrounded by vacuum has been evaluated. The initial molecular geometry was adopted as Pople's standard data,[11] and subsequently fully optimized using an energy gradient method. The conformations were designated by Klyne-Prelog terms,[12] using *s* = syn, *a* = anti, *p* = peri-planar (0 ± 30^0 & 180 ± 30^0) and all other angles *c* = clinal. The various properties of molecules are to be predicted like electronic properties, conformational changes, stability, reactivity, pharmacological action and exact position of protonation centre in the molecule for solving chemical problems,. In this way quantum chemistry simulates chemical structure and reactions numerically and allows studying chemical phenomena by running calculations on computer rather than by examining reactions experimentally. Theoretical investigations on phenethicillin tautomerism,[7,13] has been fascinated much to carry out the present review on phenethicillin tautomers.

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

Rajeev Sing et al reported,[14] 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. They are compared the calculated bond lengths and angles for the S-benzyldithiocarbazate with those of experimentally available x-ray diffraction data. The calculated bond lengths are in good agreement with experimental values. The most suitable method was found by plotting the experimental values versus calculated values and the

obtained correlation coefficients were analysed and found that correlation coefficients (CC) are not equal for different methods. For bond length, the correlation coefficient obtained for AM1 and PM3 are most satisfactory correlation (CC=0.966) between experimental and calculated bond lengths. 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,[15] carried out computational study by AM1 semi-empirical method of isomer structures of four calix[4]resorcinarenes functionalized with organic phosphorus groups. The chemical systems based on calix[4]resorcinarenes functionalized with organic- hosphorus 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,[16] have studied the effect of solvents on the formation ability of ligand to form metal complexes. M. J. S. Dewar et al,[10] 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[17] 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[18] 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.[19] 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,[20] 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- mpirical 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,[21] 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,[22] 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,[23] reported AM1-type semi- mpirical 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,[24] 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,[25] 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,[26] 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.

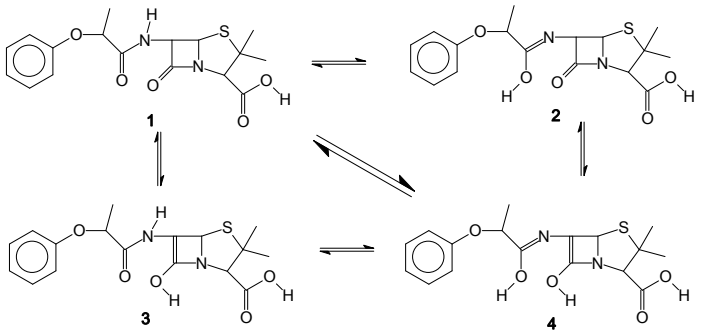
Peter et al,[27] used the semi-empirical AM1 SCF-MO method to study the benzyne mechanism for aromatic nucleophilic substitution of various *m*-substituted Chloro-benzenes would induce the formation of 2, 3-arynes through their electron-withdrawing resonance or inductive effects. The geometry and electronic structure of the 2, 3- and 3, 4- rynes 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,[28] 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 tautomerism in phenethicillin

At the time of tautomerism,[7] equilibrium is established in the shifting of protons in phenethicillin (**1**) as per Scheme-1. Lactam-lactim tautomerism **(1 ↔ 2)** & **(3 ↔ 4)** of phenethicillin involves the shifting of hydrogen atom from nitrogen atom of lactam (-HN-C=O) group to the oxygen atom in the formation of lactim (-N=C-O-H) group. Keto-enol tautomerism **(1 ↔ 3)** & **(2 ↔ 4)** of phenethicillin involves the shifting of hydrogen atom from α-carbon atom of keto (-HC-C=O) group to the oxygen atom in the formation of enol

(-C=C-O-H) group and both shifts (**1** ↔ **4)** involve in the formation of lactim-enol tautomer. As per electron excitation energies (ΔE) (in eV), the reactivity of phenethicillin tautomers is decreased in the order of **4** > **3** > **2** > **1**. This tautomerism is involved by

a) The shifting of H_{35}-proton and H_{34}-proton of phenethicillin (**1**) to respective O_{36}-atom and O_{32}-atom for the formation of respective lactim-form (**2**) and enol-form (**3**).
b) The simultaneous shifting of H_{35}-proton and H_{34}-proton of phenethicillin (**1**) to respective O_{36}-atom and O_{32}-atom for the formation of lactim-enol form (**4**) of phenethicillin.



Scheme -1: Tautomerism in Phenethicillin
1 - 2 & 3 - 4: lactam - lactim tautomerism.
1 - 3 & 2 - 4: keto - enol tautomerism.
1 - 4 : lactam - lactim & keto - enol tautomerism.

The tautomeric equilibrium constants $\log K_T$ was calculated,[29] according to the equation (1) from AM1 calculated heat of formation:

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

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 the tautomeric species participating in this equilibrium. R is the gas constant and T is the absolute temperature.

5. Importance of Protonation or Proton affinity (PA) in phenethicillin

The proton affinity (PA),[30] values were calculated by using the equation (2) from the heat of formation (ΔH_f^{o}) phenethicillin tautomers and their protonated forms.

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

Where PA is the proton affinity, $\Delta H_f^{o}(B)$ is the heat of formation for phenethicillin, $\Delta H_f^{o}(BH^+)$ is the heat of formation for the cation, and $\Delta H_f^{o}(H^+)$ is heat of formation for the

proton (367.2 kcal/mol). From this, the mechanism of protonation has been studied by comparison of the relative stabilities of cations using semi-empirical molecular orbital AM1 method.

5.1. Computational study on Electronic structure of phenethicillin tautomers (1 to 4)

The optimized electronic structure of phenethicillin (**1**) and its tautomers; lactim-form (**2**) enol-form (**3)** and lactim-enol form (**4**) are shown in Scheme-1 and the numbering of phenethicillin (**1)** (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 **4**) are presented in Table-I. The net charges on N_7- and N_{12}- atoms are -0.2402 and -0.3507 respectively atoms in the order of N_7 < N_{12} in phenethicillin (**1**). From the equation (1), $logK_T$ values and the change of net charges were calculated and incorporated in Table - III. The tautomeric equilibrium is increased in the order of $logK_{T4}$ < $logK_{T3}$ < $logK_{T1}$ < $logK_{T2}$ < $logK_{T5}$, at the time of tautomeric conversion of **3** ↔ **4, 2** ↔ **4, 1** ↔ **2, 1** ↔ **3,** and **1** ↔ **4** respectively. The net charges are increased at O_{31}- for the conversion of **1** ↔ **2,** O_{10}-, O_{31}- for **1** ↔ **3,** N_{12}-, O_{10}-, O_{36}- for **2** ↔ **4,** N_{17}-, O_{15}- for **3** ↔ **4,** O_{10}- for **1** ↔ **4** and decreased at all other hetero-atoms. The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **2** 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 **2** and **4**, due to the presence of same sign and other molecules undergo antara- facial path way is allowed due to the opposite sign.[31] The dipole moment is increasing in the order of molecules **4** < **2** < **3** < **1**. Phenethicillin (**1**) 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[32] (μ_{ind}) of molecules is in the order of $\Delta\mu_{ind}$ (**2**) 0.2311 D < $\Delta\mu_{ind}$ (**3**) 0.6352 D < $\Delta\mu_{ind}$ (**1**) 1.0129 D. According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **4** < **3** < **2** < **1**. From the Table-II, Table-III and Scheme - 1, phenethicillin (**1**) had undergone lactam-lactim tautomerism for the formation of lactim form of phenethicillin (**2**) with increasing bond length of O_{36}-C_{13} (1.3802 Å) and decreasing bond length of C_{13}-N_{12} (1.2934 Å) with the formation of single bond length of H-O_{36} (0.9699 Å). In the case of keto-enol tautomerism, phenethicillin-enol (**3**) is formed with increasing bond length of O_{32}-C_9 (1.3535 Å) and decreasing bond length at C_{11}-C_9 (1.3734 Å) with the formation of single bond length of H-O_{32} (0.9749 Å). But the phenethicillin lactim- nol (**4**) is

produced with increasing bond lengths of O_{32}-C_9 (1.3479 Å), O_{36}-C_{13} (1.3772 Å) and decreasing bonds of C_{11}-C_9 (1.3799 Å), C_{13}-N_{12} (1.2992 Å) with the formation of single bonds of H-O_{32} (0.9745 Å), H-O_{36} (0.9772 Å).

5.2. Computational study on the conformations of phenethicillin tautomers (1 to 4)

The spatial arrangement of atoms in a molecule is considered to study the conformations of phenethicillin (**1**), and its lactim form (**2**), enol form (**3)** and lactim-enol form (**4**) of phenethicillin with a view to investigate in *anti*- or *syn*- conformations, according to the position of atoms. In this context, the change in energy content of tautomerism may depend on the changes in the parameters of dihedral angles (Table-IV) of molecules (**1** to **4**). As per Scheme - 1, the shifting of H_{35}- atom from N_{12}- atom of lactam (-HN-C=O) group to the O_{36}-atom in the same molecule to form lactim (-N=C-O-H) group in the case of phenethicillin lactim form (**2**). The conformations of $C_{13}N_{12}C_{11}C_9$, $O_{15}C_{14}C_{13}N_{12}$, $C_{16}C_{14}C_{13}N_{12}$, $C_{17}O_{15}C_{14}C_{13}$, $H_{33}O_{10}C_8C_4$, and $O_{36}C_{13}N_{12}C_{11}$ are changed respectively from *-ac* to *+ap*, *+sc* to *+ac*, *-sc* to *+sp*, *+ac* to *+sc*, *+ap* to *–ap*, and *+sp* to *-sp* conformations and all other conformations are moderately changed. It is observed that the shifting of proton from N_{12}-atom to O_{36}-atom in the formation of $HO_{36}C_{13}N_{12}$ is shown *-sp* conformation. Enol form of phenethicillin (**3**) is created with the shifting of H_{34}-atom from α-carbon atom (C_{11}-atom) of keto (-HC-C=O) group to the O_{32}-atom in the same molecule to form enol (-C=C-O-H) group in phenethicillin (**1**). At the time of tautomeric change, the conformation from *–ap* of $O_{10}C_8C_4C_3$, *–ac* of $C_{13}N_{12}C_{11}C_9$, *+ap* of $C_{14}C_{13}N_{12}C_{11}$, *+sc* of $O_{15}C_{14}C_{13}N_{12}$, *-sc* of $C_{16}C_{14}C_{13}N_{12}$, *+sp* of $O_{31}C_8C_4C_3$, *+ap* of $H_{33}O_{10}C_8C_4$ and *+sc* of $H_{35}N_{12}C_{11}C_9$, are observed respectively to *–ac*, *+ac*, *-ap*, *+ac*, *-sp*, *+sc*, *-ap* and *-sp* conformations. It is investigated that the shifting of proton from C_{11}-atom to O_{32}-atom in the case of $HO_{32}C_9N_7$ is shown *+sp* conformation and all other conformations are more or less changed. The shifting of H_{35}- atom from N_{12}- atom and H_{34}-atom from C_{11}-atom of phenethicillin (**1**) simultaneously to respective O_{36}- atom and O_{32}- atom is involved for the formation of lactim-enol form of phenethicillin (**4**) with formation of ***–sp*** and ***+sp*** conformations in the case of $HO_{36}C_{13}N_{12}$ and $HO_{32}C_9N_7$ respectively. The change of dihedral angle of $O_{10}C_8C_4C_3$, $O_{15}C_{14}C_{13}N_{12}$, $C_{16}C_{14}C_{13}N_{12}$, $O_{31}C_8C_4C_3$ and $H_{33}O_{10}C_8C_4$ are converted from *-ap* to *-ac*, *+sc* to *+ac*, *-sc* to *+sp*, *+sp* to *+sc* and *+ap* to *–ap* conformations respectively and rest of positions have moderate changes.

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 phenethicillin(1) and its tautomers lactim (2), enol (3) and lactim- enol (4) forms from AM1 calculations.

Parameters	1	2	3	4
ΔH_f^o (kcal/mol)	-125.9658	-109.6539	-100.8945	-94.1242
Ionization potential (eV)	9.1224	9.1089	8.4589	8.6172
μ (Debye)	3.109	2.327	2.731	2.096
E_{HOMO} (eV)	-9.122	-9.109	-8.459	-8.617
E_{LUMO} (eV)	+0.099	-0.081	+0.083	-0.212
Electron excitation energies (eV)	9.221	9.028	8.542	8.405
S_2 (atomic charge)	+0.0526	+0.0281	+0.1012	+0.0578
N_7	-0.2402	-0.2249	-0.1449	-0.1624
N_{12}	-0.3507	-0.2517	-0.2760	-0.1877
O_{10}	-0.2865	-0.2807	-0.3232	-0.3227
O_{15}	-0.2261	-0.2020	-0.2014	-0.2096
O_{31}	-0.3519	-0.3561	-0.3535	-0.3511
O_{32}	-0.2363	-0.2280	-0.2243	-0.2000
O_{36}	-0.3522	-0.2751	-0.3510	-0.2945

Bond lengths of phenethicillin(**1**) and its tautomers lactim (**2**), enol (**3**) and lactim- enol (**4**) forms from AM1 calculations.

Table –II: Bond lengths of phenethicillin(1) and its tautomers lactim (2), enol (3) and lactim- enol (4) forms from AM1 calculations.

Bond lengths (A^0)	1	2	3	4
C_9-N_7	1.4491	1.4505	1.4617	1.4635
C_{11}-C_9	1.5696	1.5621	1.3744	1.3799
N_{12}-C_{11}	1.4125	1.4176	1.3727	1.3717
C_{13}-N_{12}	1.3831	1.2934	1.3862	1.2992
O_{32}-C_9	1.2176	1.2164	1.3535	1.3471
O_{36}-C_{13}	1.2443	1.3802	1.2450	1.3772
H-O_{32}	- -	- -	0.9741	0.9745
H-O_{36}	- -	0.9699	- -	0.9772
H-C_{11}	1.1257	1.1264	- -	- -

Tautomeric equilibrium in Phenethicillin (**1**) with its tautomers lactim (**2**), enol (**3**) and lactim- enol (**4**) forms from AM1 calculations.

Table –III: Tautomeric equilibrium in Phenethicillin (1) with its tautomers lactim (2), enol (3) and lactim- enol (4) forms from AM1 calculations.

Equilibrium	$LogK_T$	$LogK_T$ - Values	Net charges on Hetero-atoms	
			Increasing	Decreasing
1 ↔ 2	$LogK_{T1}$	11.9558	O_{31}	N_7, N_{12}, O_{10}, O_{15}, O_{32}, O_{36}
1 ↔ 3	$LogK_{T2}$	18.3760	O_{10}, O_{31}	N_7, N_{12}, O_{15}, O_{32}, O_{36}
2 ↔ 4	$LogK_{T3}$	11.3824	N_{12}, O_{10}, O_{36}	N_7, O_{15}, O_{31}, O_{32}
3 ↔ 4	$LogK_{T4}$	4.9623	N_7, O_{15}	N_{12}, O_{10}, O_{31}, O_{32}, O_{36}
1 ↔ 4	$LogK_{T5}$	23.3380	O_{10}	N_7, N_{12}, O_{15}, O_{31}, O_{32}, O_{36}

Table – IV: Dihedral angle (°) of phenethicillin (1) and its tautomeric forms (2 to 4), from AM1 calculations.

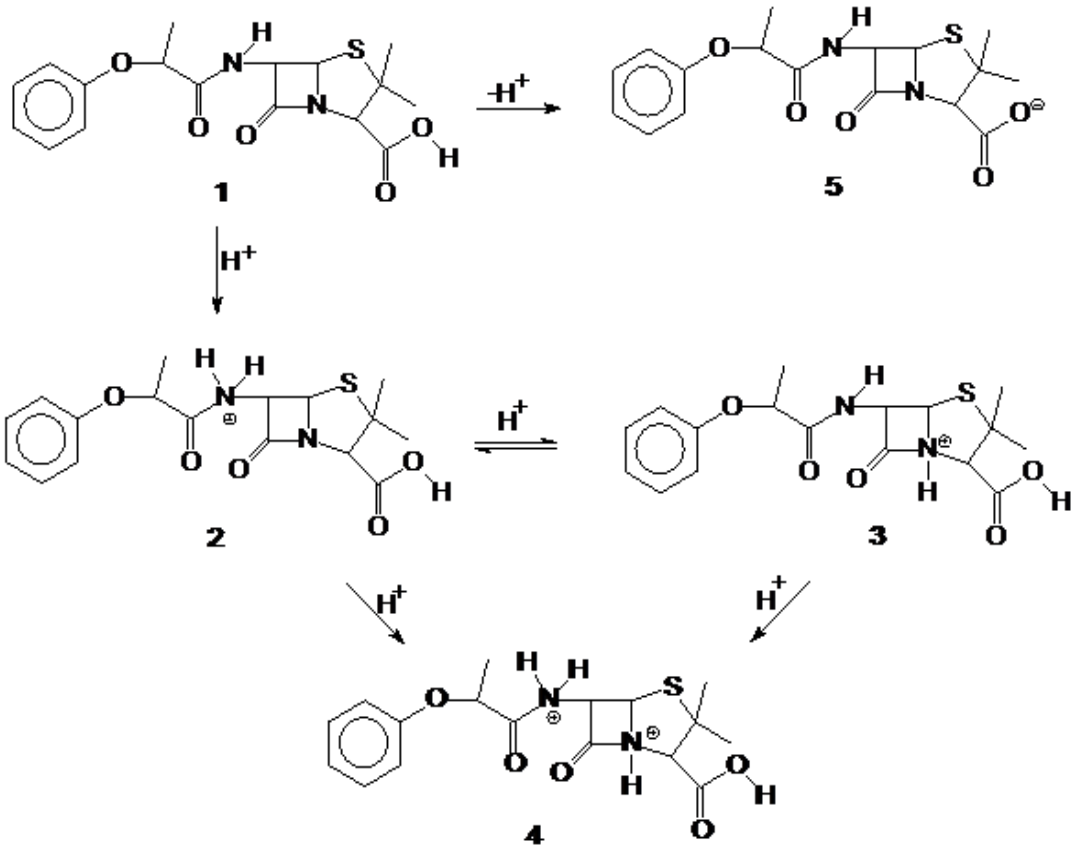
Dihedral angle (°)	1		2		3		4	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_8C_4C_3S_2$	+163.25	+*ap*	+163.15	+*ap*	+164.49	+*ap*	+162.70	+*ap*
$O_{10}C_8C_4C_3$	-173.78	-*ap*	-168.67	-*ap*	-137.39	-*ac*	-129.60	-*ac*
$C_{13}N_{12}C_{11}C_9$	-126.91	-*ac*	+160.93	+*ap*	+149.47	+*ac*	-139.40	-*ac*
$C_{14}C_{13}N_{12}C_{11}$	+179.33	+*ap*	+177.46	+*ap*	-179.49	-*ap*	+179.47	+*ap*
$O_{15}C_{14}C_{13}N_{12}$	+50.06	+*sc*	+146.27	+*ac*	+111.50	+*ac*	+124.99	+*ac*
$C_{16}C_{14}C_{13}N_{12}$	-67.73	-*sc*	+29.57	+*sp*	-7.67	-*sp*	+7.31	+*sp*
$C_{17}O_{15}C_{14}C_{13}$	+99.15	+*ac*	+85.90	+*sc*	+111.67	+*ac*	+112.27	+*ac*
$O_{31}C_8C_4C_3$	+11.66	+*sp*	+17.01	+*sp*	+46.35	+*sc*	+53.39	+*sc*
$O_{32}C_9N_7C_4$	+59.33	+*sc*	+58.15	+*sc*	+62.76	+*sc*	+65.41	+*sc*
$H_{33}O_{10}C_8C_4$	+179.98	+*ap*	-179.99	-*ap*	-177.75	-*ap*	-178.76	-*ap*
$O_{36}C_{13}N_{12}C_{11}$	+0.85	+*sp*	-0.70	-*sp*	+3.77	+*sp*	+2.04	+*sp*
$H_{35}N_{12}C_{11}C_9$	+57.52	+*sc*	- -	-	-19.99	-*sp*	- -	-
H-$O_{36}C_{13}N_{12}$	- -	-	-3.49	-*sp*	- -	-	-4.84	-*sp*
H-$O_{32}C_9N_7$	- -	-	- -	-	+27.47	+*sp*	+23.83	+*sp*

*. Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs.[12]

6.1. Computational study on electronic structure in the protonation of phenethicillin

The optimized electronic structure of Phenethicillin (**1**) and its mono-protonated (**2** & **3**), di-protonated (**4**) and anion (**5**) are shown in Scheme - 2. In this context, the numbering of phenethicillin 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. The sequence of protonation in the order of $N_7 < N_{12}$ is due to the net charges on N_7- (- .2402) and N_{12}- (-0.3507) atoms in the phenethicillin (**1**). The proton affinity (PA),[30] values were calculated by using the equation (2) and found to be 182.8360 kcal/mol and 202.1875 kcal/mol respectively in the case of mono-protonated phenethicillins (**2** and **3**). Di-protonated

phenethicillin (**4**) was formed from either of mono-protonated phenethicillins (**2** and **3**) respectively with PA 129.8583 kcal/mol and 111. 5068 kcal/mol. The proton affinity is in the order of N_7 (202.1875 kcal/mol) > N_{12} (182.8360 kcal/mol) and mono-protonated phenethicillin (**3**) appears to be more stable.



Scheme - 2

The ionization potential values are increased in the order of molecules 5 < 1 < 3 < 2 < 4. 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 highest at N_{12}- atoms for molecules **1, 3, 4** and **5**. 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 **2** to **4**, due to the presence of same sign and antara-facial path way is allowed in the case of **1** and **5,** due to the presence of opposite sign.[31] The dipole moment is increasing in the order of molecules **1** < **4** < **2** < **3** < **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[32] (μ_{ind}) of molecules is increasing in the case of $\Delta\mu_{ind}$ (**4**) 0.352D < $\Delta\mu_{ind}$ (**2**) 2.977D < $\Delta\mu_{ind}$ (**3**) 3.042D < $\Delta\mu_{ind}$ (**5**) 16.595D. According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **4** < **2** < **3** < **1** < **5**. The protonation may take place preferably at N_{12}-atom than N_7-atom in the case of Phenethicillin (**1**), this is due to the increased bond lengths of N_{12}-C_{11} (1.4752 Å), C_{13}-N_{12} (1.5248 Å) and C_{11}-C_9 (1.5793 Å). It is found that the stability of Mono-protonated Phenethicillin **3** (ΔH_f^o, +39.0467 kcal/mol) is more stable than **2** (ΔH_f^o, +57.3982 kcal/mol). The formation of di-protonated Phenethicillin **(4),** from Mono-protonated Phenethicillin (**2** & **3**) is possible with the heat of formation (ΔH_f^o, + 294. 7399 kcal/mol).

Mono-protonated cation (**3**) is formed by the protonation of phenethicillin (**1**) at N_7-atom with increasing net atomic charges at N_{12}-, O_{10}- and O_{36}- atoms and decreasing at N_7-, O_{15}-, O_{31}- and O_{32}- atoms. The protonation site of phenethicillin (**1**) at N_{12}- atom to form mono-protonated cation (**2**) is considered by decreasing net atomic charges at N_7-, N_{12}-, O_{15}-, O_{31}-, O_{32}- and O_{36}-atoms and increasing at O_{10}- atom only. In the case of di-protonated cation (**4**), the negative atomic charges are decreased at N_7-, N_{12}-, O_{31}-, O_{32}- and O_{36}- atoms and increased at O_{10}-, and O_{15}- atoms. Anion of phenethicillin (**5**) is formed by the removal of a proton from O_{10}-atom of phenethicillin (**1**) with increasing net charges at N_{12}-, O_{10}-, O_{31}- and O_{32}- atoms and decreasing at N_7-, O_{15}- and O_{36}- atoms.

6.2. Computational study on conformational analyses in the protonation of phenethicillin

Spatial arrangement of atoms in the phenethicillin (**1**), and its mono-protonated cations (**2 & 3**), di-protonated cation (**4**) and anion (**5**) is existed in *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. Fully optimized AM1 calculations of the main data of bond lengths (Table - VI) and dihedral angles (Table - VII) of molecules (**1** to **5**) were incorporated. From the Scheme - 2, mono-protonated phenethicillin (**2**) is formed by the addition of proton at N_{12}-atom of phenethicillin (**1**), with increasing bond lengths at N_{12}-C_{11}, C_{13}-N_{12} and C_{11}-C_9 and decreasing bond lengths at C_9-N_7 and O_{36}-C_{13}. The change of conformation from *–ap* of $O_{10}C_8C_4C_3$, *-sc* of $O_{15}C_{14}C_{13}N_{12}$ and *-sc* of $C_{16}C_{14}C_{13}N_{12}$ are changed to *-ac*, *+ac* and *+sp* conformations respectively. The dihedral angle of *-ac* of $C_{13}N_{12}C_{11}C_9$, *+ap* of $H_{33}O_{10}C_8C_4$ and *+ap* of $C_{14}C_{13}N_{12}C_{11}$ are changed to *–ap* conformation. The conformations of *+ac* of $C_{17}O_{15}C_{14}C_{13}$ and *+sp* of $O_{31}C_8C_4C_3$ are changed to *+sc*

conformation. It is also observed that the protonation at N_{12}- atom is shown –*sc* conformation. If the mono-protonated phenethicillin (**3**) is formed by the addition of proton at N_7- atom of phenethicillin (**1**), with increasing bond lengths at C_{13}-N_{12} and C_9-N_7 and decreasing bond lengths at O_{31}-C_8, O_{32}-C_9 and N_{12}-C_{11}. The change of conformation from –*ac* of $C_{13}N_{12}C_{11}C_9$ and -*sc* of $C_{16}C_{14}C_{13}N_{12}$ are changed to +*sc* conformation. The dihedral angle of +*ap* of $C_{14}C_{13}N_{12}C_{11}$ and $H_{33}O_{10}C_8C_4$ are changed to –*ap* conformation. The conformations of -*sc* of $O_{15}C_{14}C_{13}N_{12}$ and +*sc* of $H_{35}N_{12}C_{11}C_9$ are changed to -*ac* conformation and all other conformations are unaltered. It is observed that the protonation at N_7-atom is shown -*ap* conformation. In the case of formation of di-protonated phenethicillin (**4**), it is found that the dihedral angles of $O_{10}C_8C_4C_3$, $C_{13}N_{12}C_{11}C_9$, $C_{14}C_{13}N_{12}C_{11}$, $O_{15}C_{14}C_{13}N_{12}$, $C_{16}C_{14}C_{13}N_{12}$, $O_{31}C_8C_4C_3$, $H_{33}O_{10}C_8C_4$ and $H_{35}N_{12}C_{11}C_9$ are changed conformations from -*ap* to -*ac*, -*ac* to +*ac*, +*ap* to -*ap*, -*sc* to +*sp*, -*sc* to -*ac*, +*sp* to +*sc*, +*ap* to -*ap* and +*sc* to +*sp* conformations respectively. It is also investigated that the protonation at N_7- atom and N_{12}-atom are shown respectively -*ac* and +*ac* conformations to form stable di-protonated phenethicillin (**4**). It can be concluded that the anion (**5**) is formed with the removal of a proton from O_{10}- atom of phenethicillin (**1**), and the dihedral angle of $O_{10}C_8C_4C_3$, $O_{15}C_{14}C_{13}N_{12}$, $C_{16}C_{14}C_{13}N_{12}$, $C_{17}O_{15}C_{14}C_{13}$, $O_{31}C_8C_4C_3$ and $O_{36}C_{13}N_{12}C_{11}$ are changed the conformations from -*ap* to +*sp*, -*sc* to +*ap*, -*sc* to +*sc*, +*ac* to +*sc*, +*sp* to -*ap* and +*sp* to -*sp* respectively to form stable anion R^- (**5**) and observed the rest of positions have moderate changes.

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) and the atomic charges on S_2, N_7, N_{12}, O_{10}, O_{15}, O_{31}, O_{32} and O_{36} of phenethicillin (1) and its mono-protonated forms (2 & 3), di-protonated form (4), and anion (5) from AM1 calculations.

Parameters	1	2	3	4	5
ΔH_f^o (kcal/mol)	-125.9658	+57.3982	+39.0467	+294.7399	-151.1979
Ionization potential (eV)	9.1224	12.0307	11.4159	14.5981	5.1143
μ (Debye)	3.109	6.086	6.151	3.461	19.704
E_{HOMO} (eV)	-9.1224	-12.0307	-11.4159	-14.5981	-5.1143
E_{LUMO} (eV)	+0.099	-4.594	-1.381	-8.474	+1.994
Electron excitation energies (E_{HOMO}-E_{LUMO})	9.221	7.437	7.035	6.124	7.108
S_2 (atomic charge)	+0.0526	+0.1404	+0.1941	+0.3183	-0.0834
N_7 (atomic charge)	-0.2402	0.2196	+0.0981	+0.0941	-0.1953
N_{12} (atomic charge)	-0.3507	-0.0902	-0.3757	-0.1230	-0.3532
O_{10} (atomic charge)	-0.2865	-0.3030	-0.3129	-0.3443	-0.5628

O_{15} (atomic charge)	-0.2261	-0.1813	-0.1925	-0.2585	-0.1956
O_{31} (atomic charge)	-0.3519	-0.3117	-0.2769	-0.2226	-0.5126
O_{32} (atomic charge)	-0.2363	-0.1799	-0.0941	-0.0271	-0.2513
O_{36} (atomic charge)	-0.3522	-0.1334	-0.3740	-0.1353	-0.3422

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

Bond lengths (Å)	1	2	3	4	5
C_3-S_2	1.8142	1.8232	1.8202	1.8097	1.8510
C_9-N_7	1.4491	1.4183	1.5552	1.5329	1.4329
N_{12}-C_{11}	1.4125	1.4752	1.4077	1.4680	1.4204
C_{13}-N_{12}	1.3831	1.5248	1.3995	1.5580	1.3786
C_{11}-C_9	1.5696	1.5793	1.5672	1.5410	1.5768
O_{10}-C_8	1.3583	1.3560	1.3565	1.3563	1.2603
O_{31}-C_8	1.2334	1.2307	1.2262	1.2224	1.2522
O_{32}-C_9	1.2176	1.2131	1.1990	1.1989	1.2192
O_{36}-C_{13}	1.2443	1.2159	1.2449	1.2101	1.2437
H_{33}-O_{10}	0.9731	0.9749	0.9765	0.9794	-
H_{35}-N_{12}	0.9946	1.0298	0.9964	1.0323	0.9922
H-N_7	-	-	1.0282	1.0247	-
H-N_{12}	-	1.0311	-	1.0331	-

Table – VII: Dihedral angle (°) of phenethicillin (1) and its mono-protonated forms (2 & 3), di-protonated form (4), and anion (5) from AM1 calculations.

Dihedral angle (°)	1		2		3		4		5	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-21.06	*-sp*	-18.34	*-sp*	-27.49	*-sp*	-28.67	*-sp*	-20.34	*-sp*
$C_8C_4C_3S_2$	+163.25	*+ap*	+180.06	*+ap*	+165.89	*+ap*	+158.71	*+ap*	+165.81	*+ap*
$O_{10}C_8C_4C_3$	-173.78	*-ap*	-110.24	*-ac*	-161.79	*-ap*	-95.35	*-ac*	+21.86	*+sp*
$C_{13}N_{12}C_{11}C_9$	-126.91	*-ac*	-156.42	*-ap*	+52.99	*+sc*	-148.31	*+ac*	-132.98	*-ac*
$C_{14}C_{13}N_{12}C_{11}$	+179.33	*+ap*	-178.43	*-ap*	-179.85	*-ap*	-175.86	*-ap*	+177.48	*+ap*
$O_{15}C_{14}C_{13}N_{12}$	+50.06	*-sc*	+144.58	*+ac*	+148.04	*+ac*	+26.88	*+sp*	+172.25	*+ap*
$C_{16}C_{14}C_{13}N_{12}$	-67.73	*+ac*	+27.49	*+sp*	+30.78	*+sc*	-91.74	*-ac*	+55.82	*+sc*
$C_{17}O_{15}C_{14}C_{13}$	+99.15	*+sp*	+83.92	*+sc*	+98.46	*+ac*	+119.68	*+ac*	+89.23	*+sc*
$O_{31}C_8C_4C_3$	+11.66	*+sc*	+69.66	*+sc*	+22.72	*+sp*	+83.63	*+sc*	-163.28	*-ap*
$O_{32}C_9N_7C_4$	+59.33	*+sc*	+65.42	*+sc*	+64.06	*+sc*	+78.28	*+sc*	+62.01	*+sc*
$H_{33}O_{10}C_8C_4$	+179.98	*+ap*	-177.95	*-ap*	-177.17	*-ap*	-179.89	*-ap*	- -	- -
$O_{36}C_{13}N_{12}C_{11}$	+0.85	*+sp*	+2.00	*+sp*	+2.02	*+sp*	+5.93	*+sp*	-1.24	*-sp*
$H_{35}N_{12}C_{11}C_9$	+57.52	*+sc*	+81.05	*+sc*	-121.88	*-ac*	-27.82	*+sp*	+45.88	*+sc*
$HN_{12}C_{11}C_9$	- -	- -	-35.08	*-sc*	- -	- -	+90.10	*+ac*	- -	- -
$HN_7C_4C_3$	- -	- -	- -	- -	-158.53	*-ap*	-145.13	*-ac*	- -	- -

* Conformational analyses using prefixes a = anti, s = syn, p = peri-planar, c = clinal, and + & - signs.[12]

7.1. Computational study on electronic structure in the protonation of phenethicillin-lactim tautomer

The optimized electronic structure of phenethicillin (**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 form of phenethicillin (**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. The net charges on N_7- (-0.2249) and N_{12}- (-0.2517) atoms of phenethicillin lactim tautomer (**2**) is observed in the order of $N_7 < N_{12}$.

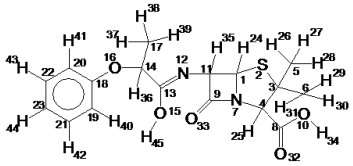


Figure - 2

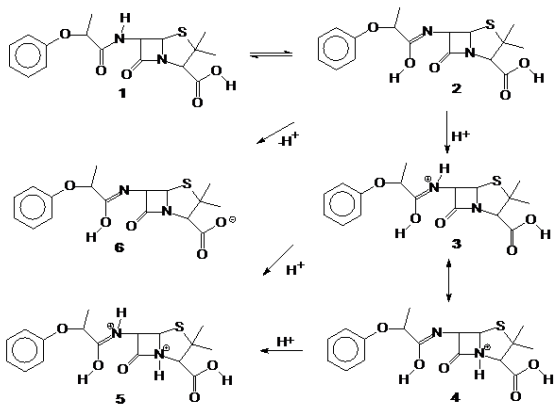
The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **1** and **6** have more electron-donor character whereas other molecules have electron-acceptor property. In the case of HOMO, the electron density is more at N_{12}- atoms for all molecules except **3** and **6**. The results so obtained reveal that the electronic properties and reactivity of molecule depend on its conformational structure. 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 **2, 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.[31] The dipole moment is increasing in the order of molecules **2** < **1** < **5** < **4** < **3** < **6**. Anion (**6**) shows higher dipole moment. The electronegative heteroatoms induce an additional dipole moment in the molecule. The magnitude of the induction effect[32] (μ_{ind}) of molecules can be estimated with respect to phenethicillin lactim form (**2**). It is found that the induction effect is increasing in the order of $\Delta\mu_{ind}$ (**1**) 0.782D < $\Delta\mu_{ind}$ (**5**) 1.571D < $\Delta\mu_{ind}$ (**4**) 3.819D < $\Delta\mu_{ind}$ (**3**) 6.01D < $\Delta\mu_{ind}$ (**6**) 20.576D. 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**. The stability of mono-protonated lactim form of phenethicillin **3** (ΔH_f^o, +36.5996 kcal/mol) is more stable than **4** (ΔH_f^o, +45.0417 kcal/mol). The formation of lactim form of di-protonated phenethicillin **(5)** is possible from mono-protonated lactim form of phenethicillins (**3** & **4**) with the heat of formation (ΔH_f^o) of

+273.5831kcal/mol. Mono-protonated phenethicillin lactim (**3**) is formed by the protonation at N_{12}-atom in the phenethicillin lactim (**2**) and decreasing net atomic charges at N_7-, N_{12}-, O_{15}-, O_{32}- and O_{33}- atoms and increasing charges at O_{10}- and O_{16}- atoms. The protonation at N_7- atom of phenethicillin lactim (**2**) is observed to form mono-protonated lactim (**4**) by decreasing net atomic charges at N_7- O_{32}- and O_{33}-atoms and increasing at N_{12}-, O_{10}-, O_{15}- and O_{16}- atoms. In the case of di-protonated form (**5**), the negative atomic charges are decreased at N_7-, N_{12}-, O_{15}-, O_{32}- and O_{33}-atoms and increased at O_{10}- and O_{16}- atom. Anion of lactim form of phenethicillin (**6**) is formed by the removal of a proton on O_{10}-atom with increasing net charges at O_{10}-, O_{15}-, O_{16}-, O_{32}- and O_{33}- atoms and decreasing at N_7- and N_{12}- atoms. The ionization potential values are decreased in the order of **5** > **3** > **4** > **1** > **2** > **6**. The proton affinity (PA)[30] values for the different nitrogen atoms of lactim form of phenethicillin RH (**2**) were calculated by using the equation (2) and found to be 220.9465 kcal/mol and 212.5044 kcal/mol respectively in the case of mono-protonated phenethicillins (**3** and **4**). Di-protonated form (**5**) was formed from either of mono-protonated phenethicillins (**3** and **4**) respectively with PA 130.2165 kcal/mol and 138.6586kcal/mol. The proton affinity is in the order of N_{12} (220.9465kcal/mol) > N_7 (212.5044kcal/mol) and mono-protonated phenethicillin (**3**) 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** > **3** > **4** > **2** > **1**. The tautomeric equilibrium constants $\log K_T$ (11.9558) was calculated[29] according to the equation (1).

7.2. Computational study on conformational in the protonation of phenethicillin lactim tautomer

The spatial arrangement of atoms in phenethicillin (**1**), and its phenethicillin lactim (**2**), mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) are existed in *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. Lactam-lactim tautomerism is also observed in phenethicillin (**1**) to form phenethicillin lactim (**2**) with increasing bond length of O_{15}-C_{13} (1.3802Å) and decreasing bond length of C_{13}-N_{12} (1.2934Å). The conformation from *–ac* of $C_{13}N_{12}C_{11}C_9$, ***-sc*** of $C_{17}C_{14}C_{13}N_{12}$ and ***–sc*** of $O_{16}C_{14}C_{13}N_{12}$ are changed respectively to *+ap*, *+sp* and *+ac* conformations. Dihedral angle of $O_{15}C_{13}N_{12}C_{11}$, $C_{18}O_{16}C_{14}C_{13}$ and $H_{34}O_{10}C_8C_4$ are changed respectively *+sp* to *-sp*, *+ac* to *+sc* and *+ap* to *-ap* conformations along with the formation of $H_{45}O_{15}C_{13}N_{12}$ with ***–sp*** conformation. From the Table - IX, Table – X and as per Scheme-3, mono-protonated phenethicillin lactim (**3**) is formed by the addition of proton at N_{12}-atom of phenethicillin lactim tautomer (**2**), with

increasing bond lengths at C_{13}-N_{12} and decreasing bond lengths at C_9-N_7, O_{15}-C_{13}, O_{32}-C_8 and O_{33}-C_9. The dihedral angle of $C_{13}N_{12}C_{11}C_9$, $O_{16}C_{14}C_{13}N_{12}$, $C_{18}O_{16}C_{14}C_{13}$ and $H_{45}O_{15}C_{13}N_{12}$ are converted from *+ap* to *–ap*, *+ac* to *+ap*, *+sc* to *+ac* and *-sp* to *-ap* conformations and all other conformations are moderately changed. It is observed that the protonation at N_{12}-atom is shown *+sp* conformation. If the mono-protonated lactim form of phenethicillin (**4**) is formed by the addition of proton at N_7- atom of lactim tautomer of phenethicillin (**2**), with increasing bond lengths at C_{13}-N_{12}, N_{12}-C_{11} and C_9-N_7 and decreasing bond lengths at O_{33}-C_9, and O_{15}-C_{13}. The dihedral angle of $C_{13}N_{12}C_{11}C_9$, $C_{17}C_{14}C_{13}N_{12}$, $O_{16}C_{14}C_{13}N_{12}$, $C_{18}O_{16}C_{14}C_{13}$ and $H_{45}O_{15}C_{13}N_{12}$ are converted from *+ap* to *–ap*, *+sp* to *+sc*, *+ac* to *+ap*, *+sc* to *+ac* and *-sp* to *-ap* conformations and all other conformations are unaltered. It is observed that the protonation at N_7-atom is shown *-ap* conformation. In the case of formation of di-protonated lactim form of phenethicillin (**5**), it is found that the dihedral angle of $O_{10}C_8C_4C_3$, $C_{13}N_{12}C_{11}C_9$, $C_{17}C_{14}C_{13}N_{12}$, $O_{16}C_{14}C_{13}N_{12}$, $C_{18}O_{16}C_{14}C_{13}$, $O_{32}C_8C_4C_3$, and $H_{45}O_{15}C_{13}N_{12}$ are changed conformation, *-ap* to *-ac*, *+ap* to *+ac*, *+sp* to *+sc*, *+ac* to *+ap*, *+sc* to *+ac*, *+sp* to *+sc* and *-sp* to *-ap* 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 phenethicillin (**5**).



Scheme - 3

It can be concluded that the anion (**6**) is formed with the removal of a proton on O_{10}- atom of lactim tautomer of phenethicillin (**2**), and the conformation from ***–ap*** of $O_{10}C_8C_4C_3$, ***+ap*** of $C_{13}N_{12}C_{11}C_9$, ***+ap*** of $C_{14}C_{13}N_{12}C_{11}$, ***+sp*** of $C_{17}C_{14}C_{13}N_{12}$, ***+ac*** of $O_{16}C_{14}C_{13}N_{12}$, ***-sp*** of $O_{15}C_{13}N_{12}C_{11}$, ***+sc*** of $C_{18}O_{16}C_{14}C_{13}$, ***+sp*** of $O_{32}C_8C_4C_3$, and ***–sp*** of $H_{45}O_{15}C_{13}N_{12}$ are changed to *+ac*, *+ac*, *-ap*, *+ac*, *-ap*, *+sp*, *+ac*, *-sc* and *+ap* conformations respectively in the formation of stable anion (**6**) and rest of positions have moderate changes.

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_{15}, O_{16}, O_{32} and O_{33} of phenethicillin(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)	-125.9658	-109.6539	+36.5996	+45.0417	+273.5831	-142.8538
Ionization potential (eV)	9.1224	9.1087	12.0112	11.6820	14.6399	4.930
μ (Debye)	3.109	2.327	8.337	6.146	3.898	22.903
E_{HOMO} (eV)	-9.122	-9.109	-12.011	-11.682	-14.640	-4.930
E_{LUMO} (eV)	+0.099	-0.081	-5.161	-4.195	-8.240	+1.726
Electron excitation energies($\Delta E = E_{LUMO} - E_{HOMO}$) (eV)	9.221	9.028	6.850	7.487	6.400	6.656
S_2 (atomic charge)	+0.0576	+0.0281	+0.1069	+0.2117	+0.2962	-0.0676
N_7 (atomic charge)	-0.2402	-0.2249	-0.2133	-0.1173	-0.0963	-0.2097
N_{12} (atomic charge)	-0.3507	-0.2517	-0.1757	-0.2972	-0.2437	-0.2067
O_{10} (atomic charge)	-0.2865	-0.2807	-0.2894	-0.3173	-0.3392	-0.5502
O_{15} (atomic charge)	-0.3522	-0.2751	-0.1914	-0.2845	-0.2138	-0.2878
O_{16} (atomic charge)	-0.2261	-0.2020	-0.2146	-0.2285	-0.2319	-0.2381
O_{32} (atomic charge)	-0.3519	-0.3561	-0.3250	-0.2753	-0.2300	-0.5361
O_{33} (atomic charge)	-0.2363	-0.2280	-0.1897	-0.0911	-0.0328	-0.2363

Table – IX: Bond lengths of phenethicillin (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_3-S_2	1.8142	1.8271	1.8280	1.8129	1.8087	1.8278
C_9-N_7	1.4491	1.4505	1.4318	1.5700	1.5374	1.4379
N_{12}-C_{11}	1.4125	1.4176	1.4385	1.4212	1.4407	1.4119
C_{13}-N_{12}	1.3831	1.2934	1.3305	1.3037	1.3391	1.2854
C_{11}-C_9	1.5696	1.5621	1.5752	1.5532	1.5483	1.5828
O_{15}-C_{13}	1.2443	1.3802	1.3423	1.3667	1.3341	1.3857
H_{45}-O_{15}	- -	0.9699	0.9877	0.9769	0.9986	0.9687
O_{32}-C_8	1.2334	1.2341	1.2317	1.2263	1.2231	1.2554
O_{33}-C_9	1.2176	1.2164	1.2139	1.1969	1.1985	1.2160
H_{34}-O_{10}	0.9731	0.9731	0.9751	0.9763	0.9790	- -
H-N_7	- -	- -	- -	1.0209	1.0235	- -
H-N_{12}	- -	- -	1.0066	- -	1.0104	- -

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

Dihedral angle (°)	1		2		3		4		5		6	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-21.06	*-sp*	-21.14	*-sp*	-19.98	*-sp*	-27.63	*-sp*	-28.94	*-sp*	-14.42	*-sp*
$C_8C_4C_3S_2$	+163.25	*+ap*	+163.15	*+ap*	+162.51	*+ap*	+162.90	*+ap*	+160.55	*+ap*	+151.55	*+ap*
$O_{10}C_8C_4C_3$	-173.78	*-ap*	-168.67	*-ap*	-156.19	*-ap*	-162.01	*-ap*	-97.28	*-ac*	+94.10	*+ac*
$C_{13}N_{12}C_{11}C_9$	-126.91	*-ac*	+160.93	*+ap*	-160.73	*-ap*	-163.19	*-ap*	+147.46	*+ac*	+108.17	*+ac*
$C_{14}C_{13}N_{12}C_{11}$	+179.33	*+ap*	+177.46	*+ap*	+179.86	*+ap*	+179.27	*+ap*	+175.36	*+ap*	-177.18	*-ap*
$C_{17}C_{14}C_{13}N_{12}$	-67.73	*-sc*	+29.57	*+sp*	+56.33	*+sp*	+57.89	*+sc*	+53.07	*+sc*	+90.74	*+ac*
$O_{16}C_{14}C_{13}N_{12}$	+50.06	*+sc*	+146.27	*+ac*	+172.07	*+ap*	+174.24	*+ap*	+170.64	*+ap*	-153.39	*-ap*
$O_{15}C_{13}N_{12}C_{11}$	+0.85	*+sp*	-0.70	*-sp*	-1.29	*-sp*	-1.88	*-sp*	-5.32	*-sp*	+1.25	*+sp*
$C_{18}O_{16}C_{14}C_{13}$	+99.15	*+ac*	+85.90	*+sc*	+94.42	*+ac*	+104.76	*+ap*	+120.57	*+ac*	+92.95	*+ac*
$O_{32}C_8C_4C_3$	+11.66	*+sp*	+17.01	*+sp*	+28.43	*+sp*	+22.87	*+sp*	+81.56	*+sc*	-83.61	*-sc*
$H_{45}O_{15}C_{13}N_{12}$	- -	- -	-3.49	*-sp*	-178.59	*-ap*	-178.07	*-ap*	-179.99	*-ap*	+126.49	*+ap*
$H_{34}O_{10}C_8C_4$	+179.98	*+ap*	-179.99	*-ap*	-177.16	*-ap*	-177.41	*-ap*	-179.55	*-ap*	- -	- -
H-$N_7C_4C_3$	- -	- -	- -	- -	- -	- -	-152.37	*-ap*	-147.64	*-ac*	- -	- -
H-$N_{12}C_{11}C_9$	- -	- -	- -	- -	+20.58	*+sp*	- -	- -	-38.81	*-sc*	- -	- -

* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs.[12]

8.1. Computational study on electronic structure in the protonation of phenethicillin-enol tautomer

The optimized electronic structure of phenethicillin (**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 phenethicillin (**2**) is shown in Figure - 3. 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-I. The net charges on N_7- (-0.1449) and N_{13}- (-0.2760) atoms of phenethicillin enol-form (**2**) are increasing in the order of $N_7 < N_{13}$. The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **3** to **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 **3** to **5**, due to the presence of same sign and **1, 2 & 6** are allowed antara-facial path way due to the opposite sign.[31] The dipole moment is increasing in the order of molecules **3** < **2** < **1** < **5** < **4** < **6**. The magnitude of the induction effect[32] (μ_{ind}) of molecules can be estimated and found increasing in the order of $\Delta\mu_{ind}$ (**3**) -0.5429 D < $\Delta\mu_{ind}$ (**1**) 0.3777 D < $\Delta\mu_{ind}$ (**5**) 4.9666 D < $\Delta\mu_{ind}$ (**4**) 5.3398 D < $\Delta\mu_{ind}$ (**6**) 15.2204 D with reference to phenethicillin enol (**2**). According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **5** < **3** < **4** < **2** < **1** < **6**. The stability of

mono-protonated phenethicillin enol (**4**) (ΔH_f^o, +55.2126 kcal/mol) is more stable than (**3**) (ΔH_f^o, +58.6352 kcal/mol). The di-protonated phenethicillin enol **(5)** is possible (with the heat of formation (ΔH_f^o) of +309.8150 kcal/mol**)** from mono-protonated enol form of phenethicillins (**3** & **4**). Mono-protonated phenethicillin enol (**3**) is formed by the protonation at N_{13}- atom in phenethicillin enol (**2**) with decreasing net atomic charges at N_{13}-, O_{10}-, O_{12}-, and O_{35}- atoms and increased at O_{16}-, and O_{32}- atoms. The protonation site of enol form of phenethicillin (**2**) at N_7- atom to mono-protonated form (**4**) is considered by decreasing net atomic charges at N_7-, O_{12}-, O_{16}-, O_{32}- and O_{35}-atoms and increasing at O_{10}-, and N_{13}- atoms. In the case of di-protonated form (**5**), the negative atomic charges are decreased at all hetero atoms except at O_{10}-atom. Anion of enol form of phenethicillin (**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_{32}-, and O_{35}-, and decreasing at O_{16}- and N_{13}- atoms.

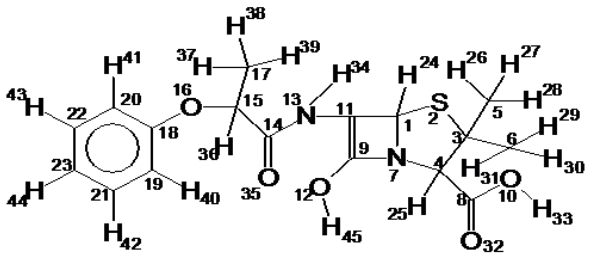
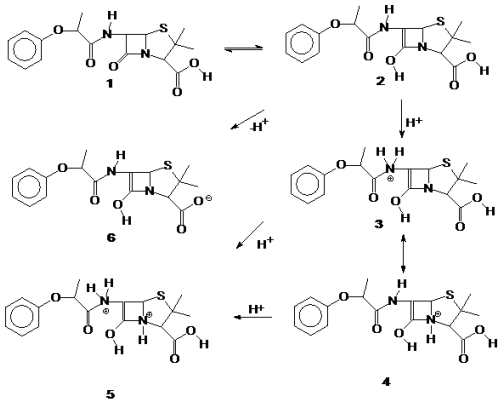


Figure - 3

Ionization potential (IP) is increasing in the order of **6** < **2** < **1** < **4** < **3** < **5**. As per electron excitation energies (ΔE) (in eV), a large gap implies high stability and small gap implies low stability. The high stability in turn indicates low chemical reactivity and small gap indicates high chemical reactivity.[33] It is also observed the reactivity, which is decreased in the order of **5** > **4** > **6** > **3** > **2** > **1**. The proton affinity (PA)[30] values for the different nitrogen atoms of enol form of phenethicillin RH (**2**) were calculated by using the equation (2) and found to be 207.6703 kcal/mol and 211.0929 kcal/mol respectively in the case of mono-protonated phenethicillins (**3** and **4**). Di-protonated form (**5**) was formed from either of mono-protonated phenethicillins (**3** and **4**) respectively with PA 116.0202 kcal/mol and 112.5976kcal/mol. The proton affinity is in the order of N_{13} (207.6703 kcal/mol) < N_7 (211.0929kcal/mol) and mono-protonated phenethicillin (**4**) appears to be more stable.

8.2. Computational study on conformational analyses in the protonation of phenethicillin enol tautomer

The spatial arrangement of atoms in phenethicillin (**1**), phenethicillin enol (**2**), Mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) are existed in *anti*- or *syn*-conformation, according to the change in energy content of the protonation. From the Table - XII, Table - XIII, Figure - 3 and Scheme - 4, phenethicillin (**1**) can undergo keto- nol tautomerism in the formation of phenethicillin enol (**2**) with increasing bond length of O_{12}-C_9 (1.3535 Å) and decreasing bond length of C_{11}-C_9 (1.3744 Å). The change of conformation from ***-ac*** of $C_{14}N_{13}C_{11}C_9$, ***+ap*** of $C_{15}C_{14}N_{13}C_{11}$, ***-sc*** of $C_{17}C_{15}C_{14}N_{13}$, ***+sc*** of $O_{16}C_{15}C_{14}N_{13}$ and ***+sp*** of $O_{32}C_8C_4C_3$ are changed respectively to ***+ac, -ap, -sp, +ac*** and ***+sc*** conformations. Dihedral angle of $H_{33}O_{10}C_8C_4$ is changed ***+ap*** to ***–ap*** conformation. After keto-enol rearrangement, the enol form of phenethicillin (**2**) is formed with the ***+sp*** conformation in the case of dihedral angle of $H_{45}O_{12}C_9N_7$.



Scheme - 4

As per Scheme - 4, mono-protonated enol form of phenethicillin (**3**) is formed by the addition of proton at N_{13}-atom of phenethicillin enol tautomer (**2**), with increasing bond lengths at N_{13}-C_{11}, C_{14}-N_{13}, C_{11}-C_9 and H_{45}-O_{12} and decreasing bond lengths at C_9-N_7, O_{35}-C_{14} and O_{12}-C_9. The conformations of ***-ac*** of $O_{10}C_8C_4C_3$, ***-ap*** of $C_{15}C_{14}N_{13}C_{11}$, ***-sp*** of $C_{17}C_{15}C_{14}N_{13}$, ***+sp*** of $O_{35}C_{14}N_{13}C_{11}$, ***+ac*** of $O_{16}C_{15}C_{14}N_{13}$, ***+sc*** of $O_{32}C_8C_4C_3$, +sp of $H_{45}O_{12}C_9N_7$ and $H_{33}O_{10}C_8C_4$ are changed respectively to ***+sc, +ap, –sc, -sp, +sc, +sc, -sp*** and ***+ap*** conformations 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 ***-sp*** conformation. If the mono-protonated enol form of phenethicillin (**4**) is formed by the addition of proton at N_7- atom of phenethicillin enol tautomer (**2**), with increasing bond lengths at C_{14}-N_{13}, H_{45}-O_{12} and C_9-N_7 and decreasing bond lengths at N_{13}-C_{11}, O_{35}-C_{14}, O_{12}-C_9 and O_{32}-C_8. The change of dihedral angle of

$C_{14}N_{13}C_{11}C_9$, $C_{15}C_{14}N_{13}C_{11}$, $C_{17}C_{15}C_{14}N_{13}$ and $H_{45}O_{12}C_9N_7$ are converted from ***+ac*** to ***+ap***, ***-ap*** to ***+ap***, ***-sp*** to ***+sp*** and ***+sp*** to ***+sc*** conformations respectively and all other conformations are unaltered. It is observed that the protonation at N_7-atom is shown *-ap* conformation. In the case of formation of di-protonated phenethicillin enol (**5**), it is found that all conformations are changed more or less comparatively. The protonation at N_7- atom and N_{13}-atom are shown ***-ac*** conformations in the formation of di-protonated phenethicillin enol (**5**). It can be concluded that the anion (**6**) is formed with the removal of a proton on O_{10}- atom of phenethicillin enol tautomer (**2**), and the change of conformation from ***-ap*** of $C_{15}C_{14}N_{13}C_{11}$, ***-sp*** of $C_{17}C_{15}C_{14}N_{13}$ are changed to ***+ap*** and ***+sc*** conformations respectively to form stable anion (**6**) and rest of positions have moderate changes.

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 phenethicillin(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)	-125.9658	-100.8945	+58.6352	+55.2126	+309.8150	-139.0668
Ionization potential (eV)	9.1224	8.4589	12.1010	11.3891	13.9812	5.3337
μ (Debye)	3.1088	2.7311	2.1882	8.0709	7.6977	17.9515
E_{HOMO} (eV)	-9.122	-8.459	-12.101	-11.389	-13.981	-5.334
E_{LUMO} (eV)	+0.099	+0.083	-4.051	-4.440	-8.418	+2.188
Electron excitation energies ($\Delta E = E_{LUMO} - E_{HOMO}$) (eV)	9.221	8.542	8.050	6.949	5.563	7.522
S_2 (atomic charge)	+0.0576	+0.1012	+0.1301	+0.2461	+0.3090	-0.0486
N_7 (atomic charge)	-0.2402	-0.1449	-0.1521	-0.0197	-0.0490	-0.0532
N_{13} (atomic charge)	-0.3507	-0.2760	+0.0266	-0.2863	-0.0356	-0.2526
O_{10} (atomic charge)	-0.2865	-0.3232	-0.2775	-0.3306	-0.3767	-0.5813
O_{12} (atomic charge)	-0.2363	-0.2243	-0.1941	-0.1634	-0.1469	-0.2508
O_{16} (atomic charge)	-0.2261	-0.2014	-0.2333	-0.1988	-0.2001	-0.2013
O_{32} (atomic charge)	-0.3519	-0.3535	-0.3877	-0.2773	-0.2168	-0.5315

Table – XII: Bond lengths of phenethicillin (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	1.4491	1.4617	1.4497	1.5092	1.5016	1.4580
N_{13}-C_{11}	1.4125	1.3727	1.4109	1.3500	1.4162	1.3756
C_{14}-N_{13}	1.3831	1.3862	1.5186	1.4195	1.5535	1.3775
C_{11}-C_9	1.5696	1.3744	1.3874	1.3795	1.3718	1.3846
O_{10}-C_8	1.3583	1.3624	1.3514	1.3605	1.3647	1.2645
O_{32}-C_8	1.2334	1.2342	1.2367	1.2265	1.2239	1.2572

O_{12}-C_9	1.2176	1.3535	1.3286	1.3388	1.3212	1.3365
O_{35}-C_{14}	1.2443	1.2450	1.2165	1.2364	1.2105	1.2459
H_{45}-O_{12}	- -	0.9741	0.9845	0.9788	0.9856	0.9871
H_{33}-O_{10}	0.9731	0.9726	0.9764	0.9759	0.9786	- -
H-N_7	- -	- -	- -	1.0191	1.0228	- -
H-N_{13}	- -	- -	1.0339	- -	1.0360	- -

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

Dihedral angle (^{o})	1		2		3		4		5		6	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-21.06	*-sp*	-23.23	*-sp*	-20.39	*-sp*	-27.42	*-sp*	-29.62	*-sp*	-18.48	*-sp*
$C_8C_4C_3S_2$	+163.25	*+ap*	+164.49	*+ap*	+156.86	*+ap*	+161.05	*+ap*	+162.73	*+ap*	+159.19	*+ap*
$O_{10}C_8C_4C_3$	-173.78	*-ap*	-137.39	*-ac*	+69.38	*+sc*	-148.15	*-ac*	-144.02	*-ac*	-101.09	*-ac*
$C_{14}N_{13}C_{11}C_9$	-126.91	*-ac*	+149.47	*+ac*	+118.19	*+ac*	+155.61	*+ap*	+116.86	*+ac*	+114.57	*+ac*
$C_{15}C_{14}N_{13}C_{11}$	+179.33	*+ap*	-179.49	*-ap*	+177.51	*+ap*	+179.36	*+ap*	-174.31	*-ap*	+178.67	*+ap*
$C_{17}C_{15}C_{14}N_{13}$	-67.73	*-sc*	-7.67	*-sp*	-67.84	*-sc*	+17.57	*+sp*	-10.50	*-sp*	+32.25	*+sp*
$O_{32}C_8C_4C_3$	+11.66	*+sp*	+46.35	*+sc*	-111.78	*-ac*	+36.37	*+sc*	+40.14	*+sp*	+77.77	*+sc*
$O_{16}C_{15}C_{14}N_{13}$	+50.06	*+sc*	+111.50	*+ac*	+49.79	*+sc*	+135.27	*+ac*	+108.15	*+ac*	+149.62	*+ac*
$H_{33}O_{10}C_8C_4$	+179.98	*+ap*	-177.75	*-ap*	+178.84	*+ap*	-178.08	*-ap*	-171.72	*-ap*	- -	- -
$H_{45}O_{12}C_9N_7$	- -	- -	+27.47	*+sp*	-13.24	*-sp*	+78.97	*+sc*	+11.49	*+sp*	-24.63	*-sp*
$O_{35}C_{14}N_{13}C_{11}$	+0.85	*+sp*	+3.77	*+sp*	-1.50	*-sp*	+1.42	*+sp*	+4.63	*+sp*	+3.67	*+sp*
$HN_7C_4C_3$	- -	- -	- -	- -	- -	- -	-151.57	*-ap*	-147.19	*-ac*	- -	- -
$HN_{13}C_{11}C_9$	- -	- -	- -	- -	-3.93	*-sp*	- -	- -	-122.14	*-ac*	- -	- -

* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs.[12]

9.1. Computational study on electronic structure in the protonation of phenethicillin lactim-enol tautomer

The optimized electronic structure of phenethicillin (**1**) and its phenethicillin lactim-enol form (**2**) mono-protonated (**3** & **4**), di-protonated (**5**) and anion (**6**) are shown in Scheme - 5. In this context, the numbering of phenethicillin lactim-enol form (**2**) is shown in Figure - 4. 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}), Electron excitation energies (eV) 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-(-0.1624) and N_{13}-(-0.1877) atoms of phenethicillin lactim-enol form (**2**) are increasing in the order of $N_7 < N_{13}$.

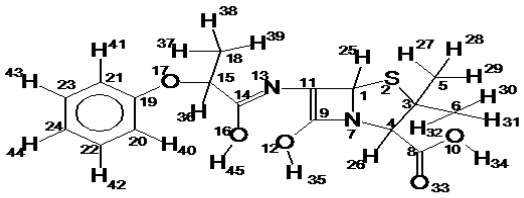


Figure - 4

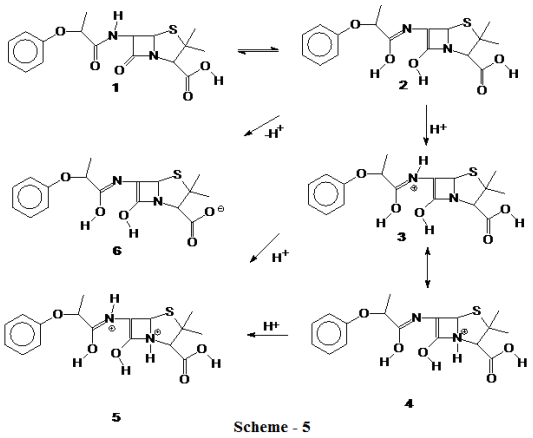
The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **1** and **6** have more electron-donor character whereas other molecules have electron-acceptor property. The promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed in the case of molecules **2** to **5**, due to the presence of same sign and other molecules undergo antara-facial path way us allowed due to the opposite sign.[31] The dipole moment is increasing in the order of molecules **2** < **1** < **3** < **4** < **5** < **6**. The magnitude of the induction effect[32] (μ_{ind}) of molecules can be estimated with respect to phenethicillin lactim-enol form (**2**) and found in the order of $\Delta\mu_{ind}$ (**1**) 1.013 D < $\Delta\mu_{ind}$ (**3**) 2.151 D < $\Delta\mu_{ind}$ (**4**) 6.049 D < $\Delta\mu_{ind}$ (**5**) 7.519 D < $\Delta\mu_{ind}$ (**6**) 13.214 D. 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**. The stability of Mono-protonated phenethicillin lactim-enol form **3** (ΔH_f^o, +43.9985 Kcal/mol) is more stable than **4** (ΔH_f^o, +63.6192 Kcal/mol). The di-protonated phenethicillin lactim-enol form **(5)** is possible (with the heat of formation (ΔH_f^o) of +287.1238 Kcal/mol**)** from Mono-protonated enol form of phenethicillin lactim-enol form (**3** & **4**). The proton affinity (PA)[30] values for the different nitrogen atoms of phenethicillin lactim-enol form RH (**2**) were calculated by using the equation (2) and found to be 229.0773 kcal/mol and 209.4566 kcal/mol respectively in the case of mono-protonated phenethicillin lactim-enol form (**3** and **4**). Di-protonated form (**5**) was formed from either of mono-protonated phenethicillin lactim-enol form (**3** and **4**) respectively with PA 124.0747 kcal/mol and 143. 6954 kcal/mol. The proton affinity is in the order of N_{13} (229.0773 kcal/mol) > N_7 (209.4566 kcal/mol) and mono-protonated phenethicillin lactim-enol form (**4**) 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** > **3** > **6** > **4** > **2** > **1**. The protonation at N_{13}-atom in the case of phenethicillin lactim-enol form (**2**) to Mono-protonated form (**3**) is considered by decreasing net atomic charges at N_7-, N_{13}-, O_{12}-, O_{16}-, and O_{33}- atoms and increased at O_{10}- atom. If it is protonated at N_7- atom of phenethicillin lactim-enol form (**2**) in the formation of mono-protonated form (**4**) is considered by decreasing net atomic charges at

N_7-, O_{12}-, O_{16}-, O_{17}- and O_{33}-atoms and increasing at N_{13}- atom. In the case of di-protonated form (**5**), the negative atomic charges are decreased at all hetero atoms except at O_{10}- and O_{17}- atom. Anion of phenethicillin lactim-enol form (**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_{33}-, and decreasing at N_{13}- atom.

9.2. Computational study on conformational analyses in the protonation of phenethicillin lactim-enol tautomer

From the Table-XV, Table-XVI, Figure-4 and Scheme-5, it is observed that phenethicillin (**1**) would undergo lactam-lactim and keto-enol tautomerism simultaneously and form phenethicillin lactim-enol form (**2**) with increasing bond lengths of O_{12}-C_9 (1.3471 Å) and O_{16}-C_{14} (1.3772 Å) and decreasing bond lengths of C_{11}-C_9 (1.3799 Å) and C_{14}-N_{13} (1.2992 Å). The dihedral angle of $O_{10}C_8C_4C_3$, $C_{18}C_{15}C_{14}N_{13}$, $O_{17}C_{15}C_{14}N_{13}$, $O_{33}C_8C_4C_3$ and $H_{34}O_{10}C_8C_4$ are changed respectively *-ap* to *-ac*, *–sc* to *+sp*, *+sc* to *+ac*, *+sp* to *+sc* and *+ap* to *-ap* conformations. After lactam-enol rearrangement, the phenethicillin lactim-enol form (**2**) is formed with the *+sp* and *-sp* conformation in the case of dihedral angle of $H_{35}O_{12}C_9N_7$ and $H_{45}O_{16}C_{14}N_{13}$ respectively. The spatial arrangement of atoms in phenethicillin (**1**), phenethicillin lactim-enol form (**2**), Mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) have formed in *anti*- or *syn*- conformation, according to the position of atoms. From the Table - XV, and Table - XVI, it is observed that as per Scheme - 5, mono-protonated phenethicillin lactim-enol form (**3**) is formed by the addition of proton at N_{13}-atom of phenethicillin lactim-enol form (**2**), with increasing bond lengths at C_{14}-N_{13}, O_{16}-C_{14}, H_{45}-O_{16}, N_{13}-C_{11} and C_{11}-C_9 and decreasing bond lengths at C_9-N_7 and O_{33}-C_8. The conformations of $C_{15}C_{14}N_{13}C_{11}$, $C_{18}C_{15}C_{14}N_{13}$, $O_{16}C_{14}N_{13}C_{11}$, $O_{17}C_{15}C_{14}N_{13}$, and $H_{35}O_{12}C_9N_7$ are changed respectively from *+ap* to *-ap*, *+sp* to *-sc*, *+sp* to *-sp*, *+ac* to *+sc* and *+sp* to *–sp* conformations 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 *+sc* conformation. If the mono-protonated phenethicillin lactim-enol form (**4**) is formed by the addition of proton at N_7- atom of phenethicillin lactim-enol form (**2**), with increasing bond lengths at C_9-N_7, H_{35}-O_{12} and C_{14}-N_{13} and decreasing bond lengths at N_{13}-C_{11}, O_{16}-C_{14}, and O_{33}-C_8. The change of dihedral angle of $O_{10}C_8C_4C_3$, $C_{15}C_{14}N_{13}C_{11}$, $C_{18}C_{15}C_{14}N_{13}$, $O_{17}C_{15}C_{14}N_{13}$ and $H_{35}O_{12}C_9N_7$ are converted from *-ac* to *-ap*, *+ap* to *-ap*, *+sp* to *-sc*, *+ac* to *+sc* and *+sp* to *+ac* conformations respectively and all other conformations are unaltered. It is observed that the protonation at N_7-atom is shown *-ap* conformation. In the case of formation of di-protonated phenethicillin

lactim-enol form (**5**), it is found that the dihedral angle of $C_{18}C_{15}C_{14}N_{13}$, $O_{16}C_{14}N_{13}C_{11}$ and $O_{17}C_{15}C_{14}N_{13}$ are changed conformation from +*sp* to -*sc*, +*sp* to -*sp* and +*ac* to +*sc* conformations respectively. It is also investigated that the protonation at N_7- atom and N_{13}-atom are shown respectively -*ac* and +sc conformations to form stable di-protonated phenethicillin lactim-enol form (**5**).



Scheme - 5

The anion (**6**) is formed with the removal of a proton on O_{10}- atom of phenethicillin lactim-enol tautomer (**2**), and the change of conformation from -*ac* of $C_{14}N_{13}C_{11}C_9$, +sp of $C_{18}C_{15}C_{14}N_{13}$, +*sp* of $O_{16}C_{14}N_{13}C_{11}$, +*ac* of $O_{17}C_{15}C_{14}N_{13}$, +*sp* of $H_{35}O_{12}C_9N_7$ and –*sp* of $H_{45}O_{16}C_{14}N_{13}$ are changed to –*ap*, -*sc*, -*ap*, +*sc*, -*sp* and +*sp* conformations respectively to form stable anion (**6**) and rest of positions have moderate changes.

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 phenethicillin(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)	-125.9658	-94.1242	+43.9985	+63.6192	+287.1238	-138.2300
Ionization potential (eV)	9.1224	8.6172	12.0709	11.4400	14.1965	5.1978
μ (Debye)	3.1088	2.0959	4.2465	8.1453	9.6145	15.3094
E_{HOMO} (eV)	-9.122	-8.617	-12.071	-11.440	-14.196	-5.198
E_{LUMO} (eV)	+0.099	-0.212	-4.617	-4.610	-8.476	+2.348
Electron excitation energies (eV)	9.221	8.405	7.354	7.830	5.720	7.546
S_2	+0.0576	+0.0578	+0.0915	+0.1734	+0.2231	-0.0749
N_7	-0.2402	-0.1624	-0.1530	-0.0190	-0.0352	-0.1685
N_{13}	-0.3507	-0.1877	-0.1096	-0.2391	-0.1794	-0.0969
O_{10}	-0.2865	-0.3227	-0.3555	-0.3211	-0.3719	-0.5793

O_{12}	-0.2363	-0.2000	-0.1792	-0.1527	-0.1397	-0.2224
O_{16}	-0.3522	-0.2945	-0.2046	-0.2586	-0.1660	-0.3293
O_{17}	-0.2261	-0.2096	-0.2100	-0.1983	-0.2109	-0.2038
O_{33}	-0.3519	-0.3511	-0.2963	-0.2778	-0.2181	-0.5297

Table – XV Bond lengths of phenethicillin(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	1.4491	1.4635	1.4533	1.5179	1.5059	1.4514
C_{11}-C_9	1.5696	1.3799	1.3892	1.3761	1.3772	1.3975
N_{13}-C_{11}	1.4125	1.3717	1.3836	1.3484	1.3819	1.3624
C_{14}-N_{13}	1.3831	1.2992	1.3348	1.3048	1.3469	1.5218
O_{16}-C_{14}	1.2443	1.3772	1.3335	1.3634	1.3242	1.3931
O_{12}-C_9	1.2176	1.3471	1.3301	1.3343	1.3237	1.3297
O_{33}-C_8	1.2334	1.2343	1.2289	1.2263	1.2218	1.2569
O_{10}-C_8	1.3583	1.3611	1.3628	1.3587	1.3622	1.2631
H_{34}-O_{10}	0.9731	0.9729	0.9753	0.9762	0.9809	- -
H_{35}-O_{12}	- -	0.9745	0.9807	0.9798	0.9863	0.9886
H_{45}-O_{16}	- -	0.9772	1.0006	0.9773	0.9945	0.9741
H-N_7	- -	- -	- -	1.0193	1.0256	- -
H-N_{13}	- -	- -	1.0085	- -	1.0159	- -

Table – XVI: Dihedral angle (^{o}) of phenethicillin (1) and its lactim-enol form (2), mono-protonated forms (3 & 4), di-protonated form (5), and anion (6) from AM1 calculations.

Dihedral angle (^{o})	1		2		3		4		5		6	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-21.06	*-sp*	-21.17	*-sp*	-20.35	*-sp*	-26.29	*-sp*	-23.03	*-sp*	-18.99	*-sp*
$C_8C_4C_3S_2$	+163.25	*+ap*	+162.70	*+ap*	+162.52	*+ap*	+160.46	*+ap*	+159.59	*+ap*	+159.72	*+ap*
$O_{10}C_8C_4C_3$	-173.78	*-ap*	-129.60	*-ac*	-110.51	*-ac*	-154.13	*-ap*	-144.71	*-ac*	-110.84	*-ac*
$C_{14}N_{13}C_{11}C_9$	-126.91	*-ac*	-139.40	*-ac*	-126.03	*-ac*	-139.44	*-ac*	-122.18	*-ac*	-163.28	*-ap*
$C_{15}C_{14}N_{13}C_{11}$	+170.33	*+ap*	+179.47	*+ap*	-179.63	*-ap*	-174.36	*-ap*	+176.48	*+ap*	+179.61	*+ap*
$C_{18}C_{15}C_{14}N_{13}$	-67.73	*-sc*	+7.31	*+sp*	-40.78	*-sc*	-51.33	*-sc*	-63.79	*-sc*	-53.45	*-sc*
$O_{16}C_{14}N_{13}C_{11}$	+0.85	*+sp*	+2.04	*+sp*	-1.11	*-sp*	+7.04	*+sp*	-2.91	*-sp*	-1.55	*-ap*
$O_{17}C_{15}C_{14}N_{13}$	+50.06	*+sc*	+124.99	*+ac*	+76.75	*+sc*	+65.75	*+sc*	+53.53	*+sc*	+63.29	*+sc*
$O_{33}C_8C_4C_3$	+11.66	*+sp*	+53.39	*+sc*	+69.23	*+sc*	+30.68	*+sc*	+38.41	*+sc*	+69.36	*+sc*
$H_{35}O_{12}C_9N_7$	- -	- -	+23.83	*+sp*	-5.16	*-sp*	+90.06	*+ac*	+16.45	*+sp*	-18.94	*-sp*
$H_{34}O_{10}C_8C_4$	+179.98	*+ap*	-178.76	*-ap*	-179.51	*-ap*	-178.91	*-ap*	-171.85	*-ap*	- -	- -
$H_{45}O_{16}C_{14}N_{13}$	- -	- -	-4.84	*-sp*	-3.26	*-sp*	-3.90	*-sp*	-1.10	*-sp*	+18.55	*+sp*
$HN_7C_4C_3$	- -	- -	- -	- -	- -	- -	-152.35	*-ap*	-148.61	*-ac*	- -	- -
$HN_{13}C_{11}C_9$	- -	- -	- -	- -	+53.49	*+sc*	- -	- -	+57.79	*+sc*	- -	- -

* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs.[12]

10. CONCLUSIONS

Phenethicillin is widely used in the treatment of gonorrhoea and the chemotherapy of bacterial infections compared with other semi-synthetic penicillin derivatives. It has a high order of selective toxicity to micro-organisms which are pathogenic to human beings without obvious side effects. Austin Model-1 (AM1) is one of the semi-empirical method, which includes experimental parameters and extensive simplification of the Schrodinger's equation ($H\Psi=E\Psi$) 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 phenethicillin (**1**), and its lactim form (**2**), enol form (**3)** and lactim-enol form (**4**) of phenethicillin are existed in *anti*- or *syn*- conformations. The tautomeric equilibrium is increased in the order of $\log K_{T4} < \log K_{T3} < \log K_{T1} < \log K_{T2} < \log K_{T5}$, at the time of tautomeric conversion of **3** ↔ **4, 2** ↔ **4, 1** ↔ **2, 1** ↔ **3,** and **1** ↔ **4** respectively. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **4 < 3 < 2 < 1**. . Ionization potential (IP) is increased in the order of **3 < 4 < 2 < 1**. The dipole moment is increased in the order of molecules **4** < **2** < **3** < **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 > **3** > **2** > **1**.
2. Phenethicillin (**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 **1** < **4** < **2** < **3** < **5**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of 4 > **3** > **5** > **2** > **1**.
3. Phenethicillin (**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** < **5** < **4** < **3** < **6**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **5** > **6** > **3** > **4** > **2** > **1**.
4. Phenethicillin (**1**) and its phenethicillin-enol (**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** < **4** < **3** < **5**. The dipole moment is increased in the order of **3** < **2** < **1** < **5** < **4** < **6**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **5** > **4** > **6** > **3** > **2** > **1**.

5. Phenethicillin (**1**) and its phenethicillin lactim-enol (**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 **2** < **1** < **3** < **4** < **5** < **6**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **5** > **3** > **6** > **4** > **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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