Research Article

AM1 Study on Electronic Structure and Conformations of Keto-enol Tautomerism in Phenethicillin

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ABSTRACT

The keto-enol tautomerism in phenethicillin and its geometry, conformation and electronic structure have been optimized and calculated in the gas phase using semi-empirical molecular orbital AM1 method usually considering an isolated molecule surrounded by vacuum. The mechanism of protonation in enol tautomer of phenethicillin has been studied by comparison of the different positions of net charges at nitrogen atoms in the molecule. Further, 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 performed. The conformational analyses of optimized mono- and di-protonated enol tautomers have also been discussed.

Keywords: AM1, keto-enol tautomerism, phenethicillin, induction effect.

INTRODUCTION

Phenethicillin has been used for the prevention of bacterial infections in patients with haematological malignancies[1,2]. Phenethicillin is readily soluble in water, stable in acid media, and well absorbed from the gastrointestinal tract[3]. Phenethicillin is less rapidly inactivated by staphylococcal penicillinase[4,5]. The tautomeric equilibria have been recognised for the study of the processes of both organic chemistry and biochemistry[6]. The tautomerism of organic compounds was reported extensively theoretical and statistical-physical approaches[7]. Theoretical models of the salvation energies of tautomers[8], the stability of tautomers[9,10] and equilibrium constants in electrostatic reaction field for heterocyclic compounds in aqueous solution[11] were studied. It is assumed that dipolar character of the drug could improve oral absorption[12].

Austin Model-1 (AM1) is one of the semi-empirical quantum calculation methods[13,14] based on the neglect of differential diatomic overlap integral approximation. It includes experimental parameters with extensive simplification of the Schrodinger's equation (HΨ=EΨ) to optimize molecules for simulating chemical structure and reactions numerically studying chemical phenomena through the calculations on computer instead of examining reactions experimentally[15]. It is important to know the conformational changes in the molecule for the prediction of its reactivity and pharmacological action. These observations together with earlier work on phenethicillin conformational analyses and electronic structure[16,17] prompted us to incorporate the study of keto-enol tautomerism in phenethicillin. It has attracted much to carry out optimization of protonated forms with a view to evaluate the polarity.

The present study reveals on molecular conformation and electronic properties of phenethicillin (**1**) and its tautomerism in gas phase usually considering an isolated molecule surrounded by vacuum has been evaluated by AM1 method. Keto-enol tautomerism of phenethicillin involves the shifting of hydrogen atom from α-carbon atom of keto (-HC-C=O) group to the oxygen atom in the same molecule to form enol (-C=C-O-H) group as shown in Figure-2. It is also observed that the keto-form is less stable than phenethicillin. From the obtained optimized electronic structure of keto-enol tautomerism of phenethicillin, the mechanism of protonation has been studied by comparison of the relative values of net charges at nitrogen atoms in different positions of the molecule. Taking enol

form of phenethicillin as a neutral molecule (**2**), the molecular geometry and conformations of mono-protonated (**3** & **4**), di-protonated (**5**) and anion (**6**) systems have been determined by full optimization calculations using semi-empirical molecular orbital AM1 method.

Computational methods[13,14]

Semi-empirical molecular orbital calculations (Austin Model-1, (AM1)) were performed on the molecules shown in Scheme-1 using the MOPAC93 in WinMOPAC ver 5.13 program by means of Intel Dualcore D102GGC2 DDR2 1GB SDRAM PC. 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 lowest energy conformation was found. The position of the atom in the molecule is mentioned as subscript (as shown in Figure-1). The initial molecular geometry was adopted as Pople's standard data[18,19], and subsequently fully optimized using an energy gradient method. The conformations were designated by Klyne-Prelog terms[20] using *s* = syn, *a* = anti, *p* = peri-planar (0 ± 30^0 & 180 ± 30^0) and all other angles *c* = clinal.

RESULTS AND DISCUSSION

Electronic structure of phenethicillin (1) and its enol tautomer (2) mono-protonated (3 & 4), di-protonated (5) and anion (6)

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-1. In this context, the numbering of enol form of phenethicillin (**2**) 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 **6**) are presented in Table-I. It is observed that the net charges on N_7- and N_{13}- atoms are -0.1449 and -0.2760 respectively in the case of phenethicillin enol-form (**2**). It is also investigated that the sequence of protonation for nitrogen atoms of phenethicillin enol-form (**2**) is increasing in the order of $N_7 < N_{13}$. Thus, N_{13}- atom is predicted to be main protonation site of phenethicillin enol-form (**2**), according to the negative charge distribution on nitrogen atoms.

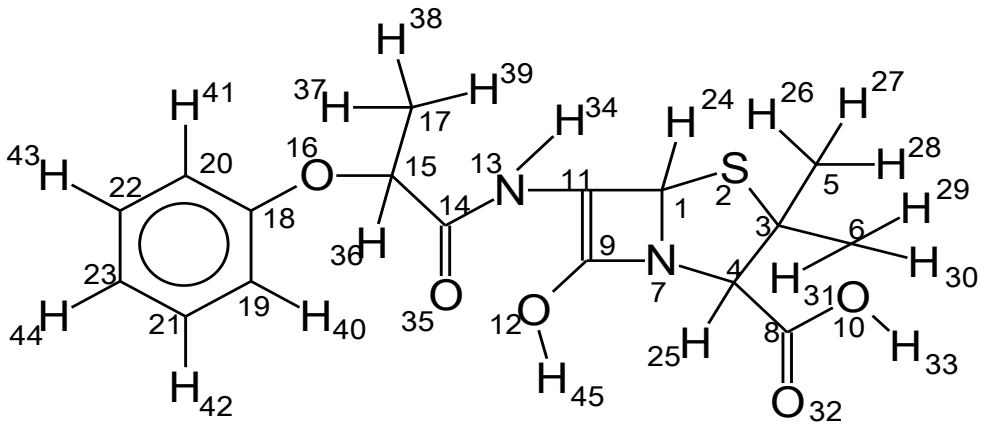


Figure - 1

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 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 **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[21].The dipole moment of molecules depends on the nature of bonds and the atoms arrangement in the molecule. The dipole moment is increasing in the order of molecules **3** < **2** < **1** < **5** < **4** < **6**. Anion (**6**) shows higher dipole moment. The electronegative heteroatoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[22] (μ_{ind}) of molecules can be estimated with respect to

phenethicillin enol form (**2**). It is found that the induction effect is 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 form (**1**). 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**. But geometry calculations in the ground state were completely optimized until the lowest energy conformation was found in the individual ions or molecules. It can be assumed that the electronic properties and reactivity of the molecule depend on its conformational structure. It is predicted that the protonation would take place preferably at N_{13}-atom than N_7-atom in the case of phenethicillin enol-form (**2**). It is confirmed that the stability of mono-protonated enol form of phenethicillin **4** (ΔH_f^o, +55.2126 kcal/mol) is more stable than **3** (ΔH_f^o, +58.6352 kcal/mol). The enol form of di-protonated phenethicillin **(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**). The protonation at N_{13}- atom in the case of enol form of phenethicillin (**2**) to mono-protonated form (**3**) is considered by 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.

The acid – base equilibrium of phenethicillin enol form and its protonated forms

Equilibrium is normally established in polar solvents, in order to investigate the basicity and it is found out the protonation sites of enol form of phenethicillin (**2**) as per Scheme-1. N_{13}-atom is main basic centre in accordance with the negative charge distribution on nitrogen atoms (Table-1). Ionization potential (IP) is increasing in the order of **6 < 2 < 1 < 4 < 3 < 5**. To determine the exact protonation centres of enol form of phenethicillin (**2**), the proton affinities (PA) for the different nitrogen atoms of the molecule have been calculated through AM1 method. 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[23]. It is also observed the reactivity, which is decreased in the order of **5** > **4** > **6** > **3** > **2** > **1**. It is confirmed that phenethicillin (**1**) is more stable than its enol-form (**2**). It indicates that di-protonated enol form of phenethicillin (**5**) is more reactive. The stable conformation of the cations formed by the protonation of each nitrogen atom of the molecule is determined; the heats of formation are calculated with full geometry optimization. The cations formed by the protonation at N_7- or N_{13}- atoms of enol form of phenethicillin (**2**) can exist in *anti*- or *syn*-conformations, according to the position of nitrogen atoms as shown in Scheme-1. Its conformation can be assigned by comparison of its geometry and electronic structure. The proton affinity (PA)[24] values for the different nitrogen atoms of enol form of phenethicillin RH (**2**) were calculated by using the equation (1) 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.

$$\mathbf{PA=\Delta H_f^o(H^+) + \Delta H_f^o(B) - \Delta H_f^o(BH^+) \; . \; (1)}$$

Where PA is the proton affinity, ΔH_f^o(B) is the heat of formation for enol form of phenethicillin (**2**), $\Delta H_f^o(BH^+)$ 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 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. All cations are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium.

The conformations of keto-enol tautomerism in phenethicillin

Figure - 2 illustrates the formation of two tautomeric forms of phenethicillin (**1**), which may possible at chemical equilibrium under ordinary conditions. Instances are known when tautomeric forms are stable under ordinary conditions, which are capable of inter-conversion at higher temperatures, frequently in the presence of catalyst. Fully optimized AM1 calculations scrutinize only the main data of bond lengths (Table-II) and dihedral angles (Table-III) of molecules (**1** to **6**) for the sake of simplicity.

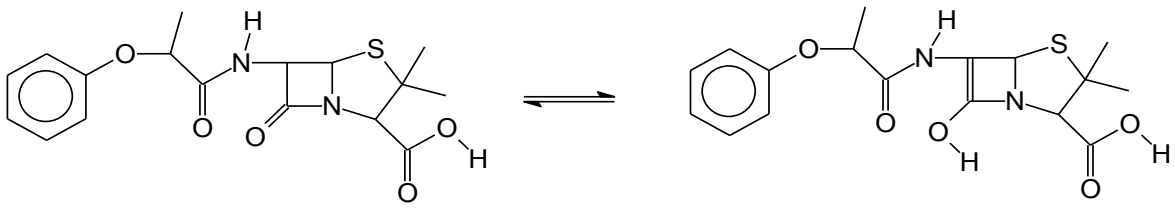


Figure - 2 : Keto - enol tautomerism in Phenethicillin

The tautomeric equilibrium constants $\log K_T$ was calculated[25,26] from the heat of formation, according to the equation (2):

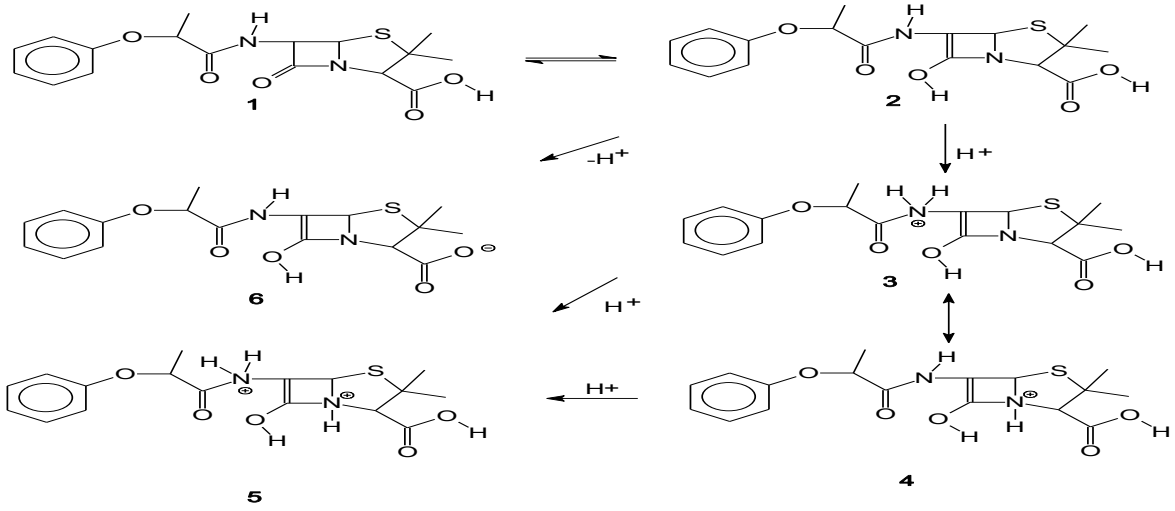
$$\log K_T = \frac{\Delta GT}{2.303\ R\ T} \approx \frac{\delta \Delta H_f^{o}}{2.303\ R\ T} \quad \text{- - - (2)}$$

Where ΔGT 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. From this equation (2), $\log K_T$ value was calculated as 18.376.

From the Table-II, Table-III, Figure-2 and Scheme-1, it is observed that phenethicillin (**1**) would undergo keto-enol tautomerism and form enol of phenethicillin (**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 $H_{45}O_{12}C_9N_7$.

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

The spatial arrangement of atoms in a molecule was considered to study the conformations of phenethicillin (**1**), and its enol form of phenethicillin (**2**), mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) with a view to investigate molecular deformations. These can exist in *anti-* or *syn-*conformation, according to the change in energy content of the protonation and also depend on the changes in the parameters of dihedral angles. Fully optimized AM1 calculations scrutinize only the main data of bond lengths (Table-II) and dihedral angles (Table-III) of molecules (**1** to **6**) for the sake of simplicity.



Scheme - 1

From the Table-II, and Table-III, it is observed that as per Scheme-1, mono-protonated enol form of phenethicillin (**3**) is formed by the addition of proton at N_{13}-atom of enol tautomer of phenethicillin (**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. It is also investigated that the protonation at N_7- atom and N_{13}-atom are shown ***-ac*** conformations to form stable 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.

CONCLUSION

Semi-empirical AM1 calculations have been actually done on the single molecule in the gaseous state contrary to the experimental values in the presence of intermolecular interactions in the case of keto-enol tautomerism of phenethicillin and its protonated forms with the sequence of proton transfer at nitrogen atom is N_{13}> N_7. Electronic excitation energies between different forms suggest that significant conformations inter-conversions can take place. In order to gain insight into the structure of phenethicillin, it is desirable to perform rigorous theoretical analysis on the different geometries of phenethicillin and its protonated forms. The utility of theoretical predictions is important for evaluating the biochemical mechanism to prevent cell wall synthesis and binding to plasma protein. This study reveals about the stability of keto-enol tautomerism, conformations and molecular deformations.

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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 (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
O_{35} (atomic charge)	-0.3522	-0.3510	-0.1502	-0.3005	-0.1558	-0.3578

Table II: 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 III: Dihedral angle (°) 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 (°)	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*
$C_5C_4C_3S_2$	+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[35].

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