Bull. Chem. Soc. Ethiop. **2023**, 37(1), 91-100.
DOI: https://dx.doi.org/10.4314/bcse.v37i1.8

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

PREPARATION AND CHEMICAL STUDY ON METALLIC BONDING WITH ANTIBIOTICS IN NANOMETER FORM TO RAISE THE THERAPEUTIC EFFICIENCY: STRUCTURAL CHARACTERIZATIONS OF Cu(II), Co(II) AND Ni(II) 6-AMINOPENICILLINIC ACID COMPLEXES

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(Received July 20, 2022; Revised August 8, 2022; Accepted August 9, 2022)

ABSTRACT. The medical and pharmaceutical areas are interested in the production of metal-biomolecule complexes because to their strong biological activity against bacteria, fungus, and cancers. The purpose of this paper was to synthesise of three coloured transition metal complexes of copper(II), cobalt(II), and nickel(II) with a 6-aminopenicillinic drug ligand (6-apen). The analyses of elemental analysis (carbon, hydrogen, and nitrogen), molar conductivity, magnetic susceptibility, infrared (FTIR), and electronic (UV-Vis) spectroscopy provided a thorough characterization of the solid complexes. The low molar conductance values of the produced solid complexes suggested that they were not electrolytic. The 6-apen drug ligand has more than one site of coordination as carboxylic group, β-lactam nitrogen, nitrogen of $–NH_2$ group, oxygen of carbonyl β-lactam group and sulfur of the five–member ring. The microanalytical, spectroscopic and thermogravimetric analyses indicated a 1:2 (Metal : Ligand) stoichiometry of all complexes. The 6-apen ligand acts as a bidentate ligand that the coordination site occurs through the oxygen of deprotonated carboxylic group and β-lactam nitrogen. The thermogravimetric analysis confirmed the absence of crystalline water molecules outside the coordination sphere. The anticancer availability of the 6-apen free drug was checked against of 2Q7K receptor prostate cancer using a molecular docking calculation.

KEY WORDS: 6-Aminopenicillinic, Coordination, Metals ions, FTIR, Molecular docking

INTRODUCTION

Consideration has been given to the use of some inorganic compounds as therapeutic agents and their incorporation into drugs [1], as well as the effect of metal ions on cells and living tissues. As a result, many experiments and research have been conducted to control the toxic and non-toxic concentrations of metal ions in living organisms by converting them to metallic complexes [2]. This requires knowledge of the shared properties of the metal ion and the ligand. These investigations set the stage for some biologically significant applied research [3]. The fundamental structure of penicillins consists of a five–member thiazolidine ring connected to a four–member β-lactam ring with an attached side chain group [4]. The resistance of bacteria to β-lactam antibiotics is a complicated function of the formation of β-lactamases, the capacity of some bacteria to exclude these antibiotics from their cells and lower the likelihood of β-lactams to attach to penicillin-binding proteins in target cells. The production of β-lactamase enzymes that break down penicillin and cephalosporins before they reach receptors renders bacteria resistant to β-lactams. As a result, the antibiotic cannot attach to the peptidoglycan layer [5]. 6-Aminopenicillinic acid is a crucial component in the -classes of lactam and is traded in the first antibiotics [4]. Minerals have a respected place in medical chemistry, and most antibiotics do not require mineral ions for their biological activities. However, there are a few antibiotics, such as bleomycin and bacitracin drugs, that require mineral ions to function properly and are more effective than pure medications [6]. This is because metal ions may interact with several types of biomolecules, including DNA, RNA, proteins, and lipids, resulting in their distinct and biological

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functions [7]. The octahedral geometry cloxacillin complexes of four transition metal ions Co, Ni, Cu, and Zn(II) have been synthesised, and spectroscopic investigation has verified that chelation occurs via oxygen of carboxylate and nitrogen of lactam groups. It was discovered that the efficacy of free cloxacillin increased following complexation with metal ions [8]. Early in the twenty-first century, Weiss *et al.* using a penicillin benzyl drug ligand, were the first to create mineral drug complexes to determine their activity against organisms and their mode of action based on the link between structure and activity. It has been postulated that the oxygen of the carboxyl group and the nitrogen of the β-lactam group coordinate these complexes [9]. This study identified the hypothesised structures of copper, cobalt, and nickel(II) complexes of 6-aminopenicillinic drug ligand using various micro analytical and spectroscopic techniques. Additionally, transmission electron microscopy was used to examine the nanostructured form of the isolated solid complexes.

EXPERIMENTAL

Materials and instruments

The pure analytical grade of drug ligand 6-aminopenicillinic drug ligand (6-apen) and three anhydrous transition metal chloride salts ($CuCl_2$, $CoCl_2$ and $NiCl_2$) were obtained from Sigma-Aldrich Chemical Company in the United States. Table 1 is a summary of the instrumental analyses and their accompanying models.

Table 1. Types of instruments and their models.

Type of analysis	Models
Elemental analyses	Perkin Elmer CHN 2400
Conductance	Jenway 4010 conductivity meter
FTIR spectra	Bruker FTIR Spectrophotometer
Electronic spectra	UV2 Unicam UV/Vis Spectrophotometer
Magnetic moment	Magnetic Susceptibility Balance
Thermo gravimetric	TG/DTG–50H, Shimadzu thermo-gravimetric analyzer
TEM	JEOL 100s microscopy

Synthesis Cu(II), Co(II) and Ni(II) complexes

For the synthesis of Cu(II), Co(II) and Ni(II) complexes, a 6-apen ligand (0.433 g, 1 mmol) methanolic solution (25 mL) was mixed with 1 mmol of (0.135 g, 0.130 g and 0.130 g) of $CuCl_2$, $CoCl_2$ and $NiCl_2$, respectively in 10 mL of distilled water. The mixture was refluxed for three hours on a hotplate, and the pH was adjusted to 8 with a few drops of 10% ammonia. After cooling to room temperature, the colourful complexes were precipitated, filtered, and washed with methanol and distilled water. In desiccators, the complexes were dried over anhydrous calcium chloride. The elemental analysis and physical characteristics of the produced compounds were listed in Table 2. Solid isolated complexes have thermally stable qualities with melting points more than 250 degrees Celsius.

The analytical and molar conductance data of the 6-apen complexes are provided in Table 2. The analytical and conductance data indicate that all the metal complexes synthesized are non-electrolytes. The non-electrolytic nature of the metal complexes suggests that the anions of the salts have absent in the associations of metal complexes. The elemental analyses data agree well with the proposed formulae for the suggested 6-apen complexes. This is because the theoretically calculated percentage values are in close agreement with the experimental values obtained from elemental analysis. The elemental analysis agree well with a 1:2 metal:ligand stoichiometry for all the complexes (Table 2).

Table 2. Elemental analysis and physical properties of Cu, Co and Ni(II) complexes.

Complex	Colour	M.P (ºC)	Conductance ($ohm^{-1}.cm^2.mol^{-1}$)	Element	Calc.	Found
Cu(II)	Blue	>250	9	%C	36.25	36.19
				%H	4.94	4.91
				% N	10.57	10.50
				%Cu	11.99	11.94
Co(II)	Dark pink	>250	12	%C	36.57	36.45
				%H	4.99	4.69
				% N	10.66	10.56
				%Co	11.22	11.17
Ni(II)	Green	>250	10	%C	36.59	36.48
				%H	4.99	4.87
				% N	10.67	10.54
				%Ni	11.17	11.12

RESULTS AND DISCUSSIONS

Elemental analysis and molar conductance studies

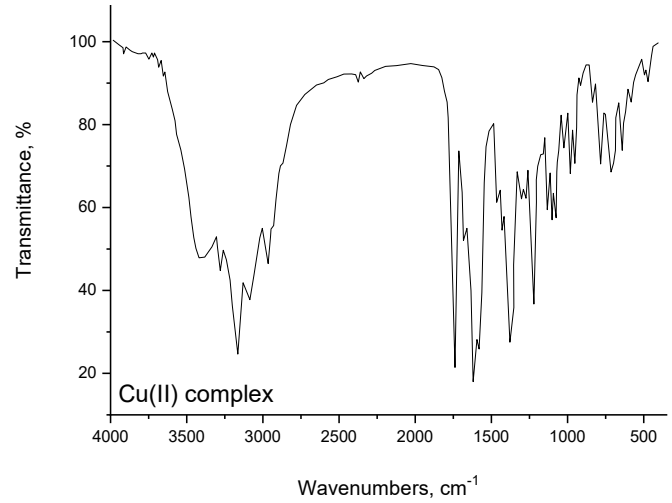
The elemental analysis Percentages (carbon, hydrogen, and hydrogen) of the solid produced Cu(II), Co(II), and Ni(II) 6-apen complexes played a crucial part in hypothesising the hypothesised structure and molecular formula alongside the metal analysis utilising an atomic absorption spectrometer. Table 2 demonstrates that computed results correspond to experimental data. Conductance behaviour (electrolyte/non-electrolyte) is identified by measuring the molar conductance of dissolved complexes in dimethyl sulfoxide at a concentration of 10^{-3} M. In DMSO solvent, all produced complexes are non-electrolytic. Table 2 lists these values, which fall within the non-electrolytic property range of 9-12 $ohm^{-1}.cm^2.mol^{-1}$ as described in the literature [10].

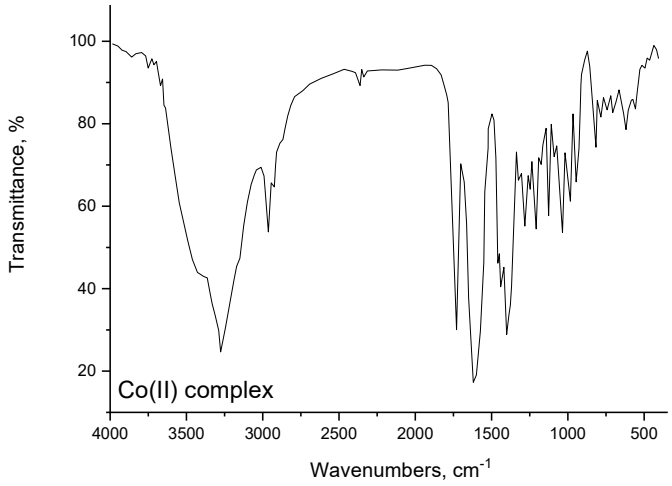
FTIR spectral study

Table 3 lists the IR frequencies of free 6-apen ligand and its complexes. The spectra of Cu(II), Co(II), and Ni(II) complex are shown in Figure 1. This band is absent in the spectra of Cu(II), Co(II), and Ni(II) complexes due to deprotonation. The infrared spectrum of the 6-apen free ligand exhibits a vibration band at 3400 cm^{-1} owing to the ν(OH) of the carboxylic group (Figure 1). In case of free ligand, the very strong and strong intensities bands at 1626 and 1740 cm^{-1} were assigned to the stretching vibrations of $\nu(C{=}O)_{carboxylic} + \delta(NH_2)$ and ν(C=O) of β-lactam moieties respectively, after chelations the band regarding ν(C=O) of β-lactam ring didn't affected but the absorption band of ν(C=O) carboxylic is absent in the spectra of all complexes indicating that carboxylic group was involved in complex formation. In all complexes, the stretching vibration of terminal amino ν(N-H) is moved to a higher frequency of 3090 cm^{-1}, indicating that the nitrogen atom of the amino group is far from the coordination site. Therefore, oxygen of β—lactam ring is not engaged in complexation; rather, coordination occurs via nitrogen atom of β-lactam ring, which exists at a favourable spot to form a stable five-membered ring due to its out-of-plane orientation relative to the other three carbon atoms in β-lactam ring [8, 9, 11]. The complexation through oxygen of deprotonated carboxylic group and nitrogen of β-lactam ring is verified by the existence of new stretching vibration bands (M-O) and (M-N) at 633-469 cm^{-1} in the spectra of complexes, in accordance with published findings [11, 12].

Table 3. Infrared spectral data (cm^{-1}) of Cu, Co and Ni(II) 6-apen complexes.

Compounds	Frequencies, cm^{-1}							
	ν(OH)	ν(NH)	ν(C=O) β-lactam	ν(C=O) carboxylic + δ(NH_2)	ν_{as}(COO)	ν_{s}(COO)	ν(M-O)	ν(M-N)
6-apen	3400	3012	1740	1626	-	-	-	-
Cu(II)	3281 3167	3091	1741	1621	1589	1377	583	469
Co(II)	3276	3090	1733	1621	1435	1282	618	555
Ni(II)	3326 3269	3088	1738	1616	1391	1210	633	557





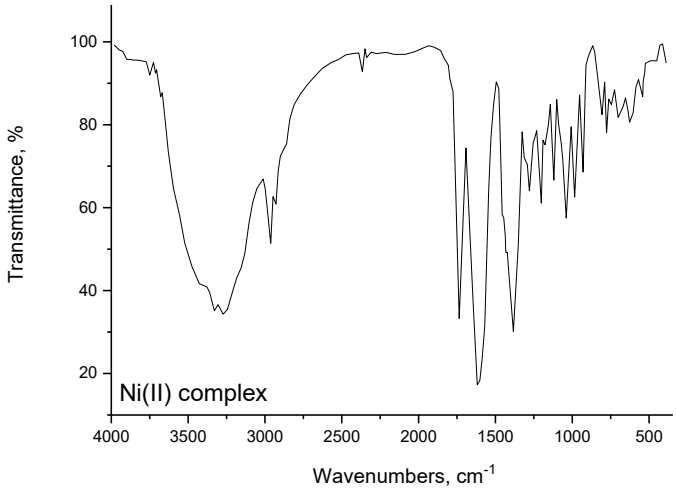


Figure 1. Infrared spectra of 6-apen complexes.

Electronic spectra and magnetic susceptibility

Within the region of 200-800 nm, the UV-Vis electronic spectra of the 6-apen ligand and copper, cobalt, and nickel(II) complexes was scanned in DMSO solvent. The absorption spectrum of free ligand has two peaks at 265 and 285 nm, which correspond to π-π* and n-π* transitions for aromatic ring and non-bonding electron pair of nitrogen, oxygen, and sulphur atoms of four- or five-member rings, respectively [13]. Due to the $^2E_g \rightarrow {}^2T_{2g}$ electronic transition the copper(II) complex possesses an absorption band at 16474 cm^{-1} in its electronic spectrum [13]. This finding confirmed the Jahn-Teller distortion of the octahedral geometry of the Cu(II) complex [14]. The experimental magnetic susceptibility of copper(II) complex is 1.68 B.M. confirming the deformed octahedral structure (Figure 2). Two absorption bands at 18248 and 16181 cm^{-1} may be ascribed to $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ and $^4T_{1g}(F) \rightarrow {}^4A_{2g}$ (F), respectively, in the electronic spectra of cobalt(II) complex. The magnetic susceptibility of cobalt(II) complex is 4.96 B.M., which corresponds to an octahedral geometry as seen in Figure 2 [15]. Due to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ and $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ electronic transition, nickel(II) complex showed two absorption bands at 23256 and 14598 cm^{-1} in its electronic spectra [16]. The magnetic susceptibility of the nickel(II) complex was determined to be 3.02 B.M. which is within the typical range for Ni(II) complexes with an octahedral geometry (Figure 2).

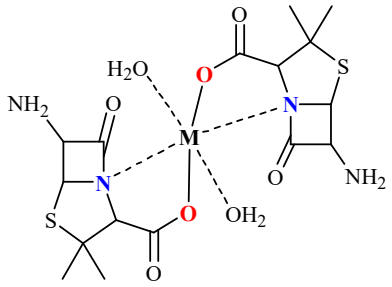
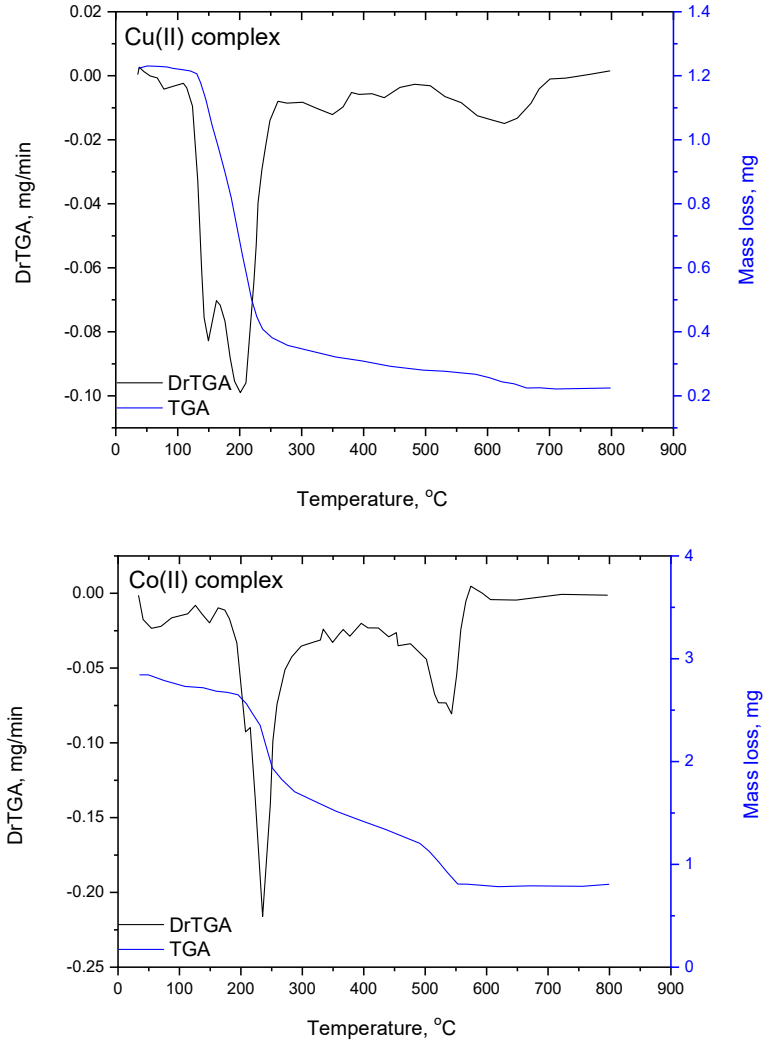


Figure 2. Speculated structures of Cu(II), Co(II) and Ni(II) 6-apen complexes.

Thermogravimetric analysis

In the current investigation, heating rates were appropriately controlled at 10 ºC/min under inert N_2 gaseous atmosphere, and the mass losses were calculated from the room temperature till 800 ºC. The loss of the two coordinated water molecules are observed at high temperatures within range of 150-300 ºC with endothermic differential thermo gravimetric peaks at 149, 152, and 257 ºC for Cu(II), Co(II) and Ni(II) complexes respectively. In the thermograms of TGA and DrTGA of complexes $[Cu(6\text{-apen})_2(H_2O)_2]$, $[Co(6\text{-apen})_2(H_2O)_2]$, and $[Ni(6\text{-apen})_2(H_2O)_2]$ (Figure 3) an endothermic peak with mass loss in the range of 120-300 ºC is assigned to loss of coordinated water molecules and gradual mass loss in the range of 300-800 ºC is assigned to the start of decomposition of 6-apen ligand molecules. The residue at 800 ºC indicates the set of metal oxides polluted with few nonoxidized carbon atoms.



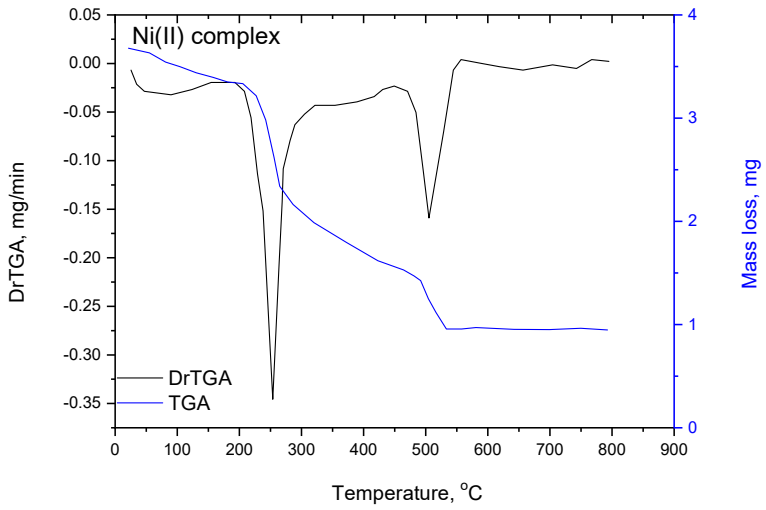
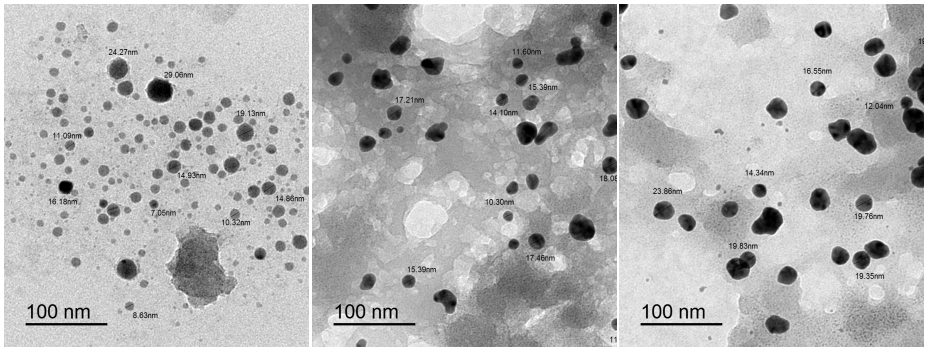


Figure 3. TG-DrTGA curves of 6-apen complexes.

Morphological analysis

The transmission electron microscopy TEM images of the Cu(II), Co(II) and Ni(II) 6-apen complexes are showed in Figure 4, these nanostructured complexes contains a spherical black spots particle size within 7-30 nm, 10-18 nm, and 12-29 nm, respectively.



Cu(II) complex Co(II) complex Ni(II) complex

Figure 4. TEM images of 6-apen complexes.

Molecular docking study

6-Aminopenicillinic drug ligand was used to carry out molecular docking studies against 2Q7K receptor prostate cancer to determine the best method of reaction. The binding mode of 6-aminopenicillinic acid with the corresponding protein were discussed. Docking interactions are given in Tables 4-6. In a docking study it shows a positive interaction between 6-aminopenicillinic

acid and receptor according to the calculated energy as listed in Tables 4-6. The HB plot curve indicates that, the 6-aminopenicillinic acid compound binds to the 2Q7K protein with hydrogen bond interactions and decomposed interactions energies in kcal/mole exist of 6-aminopenicillinic acid with 2Q7K (Tables 4-6). The 6-aminopenicillinic acid possesses a high pKI for the 2Q7K receptor. The computed efficiency is advantageous, the AutoDock-estimated Ki values were compared to experimental Ki values where available, and the Gibbs free energy is negative. Based on these findings, it is also plausible that the 2Q7K receptor and 6-aminopenicillinic acid interact. It is obvious from the value analysis that the binding energy of 6-aminopenicillinic acid diminishes. Therefore, a decrease in binding energy as a result of mutation will improve the binding affinity of 6-aminopenicillinic acid for the receptor. The distinctive property of these compounds is the presence of many active hydrogen-bonding sites. This property enables them to be effective protein-binding inhibitors and contributes to the production of enhanced inhibitory molecules. This interaction may initiate apoptosis in cancer cells through the energy of interactions with 6-aminopenicillinic acid. Thus, a decrease in binding energy because of mutation will improve the binding affinity of chelates to the receptor [17-22].

Table 4. Decomposed interaction energies in kcal/mol for 6-aminopenicillinic acid and 2Q7K receptor.

Polar	Other
GLN792 (-2.1123)	SER759 (-0.9361)

Table 5. Interaction between 6-aminopenicillinic acid and 2Q7K receptor.

Hydrogen bonds	Polar	Hydrophobic	Cation-pi	Other
N(13) - GLN792 [2.87] (CB, CG, NE2, O)	O(14) - GLN792 [3.51] (NE2)	CA(12) - TRP796 [3.78] (CD1)	1H(24) - TRP796(CD1, [2.73] CD2, CE2, CG)	O(14) - SER759 [3.59] (CB)
O(14) - TRP796 [3.15] (NE1)	OXT(10) - GLN792 [3.81] (NE2)		2H(25) - TRP796 [3.73] (CE2)	C(11) - ARG760 [3.84] (CG)
	N(13) - TRP796 [2.79] (NE1)			1H(24) - GLN792 [3.70] (CB)
	1H(24) - TRP796 [2.92] (NE1)			2H (25) - GLN792 [2.47] (CB, CG)
	2H(25) - TRP796 [3.63] (NE1)			C(11) - GLN792 [3.38] (CG, NE2)
				O(14) - GLN792 [3.80] (CG)
				CA(12) - GLN792 [3.59] (NE2)
				N(13) - TRP796 [3.09] (CD1, CD2, CE2, CG, CZ2)
				C(11) - TRP796 [3.33] (NE1)
				CA(12) - TRP796 [3.37] (NE1)

Table 6. Docking calculations for the 6-aminopenicillinic acid and 2Q7K receptor.

Est. free energy of binding	Est. inhibition constant, Ki	vdW + Hbond + desolv Energy	Electrostatic energy	Total intermolec. energy	Frequency	Interact. surface
-3.35 kcal/mol	3.52 mM	-3.92 kcal/mol	-.0.02 kcal/mol	-3.94 kcal/mol	40%	362.226

ACKNOWLEDGEMENTS

This work was supported by grants from Deanship of Scientific Research, Taif University, Saudi Arabia under project Grants No. 67-442-1.

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