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Synthesis, structure and bioactivity of a mononuclear octahedral [Prolinate₂-Na(MeOH)₄]⁻H⁺ complex

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ABSTRACT

The synthesis of a mononuclear octahedral [Prolinate₂-Na(MeOH)₄]⁻H⁺ complex is achieved from a [3+2] cycloadduct with excess of NaOMe in methanol. The slow crystallization provides the hexacoordinated complex stabilized by four molecules of solvent. The antibacterial, antifungal and anti-tuberculosis activity of this compound is also evaluated.

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The hydrophilic nature of sodium cation is the reason why they are frequently coordinated to water {the most common being [Na(H₂O)₆]⁺}. However, other solvents as alcohols, DMF or DMSO are not able to form by themselves pure sodium complexes. It is known that sodium cation improves the solubility and efficiency of certain drugs, for example, dried naproxen sodium-chitosan complex (NPXF) was not corrosive to the gastric mucosa.[1]

Sodium forms many complexes with oxygen-based moieties as crown ethers,[2] cryptands,[3] calixarenes[4] and other related ligands.[5] Also polydentate ligands promote supramolecular associations with sodium, for instance, benzothiazole derivatives,[6] salen ligands,[7,8] star-shaped polyethers,[9] porphyrin derivatives,[10] etc.

Although less common than transition metal complexes, sodium generates mononuclear complexes[11] with other ligands.[12] Searching in the literature, polymeric bis-tetrazole moieties generated octahedral hydrated sodium complexes,[13] DMF and water stabilized distorted octahedral sodium complex,[14] other sodium cation was surrounded by three units of 7-acetyl-2-benzo[b]furancarboxylic acid,[15] and DMSO was a crucial ligand in the stabilization of the cluster [Na₄(DMSO)₁₅]⁴⁺[(I₃)₃(I)] based on octahedral metal cations.[16]

To the best of our knowledge, a simple and mononuclear hexacoordinated sodium complex, partially complexed by alcohols are not frequently reported. So, in this work we introduce a [prolinate₂-Na(MeOH)₄]⁻H⁺ complex, which was obtained and isolated during the synthesis of new bioactive substances.[17,18] The reaction of pyrrolidine **1**, which was synthesized from 1,3-dipolar cycloaddition of the corresponding methyl arylideneaminotryptophanate with *N*-phenylmaleimide (NPM),[17,18a] in the presence of NaOMe (2.5 equiv) in refluxing methanol for 36 h allowed the access to a rearranged highly substituted structurally diverse fused bicyclic pyrrolidine **2** in a regio- and stereospecific manner. A second hydrolysis took place affording cycloadduct **3** (Scheme 1). The very slow evaporation of methanol at room temperature and final recrystallization furnished transparent crystals of the resulting mononuclear octahedral sodium complex **4**. It was isolated and analysed by X-ray diffraction analysis.

Compound **4** was crystallised as colourless prisms in the P₁ space group with half a molecule in the asymmetric unit. The molecular structure of compound **4** is shown in Figure 1 and crystal data are depicted in Table 1.[19] Selected bond lengths and angles are shown in the supporting information. The sodium atom lied on a centre of inversion and exhibited distorted octahedral geometry, with *cis*-

bonds in the literature. Na-O=C bond lengths of 1.987(2) Å were shorter than most similar bonds reported, although some short bond lengths were observed. Intramolecular hydrogen bonding between the bound methanol molecules and amide oxygen atoms was observed, with O-O distances of 2.563(3) and 2.917(3) Å. Also, hydrogen bonding between the carbonyl group of the carboxylic acid and a H of one of the methanol molecules placed in the base of the bipyramid. In order to balance the extra negative charge of the sodium atom an additional proton is necessary, such as it is indicated in the empirical formula (Table 1). This hydrogen atom did not appear in the X-ray diffraction pattern and also could not be detected in the ^1H NMR spectra (14-16 ppm).

Scheme 1. Synthesis of sodium complex 4

Intermolecular $\pi \cdots \pi$ stacking between dichlorophenyl and *N*-phenyl rings (Figure 2) led to the formation of chains of molecules along the [101] direction, with the overall solid-state structure shown in Figure 1 (see, SI). If we analyse the dotted lines marking the $\pi \cdots \pi$ stacking in this Figure 2, we can observe that the two nitrogen atoms of different pyrrolidine moieties are separated by a distance of 5 Å. This is the most basic moiety of the complex and the proton can be delocalized in this area. It is known that a shared proton by two nitrogen atoms of two cytidines is located precisely in the midpoint between them when the N-N distance is 2.5 Å. However, with higher N-N distances the proton is bonded to one nitrogen atom and undergoes fluctuations inside of this basic cage and non-hydrogen bonding is observed.²⁰

at the 50% level.

Table 1: Summary of crystal and refinement data for Compound **4**.

Empirical formula	C ₆₂ H ₆₄ Cl ₄ N ₆ NaO ₁₄
Formula weight	1282.99
Temperature/K	293(2)
Crystal system	triclinic
Space group	P-1
a/Å	11.4834(12)
b/Å	12.4494(13)
c/Å	13.2719(13)
α/°	117.096(5)
β/°	107.230(5)
γ/°	97.871(6)
Volume/Å ³	1528.6(3)
Z	1
ρ _{calc} /g/cm ³	1.394
μ/mm ⁻¹	0.272
F(000)	670.0
Crystal size/mm ³	0.934 × 0.588 × 0.528
Radiation	Mo-K _α (λ = 0.71073)
2θ range for data collection/°	3.732 to 57.132
Index ranges	-15 ≤ h ≤ 15, -16 ≤ k ≤ 15, -17 ≤ l ≤ 17
Reflections collected	35619
Independent reflections	7704 [R _{int} = 0.0471, R _{sigma} = 0.0418]
Data/restraints/parameters	7704/85/452
Goodness-of-fit on F ²	1.032
Final R indexes [I > 2σ (I)]	R1 = 0.0740, wR2 = 0.2033
Final R indexes [all data]	R1 = 0.1130, wR2 = 0.2333
Largest diff. peak/hole / e Å ⁻³	0.57/-0.47

Antibacterial Activity: For testing the antibacterial activity of **1** and **4** against five bacterial strains (*Bacillus subtilis* [ATCC 6633], *Staphylococcus aureus* [ATCC 25925], *Escherichia coli* [ATCC 25923], *Acinetobacter baumannii* [ATCC 02026], and *Aeromonas hydrophila* [ATCC 95080]), provided from Refik Saydam Hifzissihha Institute (Ankara, Turkey), were used. As a standard reference drug ampicillin was employed. According to the literature method, broth microdilution method was used to determine the minimum inhibitory concentrations (MIC) values of the target compounds, in duplicate.[21] The compounds were dissolved in DMSO to obtain an initial concentration of 1000 µg/mL. Serial twofold dilutions were made in Mueller-Hinton broth (in 100 µl) to obtain the concentrations of 500-0.24 µg/ml in test medium. Effects of DMSO were controlled by inoculated broth supplement at the same solutions.

The screened compound **1** inhibited the growth of bacteria at (MIC) values in the range of 125-500 µg/ml whereas the compound **4** showed activities in the range of 62.5-250 µg/ml. The control, ampicillin, showed activity against the tested bacteria with a range of 0.9–125 µg/ml. The reason to study the activity of complex **4** is the solubility in water and permeability, which are higher than the corresponding ones of its precursor **1**. Also, sodium cation has no toxicity.

Anti-tuberculosis (TB) Activity: Resazurin Microtitre Assay (REMA) method was used to determine the anti-TB activity of the compounds **1** and **4**, according to literature method in duplicate.[22] *M. tuberculosis* H37Rv was used as standard strain and provided from the Refik Saydam National Public Health Agency, National Tuberculosis Reference Laboratory, Ankara, Turkey. Isoniazid (INH) (Sigma I3377), Rifampicin (RIF) (Sigma R3501), and Ethambutol (EMB) (Sigma E4630) were used as reference anti-TB agents. Stock

so the compounds and reference anti-TB agents in 100 μl of 7H9-S medium were prepared in the plates. After inoculation of the standard strain, the tested concentration range of the compounds was 250-0.12 $\mu\text{g/mL}$. A growth control containing no antibiotic and a sterility control without inoculum were included in each plate. Effects of DMSO were controlled by inoculated broth supplement at the same solutions.

The compound **1** exhibited anti-tuberculosis activity in 62,50 $\mu\text{g/mL}$ whereas the compound **4** showed activity in 31,25 $\mu\text{g/mL}$ suggesting that better MIC values when compared their antibacterial activity. The observed detail data on the inhibited growth of bacteria at (MIC) values are given in Table 2.

Antifungal Activity: The MIC values ($\mu\text{g/mL}$) of the target compounds against to *Candida albicans* ATCC 14053, *C. tropicalis* ATCC 1369 and *C. glabrata* ATCC 15126 yeast strains were determined by microdilution broth method[23] and according to the NCCLS standard document M27-A2.[24] Standard *Candida* strains were provided from Refik Saydam Hıfzısıhha Institute in Ankara Turkey. The fluconazole (Sigma, F8929) was used as reference anti-fungal agent. According to the NCCLS document, RPMI 1640 Medium (Sigma, R6504) was buffered with 0.165 M 3-(*N*-morpholino)-propanesulfonic acid (MOPS, Sigma, M1254) to obtain the pH 7.0 value. After preparing an initial concentration of 1000 $\mu\text{g/mL}$ of the tested compounds in DMSO, serial twofold dilutions of the compounds and reference anti-fungal agent in 100 μl of RPMI 1640 broth medium were prepared in the plates. Standard *Candida* strains were suspended in 0.85% saline to achieve 0.5 McFarland standard. After this, a working suspension is made by a 1:100 dilution followed by a 1:20 dilution of the stock suspension with RPMI 1640 broth medium. A 100 μl of working suspension was added to plates and so 250-0.12 $\mu\text{g/mL}$ final concentration range was obtained. The plates were incubated at 35°C for 48 h in ambient air. The MIC is the lowest concentration of a compound that inhibits growth of the organism as detected visually. The fluconazole (Sigma, F8929) showed activity with a range of 3.90-31.25 $\mu\text{g/mL}$ when tested against the indicated yeast. The tested molecules **1** showed anti-fungal activities with 250 $\mu\text{g/mL}$ whereas the novel compound **4** showed much better activity with 62.5 $\mu\text{g/mL}$ MIC value (Table 3).

Figure 2: Chains of molecules of Compound **4** formed through $\pi\cdots\pi$ stacking. Minor disorder components have been omitted for clarity.

Table 2. The Minimum Inhibitory Concentrations (MIC) values of Na⁺ complex against the bacteria and *M. tuberculosis* H37Rv strain

Compound	<i>Staphylococcus aureus</i> (ATCC 25925)	<i>Escherichia coli</i> (ATCC 25923)	<i>Acinetobacter baumannii</i> (ATCC 02026)	<i>Bacillus subtilis</i> (ATCC 6633)	<i>Aeromonas hydrophila</i> (ATCC 95080)	<i>M. tuberculosis</i> H37RV
1	500 $\mu\text{g/mL}$	500 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$	62.50 $\mu\text{g/mL}$
4	62.5 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$	31.25 $\mu\text{g/mL}$
Ampicillin	31.5 $\mu\text{g/mL}$	15.62 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$	0.9 $\mu\text{g/mL}$	31.2 $\mu\text{g/mL}$	-
Isoniazid	-	-	-	-	-	0.2 and 1 $\mu\text{g/mL}$
Ethambutol	-	-	-	-	-	5 and 10 $\mu\text{g/mL}$
Rifampicin	-	-	-	-	-	0,97 $\mu\text{g/mL}$

Table 3 Anti-fungal activity ($\mu\text{g/mL}$)

Compound	<i>Candida albicans</i> ATCC 14053	<i>Candida tropicalis</i> ATCC 1369	<i>Candida glabrata</i> ATCC 15126
1	250 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$
4	62,5	62,5	62,5
Flukonazol	31,25 $\mu\text{g/mL}$	15,62 $\mu\text{g/mL}$	3,90 $\mu\text{g/mL}$

Conclusions

In this study the identification of an original mononuclear octahedral sodium complex has been completed by X-ray diffraction analysis. The surrounding molecules of methanol are not frequently found in these types of molecules. Matching intermolecular attractions as hydrogen bonding and $\pi \cdots \pi$ stacking/London dispersion interactions contribute to stabilize the unitary cell. It is noteworthy the strong hydrogen bonding between the amido group and a molecule of coordinated methanol emulated the typical arrangement of protein substructures. The antibacterial, antituberculosis and antifungal character of this sodium complex **4** is higher than its precursor **1** but higher doses of it are necessary in comparison with the reference drugs.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

All authors declare no conflict of interests.

(See also manuscript)

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

New mononuclear octahedral sodium complex as antimicrobial agent

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New mononuclear octahedral sodium complex as antimicrobial agent

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