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PREPARATION AND STRUCTURAL STUDYING OF THE TETRACLORIDOZINCATE (II) COMPLEX WITH METHYLENE BLUE - A POTENTIAL ANTIVIRAL AND ANTIMICROBIAL AGENT

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Abstract. *Tetrachloridozincate(II) complex* $[MB]^+_2[ZnCl_4]^{2-}$ *(where [MB]+ – methylthioninium cation; methylene blue cation) was synthesized as a result of the mechanochemical reaction of* $ZnCl_2{\cdot}2H_2O$ *hexahydrate with* $[MB]Cl{\cdot}5H_2O$ *in the molar ratio 1:2. The asymmetric unit of the studied compound consists of a* $[ZnCl_4]^{2-}$ *anion and two* $[MB]^+$ *cations. Coordination polyhedron of the central atom is regular tetrahedron square planar. The planar* $[MB]^+$ *cations are stacked in an antiparallel mode with sulfur atoms disposed alternatively on opposite sides, and exhibiting π-π stacking at the average interplanar distances of 3.482 Å and 3.478 Å.*

Keywords: *Alzheimer's disease, antifungal, antimalarial therapy, candidiasis, methylene blue, mitochondrial dysfunction, tau proteins.*

Аннотация. *Комплекс тетрахлороцинката(II) [MB]+2[ZnCl4]2- (где* $[MB]^+$ *– катион метилтиония; катион метиленового синего) синтезирован в результате механохимической реакции гексагидрата* $ZnCl_2.2H2O$ *с* $[MB]Cl.5H_2O$ *в мольном соотношении 1:2. Асимметричная единица изученного соединения состоит из аниона* $[ZnCl4]^{2-}$ *и двух катионов* $[MB]^+$*. Координационный полиэдр центрального атома представляет собой правильный тетраэдр, плоский квадрат. Плоские катионы* $[MB]^+$ *укладываются антипараллельно, при этом атомы серы расположены попеременно на противоположных сторонах и демонстрируют π-π-укладку на средних межплоскостных расстояниях 3,482 Å и 3,478 Å.*

Ключевые слова*: болезнь Альцгеймера, противогрибковая, противомалярийная терапия, кандидоз, метиленовый синий, митохондриальная дисфункция, тау-белки.*

Annotatsiya. $ZnCl_2.2H2O$ *geksagidratning* $[MB]Cl.5H_2O$ *bilan mexanik kimyoviy reaksiyasi natijasida tetraxloridozinkat(II) kompleksi* $[MB]^+_2[ZnCl_4]^{2-}$ *(bu yerda [MB]+ – metiltioniniy kationi; metilen ko'k kationi) sintez qilingan. 1:2 molyar nisbatda. O'rganilayotgan birikmaning assimetrik birligi* $[ZnCl4]^{2-}$ *anion va ikkita* $[MB]^+$ *kationdan iborat. Markaziy atomning koordinatsion ko'pburchaklari muntazam tetraedr kvadrat tekislikdir. Tekis* $[MB]^+$ *kationlari antiparallel rejimda yotqizilgan, oltingugurt atomlari muqobil ravishda qarama-qarshi tomonlarga joylashtirilgan va o'rtacha tekisliklararo 3,482 Å va 3,478 Å masofalarda π-π stackingni ko'rsatadi.*

Kalit so'zlar*: Altsgeymer kasalligi, antifungal, antimalarial terapiya, kandidoz, metilen ko'k, mitoxondriyal disfunktsiya, tau oqsillari.*

1. Introduction

Methylene blue (MB), a blue-colored aniline-based dye that was first developed for the dyeing of cotton, is a phenothiazine derivative that is soluble in water and organic solvents. Its reduced form, leucomethylene blue, is colorless and has a regulatory effect on the redox cycle [1]. This feature allows it to be used in malarial therapy by inducing glutathione reductase (GR), an enzyme of glutathione metabolism. Malaria is a disease characterized by repeated growth

cycles of Plasmodium group parasites in erythrocytes and changes in glutathione metabolism. Methylene blue was recognized as an antimalarial agent when it was observed that it reduced reactive oxygen species (ROS) by inhibiting Plasmodium falciparum glutathione reductase and by selectively inducing oxidative stress. It has also been observed that MB can reduce factors that cause aging and Alzheimer's disease (AD), such as the accumulation of tau proteins in plaques, mitochondrial dysfunction, and ROS increase due to disorders in the electron transport chain (ETC). MB has been used in Alzheimer's treatment, considering its features of tau protein inhibition, anti-ROS antioxidant properties, and ability to regulate ETC. MB, known for its antimalarial and antioxidant effects, has also been used in the treatment of Candida infections as an antifungal agent. In candidiasis infections, MB is aimed to cause mitochondrial dysfunction in yeast and treat the patient. In this review, the history of MB as an antimalarial, antioxidant and antifungal agent; malaria, Alzheimer's, and its role in the treatment of candidiasis [2, 3].

Zinc is an essential trace element that is crucial for growth, development, and the maintenance of immune function. Its influence reaches all organs and cell types, representing an integral component of approximately 10% of the human proteome, and encompassing hundreds of key enzymes and transcription factors. Zinc deficiency is strikingly common, affecting up to a quarter of the population in developing countries, but also affecting distinct populations in the developed world as a result of lifestyle, age, and disease-mediated factors. Consequently, zinc status is a critical factor that can influence antiviral immunity, particularly as zinc-deficient populations are often most at risk of acquiring viral infections such as HIV or hepatitis C virus. This review summarizes current basic science and clinical evidence examining zinc as a direct antiviral, as well as a stimulant of antiviral immunity. An abundance of evidence has accumulated over the past 50 y to demonstrate the antiviral activity of zinc against a variety of viruses, and via numerous mechanisms. The therapeutic use of zinc for viral infections such as herpes simplex virus and the common cold has stemmed from these findings; however, there remains much to be learned regarding the antiviral mechanisms and clinical benefit of zinc supplementation as a preventative and therapeutic treatment for viral infections.

Zinc is one of the essential trace elements in the human body. This element belongs to the eighth iron-group. In the biological media it occurs in the oxidation form +2. The combination of methylene blue with zinc (II) ion expands the range of the antiviral properties of methylene blue and zinc (2+) ion. However, complexes of *d*-metals with methylene blue have not yet been synthesized.

Methylene blue (Mb) is a salt of the formula [$(C_{16}H_{18}SN_3)^+Cl^-$] [IUPAC name: 3,7-bis(dimethylamino)-phenothiazin-5-ium chloride or shortly [MB]$^+$Cl$^-$]]. The [MB]$^+$ cation consists of three condensed six-membered rings and two coplanar NMe_2 substituents [6]. The aromaticity is suggested by the total 18 pπ electron count, as indicated from the resonant structures in Fig.1.

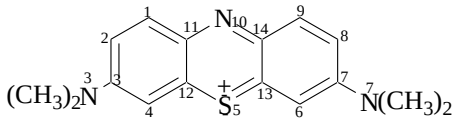


Figure 1. Molecular structure of [MB]$^+$ cation with numbering atoms

The [MB]$^+$ has N and S atoms as possible electronic donor centers. The N atoms are harder than S atom in the [MB]$^+$ structure. It is predicted that the eventual coordination of [MB]+ to a hard acid, such as Zn2+ and other two valence *d*-metals, do not happen neither through the S

and the N atoms, that in accordance to Pearson's acid–base theory [5]. However, this is not observed experimentally.

The crystal structures of Cu^+, Ag^+, and Au^+ complexes with MBCl were studied by Canossa's group [8]. The first two metal complexes showed coordination by the central N atom of $[MB]^+$, forming a distorted trigonal planar structure with $[MCl_2(MB)]$.

We decided to synthesize a salt-type complex of zinc (II) chloride with MBCl. Replacing of the chlorine ion in this complex allows to modify of the composition and receive new bioactive complexes. In this paper, crystal structure of zinc (II) complex with MB is described.

2. Preparation Syntheses of complex

The complex has been obtained as a result of the mechanochemical reaction of $ZnCl_2 \times 2H_2O$ with $[MB]Cl \times 5H_2O$ that eliminate the adverse effect of the solvent on the course of the reaction. Reagents $ZnCl_2 \times 2H_2O$ (17.2 mg, 0.1 mmol) and $[MB]Cl \times 5H_2O$ (82 mg, 0.2 mmol) in a 1:2 stoichiometric ratio were ground in an agate mortar until a gold-like thin mass was formed. 5.0 ml of DMF was gradually added dropwise to the reaction mixture and the process was continued until a homogeneous dark blue solution was formed.

Crystals available for X-ray diffraction were obtained by slow evaporation of the reaction solution for one week. Crystal of $[ZnCl_4]\times[MB]_2$ (**I**) is monoclinic in the space group P21/n, crystal parameters and details of the X-ray study are given in Tables 1.

Table 1

Crystal data and structure refinement for $[ZnCl_4]\times[MB]_2$.

Empirical formula	$C_{32}H_{36}N_6O_4ClZnS$
Formula weight	666.142
Temperature/K	278 K
Crystal system	triclinic
Space group	$P\bar{1}$
a/Å	9.7734(2)
b/Å	11.5185(2)
c/Å	16.6554(3)
α/°	108.0150(16)
β/°	100.2220(16)
γ/°	92.0898(16)
Volume/$Å^3$	1746.37(6)
Z	1
$\rho_{calc}g/cm^3$	1.239
μ/mm^{-1}	1.235
F(000)	683.9
Crystal size/mm^3	0.3 × 0.3 × 0.3
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	5.7 to 142.82
Index ranges	$-11 \le h \le 11$, $-14 \le k \le 14$, $-19 \le l \le 20$
Reflections collected	16626
Independent reflections	6704 [R_{int} = 0.0366, R_{sigma} = 0.0482]
Data/restraints/parameters	6704/0/423
Goodness-of-fit on F^2	1.028

Final R indexes [I>=2σ (I)] $R_1 = 0.0630$, $wR_2 = 0.1835$
Final R indexes [all data] $R_1 = 0.0812$, $wR_2 = 0.1972$
Largest diff. peak/hole / e Å^{-3} 1.30/-0.99

The X-ray diffraction experiment was carried out on an 'XtaLAB Synergy, HyPix3000' diffractometer (CuK_α - radiation, λ = 1.54184 Å, ω scan mode, mirror monochromator) [15].

The crystal structures were solved with OLEX2 [16] using the program SHELXT [17], and refined by full-matrix least-squares method on F^2 using the SHELXL refinement package [18].

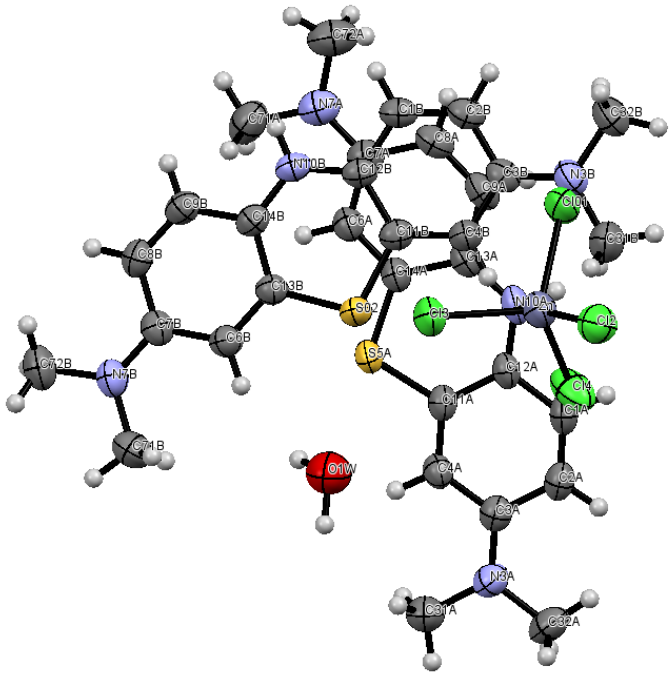
All non-hydrogen atoms were refined anisotropically. The H atoms of the CH_3-groups were included in calculated positions and refined as riding: C–H = 0.95–0.98 Å with U_{iso}(H) = 1.5U_{eq}(C-methyl) and 1.2U_{eq}(C) for other H atoms. The positions of all disordered Cl atoms defined from the difference Fourier map of the electron density and refined isotropically.

3. Results and discussions

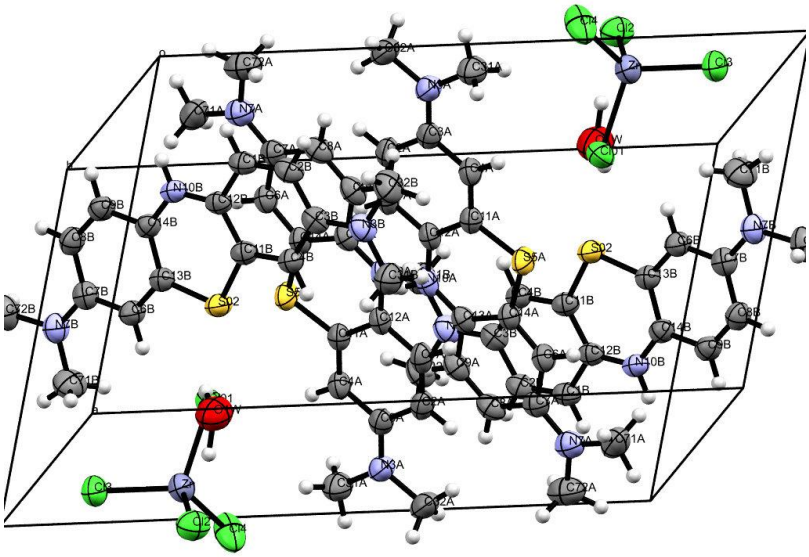
The crystalline structure of **1** composed of $[ZnCl_4]^{2-}$ anion and two symmetry-independent Mb^+ cations is shown in Fig. 1. The chlorine ions in the $[ZnCl_4]^-$ anion regular tetrahedral configuration. The Zn – Cl distances lie in the range of 2. 2.288(1) - 2.300(1) Å and have an averaged value 2.49 Å. It is shorter than the sum of the ionic radii of Zn(2+) (0.72 Å) and Cl^- (1.84 Å) atoms. The bond angles Cl – Zn – Cl lay in the range of 88 - 141.1(1)° (Table 2) and show the degree of the distortion of the $[ZnCl_4]^{2-}$ tetrahedron.

The $[ZnCl_4]^{2-}$ anion linked with eight Mb^+ cations owing to short contacts of the type C-H…Cl with hydrogen atoms of the aromatic cycles and terminal methyl group. Geometrical parameters these contacts are presented in Table 2. The $[ZnCl_4]^{2-}$ anions linked with seven $[Mb]^+$ cations through interaction of the types C-H…Cl with terminal methyl groups and aromatic cycles.

The [Mb]+ cations are planar and mutually packed in an antiparallel orientation, forming intermolecular nonclassical hydrogen bonds with each other, the geometrical parameters of which are given in Table 2. The C1– C2 bonds, C4 – C12, C8 – C9 and C6 – C13 in both crystals are shortened compared with other C – C bonds (average length 1.425(6) Å) in the ring.



(a)



(b)

Figure 2: The asymmetric unit of crystal **1** (a) and crystal packing of the subunits **(b)**. Displacement ellipsoids are drawn at the 50% probability level.

Table 2 Bond Lengths for $[ZnCl_4](Mb)_2$

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Zn	Cl1	2.288(1)	C3A	C4A	1.420(6)
Zn	Cl2	2.3000(15)	C3A	C2A	1.438(6)
Zn	Cl3	2.2474(14)	C12B	C1B	1.418(6)
Zn	Cl4	2.2569(16)	C12B	C11B	1.432(6)
S02	C13B	1.742(4)	C14A	C13A	1.442(6)
S02	C11B	1.729(5)	C14A	C6A	1.378(6)
S5A	C14A	1.724(4)	C4A	C11A	1.368(6)
S5A	C11A	1.725(4)	C13B	C14B	1.425(6)
N10A	C13A	1.328(6)	C13B	C6B	1.369(6)
N10A	C12A	1.330(6)	C4B	C3B	1.402(6)
N7B	C7B	1.344(6)	C4B	C11B	1.378(6)
N7B	C71B	1.475(7)	C13A	C9A	1.409(6)
N7B	C72B	1.463(6)	C1B	C2B	1.353(7)
N3A	C3A	1.339(6)	C1A	C2A	1.341(7)
N3A	C32A	1.451(6)	C1A	C12A	1.429(6)
N3A	C31A	1.468(7)	C7A	C6A	1.402(6)
N7A	C7A	1.342(6)	C7A	C8A	1.425(7)
N7A	C71A	1.469(7)	C3B	C2B	1.432(7)
N7A	C72A	1.448(7)	C9B	C14B	1.427(6)
N10B	C12B	1.342(6)	C9B	C8B	1.352(7)
N10B	C14B	1.326(6)	C12A	C11A	1.438(6)

Table 2 Bond Lengths for $[ZnCl_4](Mb)_2$

Atom	Atom	Length/Å	Atom	Atom	Length/Å
N3B	C3B	1.346(6)	C6B	C7B	1.408(6)
N3B	C32B	1.460(6)	C9A	C8A	1.356(7)
N3B	C31B	1.457(7)	C8B	C7B	1.437(7)

The centroid distances between adjacent $MB(A)^+$ and $MB(B)^+$ cations is 4.015 Å, between two adjacent $MB(A)^+$ cations is 4.077 Å, and between two adjacent $MB(B)^+$ cations is 4.476 Å.

The dihedral angle between average planes (except of H atoms) of the $[MB(A)]^+$ and $[MB(B)]^+$ cations in the π–π stack is 2.1º.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A),	Symmetry
C4A-H… Cl1	0.93	2.755	156.89	3.157	[x, y+1, z]
O1W-H…Cl2	1.053	2.162	156.66	3.157	[x, y+1, z]
$O1_W$-H2…Cl1	1.080	2.404	138.75	3.294	[x, y+1, z]
C1B-H…Cl3	0.930	2.913	139.39	3.669	[x-1, y, z]
C1A-H…Cl2	0.930	2.863	138.93	3.616	[-x+2, -y, -z+1]
C9B-H…Cl3	0.930	2.967	131.94	3.655	[-x+1, -y, -z]
C6B-H… O1W	0.930	2.499	149.20	3.332	
C8B-H…Cl1	0.930	2.873	175.10	3.801	1 [-x+1, -y, -z]
C8A-H…Cl4	0.930	2.719	149.48	3.552	[x-1, y, z]
C32A-H…O1W	0.960	2.488	149.66	3.352	[-x+2, -y+1, -z+1]
C72B-H…Cl1	0.960	2.904	136.92	3.664	[-x+1, -y, -z]
C71A-H…Cl3	0.960	2.863	113.15	3.359	[-x+1, -y, -z]
C72A-H…Cl3	0.960	2.841	132.07	3.557	[x-1, y, z]
C72A-H…S5A	0.960	2.893	148.94	3.749	[x-1, y, z]
O1W-H1…Cl2	1.053	2.162	156.66	3.157	[x, y+1, z]

Conclusion

During the solution-assistance mechanochemical synthesis of cobalt chloride with methylene blue has been obtained a salt-type complex $[MB]^+_2[ZnCl_4]^{2-}$. Chemical composition and structure of the complex were determined by the single crystal X-ray diffraction crystallography. The $[MB]^+$ cations are planar and stacked in an antiparallel fashion with the

sulfur atom disposed alternatively on opposite sides, and exhibiting π–π stacking at an with average intercationic separations 3.482 and 3.47 Å.

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