Synthesis, characterization, thermal behavior, and antitumor activities of an Ag(I) complex based on 4-(2-hydroxyphenyl)-2-methylpyrimidine

Wu-Wu Li¹, Min-Yan Zheng¹, Yong-Hui Shang¹, Jin-Qiong Xu¹, Zun-Ting Zhang², Hao-Nan Zheng¹, Xiao-Peng Li¹, A-Tong Weng¹, Ling-Ying Feng¹ and Lu Liu¹

Abstract
A new Ag(I) coordination complex, Ag(C₁₁H₁₀N₂O)₂ NO₃ (C₁₁H₁₀N₂O = 4-(2-hydroxyphenyl)-2-methylpyrimidine) is successfully synthesized and characterized by infrared spectroscopy, elemental analysis, and single-crystal X-ray diffraction analysis. This complex features a three-dimensional framework consisting of hydrogen bonds, π–π stacking interactions, coordination interactions, and electrostatic interactions. Moreover, the thermal stability and non-isothermal thermal decomposition reaction kinetics of the complex are well investigated by the methods of Kissinger and Ozawa. Finally, the antitumor ability of the complex is evaluated against human lung cancer cells (NCI-H460), human hepatocellular cancer cells (HepG2), and human breast cancer cells (MCF7). The complex exhibits potent antitumor activities against HepG2 and MCF7 cancer cells.

Keywords
4-(2-hydroxyphenyl)-2-methylpyrimidine, Ag(I) complexes, antitumor activities, crystal structure, synthesis, thermal behavior

Date received: 2 March 2022; accepted: 11 May 2022

Presented here is a new Ag(I) coordination framework, namely Ag(C₁₁H₁₀N₂O)₂ NO₃ (I, C₁₁H₁₀N₂O = 4-(2-hydroxyphenyl)-2-methylpyrimidine). Complex 1 features three-dimensional (3D) network structure in which Ag ions and ligands are linked by Ag–N coordinate bonds, hydrogen bonds, π–π stacking interactions and electrostatic interactions. The thermal decomposition activation energy of complex 1 is 261.83kJ•mol⁻¹ (Ozawa’s method). Complex 1 shows better vitro antitumor effect than carboplatin against human hepatocellular cancer cells HepG2 and human breast cancer cells MCF7.
Introduction

Nitrogen-containing heterocyclic compounds are very important organic frameworks and are widely present in natural products, synthetic drugs, and naturally occurring peptides. Due to their excellent electronic, catalytic, and biological properties, nitrogen-containing heterocyclic compounds have been widely applied in many fields, such as in the study of physiologically active molecules and the preparation of functional materials, and have been involved in catalysis and coordination chemistry. Pyrimidines are important heterocyclic compounds because they possess highly valuable skeletons that are widely found in many biologically active compounds and pharmaceuticals. A number of pyrimidine derivatives exhibit good biological activities, including antibacterial, antiviral, anticancer, anticonvulsant, antioxidant, and anti-inflammatory activities. However, pyrimidine heterocyclic compounds containing two N atoms have strong coordination ability, and easily form π-π stacking and hydrogen bonding. Hence, they form complexes with novel structures and unique properties when they are self-assembled with metal ions.

Silver ions have strong broad-spectrum antibacterial and bacteriostatic effects. Clinically, silver salts can be used to treat chronic ulcers, large skin burns, and prevent neonatal conjunctivitis and other bacterial infections. It has been reported that silver complexes have useful antibacterial activity against Staphylococcus aureus (Gram-positive), Pseudomonas aeruginosa (Gram-negative), Escherichia coli, and other pathogenic bacteria, and they have anti-proliferative activity against human colorectal cancer cells (HTC 116) and human leukemia cells (HL 60).

Based on the above research, this paper hopes to combine pyrimidines and silver ion into the same molecule by synthesizing coordination complex, so we synthesized 4-(2-hydroxyphenyl)-2-methylpyrimidine, and it was used to self-assemble with Ag(I) ions under hydrothermal conditions.

Crystal structure description

The X-ray crystallographic data of complex 1 are listed in Table 1, and the selected bond angles and bond lengths are listed in Table 2. The molecular structure of complex 1 (Figure 1) consists of an Ag(I) cation, a nitrate ion, and two 2-methyl-4-(2-hydroxyphenyl)pyrimidine ligands. The ligand consists of a benzene ring (C6–C11) and a pyrimidine ring (N1, N2, C2–C5), and the angle between the benzene ring and the pyrimidine ring is 1.73°. The ligand is basically planar with the mean deviation from the mean plane being 0.0134 Å. Ag(I) forms coordination bonds with N1 of the pyrimidine rings of the two ligands, and the Ag–N bond lengths are 2.186(3) Å (Table 2), which is consistent with the literature reports. The nitrate ion acts as the counter anion, in which N–O bond lengths ranging from 1.214(7) to 1.249(7) Å (Table 2).

The crystal-packing analysis demonstrated that there are three hydrogen bonds in complex 1 (the hydrogen bonds parameters are shown in Table 3). An intramolecular hydrogen bond O1–H1···N2 is formed between the phenolic hydroxy group and the pyrimidine nitrogen atom, resulting in a stable six-membered ring being formed (Figure 2). Another hydrogen bond C1–H1C···O4i (symmetry codes: (i) 1 − x, 1 − y, −1/2 + z) exists in complex 1. The nitro oxygen atom O4i acts as the hydrogen bond acceptor, and the hydrogen atom H1C on the methyl of the ligand acts as the hydrogen bond donor. The hydrogen bond C1–H1C···O4i assembles the neighboring molecule into one-dimensional chains along the c-axis (Figure 2).
Table 1. Crystallographic data for complex I.

| Property                        | Complex I |
|--------------------------------|-----------|
| Empirical formula              | C_{22}H_{20}AgN_{5}O_{5} |
| Formula weight                 | 542.3     |
| Temperature                    | 296(2) K  |
| Wavelength                     | 0.71073 Å |
| Crystal system, space group    | Orthorhombic, Cmc2(1) |
| Unit cell dimensions           | a = 23.523(6) Å, b = 7.4699(18) Å, c = 12.189(3) Å |
| α (°)                          | 90        |
| β (°)                          | 90        |
| γ (°)                          | 90        |
| V (Å³)                         | 2141.7(9) |
| Z                              | 4         |
| Calculated density             | 1.682 g/cm³ |
| Absorption coefficient         | 0.987 mm⁻¹ |
| F(000)                         | 1096      |
| Crystal size                   | 0.347 mm × 0.258 mm × 0.202 mm |
| Theta range for data collection| 3.114–27.498° |
| Limiting indices               | −27 ≤ h ≤ 29, −9 ≤ k ≤ 9, −13 ≤ l ≤ 15 |
| Reflections collected/unique   | 5887/1986 (R = 0.0230) |
| Completeness to theta = 25.242 | 99.60%    |
| Maximum and minimum transmission| 0.7456 and 0.6942 |
| Refinement method              | Full-matrix least-squares on F² |
| Data/restraints/parameters      | 1986/1160 |
| Goodness-of-fit on F²          | 1.025     |
| Final R indices (I > 2σ(I))    | R1 = 0.0243, wR2 = 0.0566 |
| R indices (all data)           | R1 = 0.0271, wR2 = 0.0588 |
| Largest difference peak and hole| 0.293 and −0.296 e. Å⁻³ |

2). A C₄–H₄–O¹⁵ hydrogen bond (symmetry codes: (i) 3/2 − x, 3/2 − y, 1/2 + z) exists in an adjacent ligand, with the hydroxy oxygen atom O₁ acting as the hydrogen bond acceptor, and the hydrogen atom H₄ on the phenyl group acting as the hydrogen bond donor. Complex I reveals that the cations and anions are linked into two-dimensional (2D) layers that are parallel to the ac plane via hydrogen bond C₄–H₄···O₁ (Figure 3). The crystal analysis further demonstrates that face-to-face π–π stacking interactions are observed between neighboring antiparallel ligand skeletons (Figure 4). The benzene ring of the ligand at the (x, y, z) position has π–π stacking interactions with the pyrimidine ring of the adjacent ligand at (3/2 − y, 1/2 + y, z) position (centroid-to-centroid distance (Cg₂–Cg₁), Cg₁ and Cg₂ represent the ring centroids of the benzene and pyrimidine rings, respectively) of 3.536(2) Å and a vertical distance from Cg₂ to the pyrimidine ring of 3.3940(15) Å. The pyrimidine ring of the ligand at the (x, y, z) position has π–π stacking interactions with the benzene ring of the adjacent ligand at (3/2 − x, −1/2 + y, z) position (centroid-to-centroid distance (Cg₁–Cg₂), Cg₁ and Cg₂ represent the ring centroids of 3.423(4) Å and a vertical distance from Cg₁ to the benzene ring of 3.352(13) Å). These parameters agree well with classical π–π stacking interaction data. The ligands are linked by π–π stacking interactions to form one-dimensional chains along the b-axis. These chains are further linked by coordination interactions into a 2D layer along the ab plane. Hydrogen bonds, π–π stacking interactions, coordination interactions, and electrostatic interactions assemble complex I into a 3D network (Figure 5). 

Thermal stability

Differential scanning calorimetry (DSC) was employed to evaluate the thermal stability of complex I. The heating rate was 8, 10, 15, or 20 °C min⁻¹, the atmosphere was high purity N₂, the temperature range was 25–420 °C, and the sample mass was less than 0.1 mg (considering the safety aspects of the experiment). The results of the DSC analysis are presented in Figure 6. Since there is no water of crystallization in complex I, only thermal decomposition occurs. The decomposition peak temperatures are 234.8, 236.1, 239.5, and 241.9 °C at heating rates of 8, 10, 15, and 20 °C min⁻¹, respectively. In this process, the ligand is thermally decomposed, and the framework of complex I collapses with the release of heat.

Kinetics of non-isothermal thermal decomposition

In order to study the thermal decomposition reaction kinetics of complex I and obtain its thermal decomposition kinetic parameters (apparent activation energy (E), pre-exponential factor (A), and linear correlation coefficient (R₀)), it was analyzed by DSC under an N₂ atmosphere, scanning from 25 to 420 °C with heating rates of 8, 10, 15, or 20 min⁻¹. The values of the decomposition peaks (initial temperature (T₀), peak temperature (Tₚ), and termination temperature (Tₜ)) are listed in Table 4. According to the peak temperature at different heating rates, the Kissinger²² and Ozawa²³ methods were used to fit the calculations. The values of ln(β/Tₚ) and lnβ were plotted against 1000/Tₚ, respectively, and linear regression analysis was conducted. The thermal decomposition activation energy E of complex I was obtained by calculation of the slope, and the pre-exponential factor A was obtained from the intercept. The obtained non-isothermal thermal decomposition kinetic parameters are shown in Table 4. The values of the thermal decomposition kinetic parameters obtained by the Kissinger method are consistent with those obtained by the Ozawa method. The thermal decomposition activation energy E of complex I is 266.81 kJ mol⁻¹ (the Kissinger method) and 261.83 kJ mol⁻¹ (the Ozawa method). 

In vitro antitumor activity

The in vitro antitumor activity of complex I was examined using MTT assays, the basic principle is that the succinate dehydrogenase in the mitochondria of living cells can reduce exogenous MTT to water-insoluble blue-purple formazan crystals that deposit in cells; however, dead cells do not undergo such deposition. The amount of formazan crystals is directly proportional to the number of
Table 2. Selected bond angles (°) and bond lengths (Å) of 1.

| Bond lengths | Bond lengths (Å) |
|--------------|-----------------|
| Ag(1)–N(1)   | 2.186(3)        |
| N(3)–O(2)    | 1.214(7)        |
| N(3)–O(4)    | 1.227(6)        |
| Ag(1)–N(1)a  | 2.186(3)        |
| N(3)–O(3)    | 1.249(7)        |

| Bond angles | Bond angles (°) |
|-------------|----------------|
| N(1)–Ag(1)–N(1)a | 163.72(15)   |
| O(2)–N(3)–O(3)  | 116.9(6)      |
| O(4)–N(3)–O(3)  | 120.4(6)      |
| Symmetry codes: (a) 1 − x, y, z. |

Table 3. Hydrogen-bonding parameters in complex 1.

| D–H . . A       | D–H | H . . A | D . . A | D–H . . A |
|-----------------|-----|--------|---------|----------|
| O(1)−H(1i). . .N(2) | 0.82| 1.83   | 2.557(4)| 148      |
| C(1)−H(1C). . .O(4)i | 0.96| 2.52   | 3.447(7)| 162      |
| C(4)−H(4). . .O(1)i | 0.93| 2.60   | 3.462(5)| 155      |

Symmetry codes: (i) 1 − x, 1 − y, −1/2 + z; (ii) 3/2 − x, 3/2 − y, 1/2 + z.

Figure 1. Molecular structure of complex 1.

Figure 2. One-dimensional chain structures in complex 1. Symmetry codes: (i) 1 − x, 1 − y, −1/2 + z.

Conclusion

A 3D network complex Ag(C_{11}H_{10}N_{2}O)_{2}·NO_{3} based on Ag(I) and 4-(2-hydroxyphenyl)-2-methylpyrimidine has been successfully prepared. The single-crystal structures show that the title complex exhibits a 3D network structure in which Ag ions and ligands are linked by Ag–N coordinate bonds, hydrogen bonds, π–π stacking interactions, and electrostatic interactions. In addition, we have studied the thermal stability and non-isothermal thermal...
Figure 3. Two-dimensional layers in complex 1. Symmetry codes: (ii) 3/2 − x, 3/2 − y, 1/2 + z.

Figure 4. π–π stacking interactions in complex 1. Symmetry codes: (iii) 3/2 − x, 1/2 + y, z; (iv) 3/2 − x, −1/2 + y, z.

Figure 5. Packing diagram of complex 1.
decomposition kinetics of the title complex. The results indicate that its decomposition is only a thermal process. The decomposition peak temperatures are 234.8, 236.1, 239.5, and 241.9 °C at heating rates of 8, 10, 15, and 20 °C min⁻¹, respectively. The thermal decomposition activation energy of the title complex is 266.81 kJ mol⁻¹ (the Kissinger method). The results of the in vitro antitumor activity show that the inhibition effect of the title complex against HepG2 and MCF7 cancer cells is better than that of carboplatin.

Experimental

Reagents were purchased from commercial sources and used as received, unless mentioned otherwise. Reactions were monitored by thin-layer chromatography (TLC) using UV light to visualize the course of the reaction. Purification of the reaction products was carried out by recrystallization. Chemical yields refer to those of pure isolated substances. Elemental analyses were recorded on a PerkinElmer 240C Elemental Analyzer. Infrared spectra were measured on a Nicolet 6700 FTIR spectrometer in the range of 400–4000 cm⁻¹ as KBr pellets. Single-crystal X-ray diffraction was carried out with an Oxford Xcalibur E diffractometer. The thermal behavior (DSC) was studied under an N₂ flow with a DSC-Q100 TA instrument.

4-(2-Hydroxyphenyl)-2-methylpyrimidine

The mixture of 1-(2-hydroxyphenyl)ethan-1-one (204 mg, 1.5 mmol) and DMF-DMA (536 mg, 4.5 mmol) was refluxed in N,N-dimethylformamide (50 mL) at 80 °C for 1.5 h. The reaction progress was monitored by TLC. After cooling to room temperature, the reaction mixture was poured into saturated NaCl solution (100 mL), and a yellow precipitate appeared. The precipitate was filtered and washed with saturated NaCl solution. The yellow precipitate was recrystallized from ethyl acetate to give (E)-3-(dimethylamino)-1-(2-hydroxyphenyl)prop-2-en-1-one. The thermal decomposition activation energy of the title complex is 266.81 kJ mol⁻¹ (the Kissinger method). The results of the in vitro antitumor activity show that the inhibition effect of the title complex against HepG2 and MCF7 cancer cells is better than that of carboplatin.

Table 4. Calculated parameters for complex 1 in the decomposition reactions.

| Heating rate β (°C min⁻¹) | 8  | 10  | 15  | 20  |
|---------------------------|----|-----|-----|-----|
| Initial temperature Tₑi (°C) | 233.2 | 234.7 | 237.4 | 239.8 |
| Peak temperature Tₚ (°C) | 234.8 | 236.1 | 239.5 | 241.9 |
| Termination temperature T₉ (°C) | 241.7 | 243.1 | 246.2 | 249.3 |
| ΔHₑD (J g⁻¹) | 138.5 | 111.7 | 127.5 | 160.8 |
| Kissinger’s method | Eₚ (kJ mol⁻¹) | 266.81 | lg Aₚ (s⁻¹) | 25.6 | Rₑ | 0.99 |
| | Ozawa–Doyle’s method | Eₒ (kJ mol⁻¹) | 261.83 | lg Aₒ (s⁻¹) | 25.4 | Rₒ | 0.99 |
Table 5. In vitro inhibitory activity of complex 1 and carboplatin on cancer cells.

| compound       | IC50/μmolL−1 | HepG2 | NCI-H460 | MCF7 |
|----------------|--------------|-------|----------|------|
| 1              | 6.18 ± 0.22  | 7.42 ± 0.12 | 5.19 ± 0.25 |
| Carboplatin    | 7.68 ± 0.19  | 7.29 ± 0.18 | 8.05 ± 0.37 |

C11H10N2O·NO+ [M + H]+: 187.0871; found 187.0861; Anal. calcd for C11H10N2O·NO+ (187.08): C, 48.73; N, 12.91; H, 3.72; found: C, 48.78; N, 12.98; H, 3.60. IR (KBr pellet): 3615, 1632, 1554, 1476, 1365, 1285, 1204, 1045, 1011, 835, 776, 632, 545 cm−1.

X-ray single-crystal structure determination

A suitable single crystal of 1 was carefully selected under an optical microscope and glued to thin glass fibers. Structural measurements were performed with a computer-controlled Oxford Xcalibur E diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) at T=293(2)K. Absorption corrections were made using the SADABS program. The structure was solved by direct methods and refined by full-matrix least-square methods on F2 using the SHELXL-97 program package. All non-hydrogen atoms were refined anisotropically. The H atoms attached to their parent atoms were geometrically placed and refined using the riding model. Crystal data as well as details of data collection and refinements of complex 1 are summarized in Table 1, and the selected bond lengths and angles are given in Table 2.

In vitro determination of the antitumor activity

The compounds to be tested were dissolved in DMSO and further diluted to different concentrations with culture medium. The tumor cells NCI-H460, HepG2, and MCF7 were inoculated in 96-well plates with 5 × 104 cells per well. After incubation for 12 h, different concentrations of test compounds were added to the 96-well plate, with the concentration of each compound being 2, 4, 8, 16, 32, and 40 μmol/L, respectively. The final concentration of DMSO in the culture medium should be controlled and less than 0.01%, while no drugs were added to the blank control group, only containing culture medium. Each concentration of the drug and control groups was tested with three multiple wells. After 12 h, cell-counting MTT was used to determine the viability of the NCI-H460, HepG2, and MCF7 cells, and 10 μL of a 5-mg/mL MTT solution was added to each well plate followed by incubation for 12 h at 37 °C. The absorbance of each well was recorded with an automatic microplate reader at 490 nm. The inhibition rate was calculated according to the absorbance value, and the IC50 value was calculated according to the inhibition rate.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by the Scientific Research Program Funded by Shaanxi Provincial Education Department (no. 19JK0767), the Natural Science Basic Research Plan Funded by Shaanxi Province of China (no. 2020JM1543), the Scientific Research Project Funded by Xianyang Normal University (nos XSYK21041 and XSKY19046), the University Students Research and Innovation Training Program of Ministry of Education (no. S202010722009), the Qing–Lan Talents Project Funded by Xianyang Normal University (no. XSYQL201904), and the Seventh Batch of College Student Innovation and Entrepreneurship Base Team of Xianyang Normal University (no. XSYC202118).

ORCID iD

Wu-Wu Li https://orcid.org/0000-0003-1644-4848

Supplemental material

Supplemental material for this article is available online. CCDC 2122123 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/structures.

References

1. Kalinin AA and Mamedov VA. Chem Heterocycl Compd 2011; 46: 1423–1442.
2. Giraudo A, Krall J, Bavo F, et al. J Med Chem 2019; 62: 5797–5809.
3. Doddi A, Peters M, Tamm M, et al. Chem Rev 2019; 119: 6994–7112.
4. Peris E. Chem Rev 2018; 118: 9988–10031.
5. Danopoulos AA, Simler T and Braunstein P. Chem Rev 2019; 119: 3730–3961.
6. Buron F, Merour JY, Akssira M, et al. Eur J Med Chem 2015; 95: 76–95.
7. Suresh L, Kumar PSV and Chandramouli GVP. J Mol Struct 2017; 1134: 51–58.
8. Shakya N, Vedi S, Liang C, et al. Bioorg Med Chem Lett 2014; 24: 1407–1409.
9. El Sayed MT, Hussein HAR, Elebiary NM, et al. Bioorg Chem 2018; 78: 312–323.
10. Amin LHT, Shawer TZ, El-Naggar AM, et al. Bioorg Chem 2019; 91: 103159.
11. Sahu M, Siddiqui N, Sharma V, et al. Bioorg Chem 2018; 77: 56–67.
12. Adhikari A, Kalluraya B, Sujith KV, et al. Eur J Med Chem 2012; 55: 467–474.
13. Somakala K, Tariq S and Amir M. Bioorg Chem 2019; 87: 550–559.
14. Vinogradova KA, Shekhovtsov NA, Berezin AS, et al. Dalton Trans 2021; 50: 9317–9330.
15. Senthilkumar GS, Sankarganesh M, Raja JD, et al. Monatsh Chem 2021; 152: 251–261.
16. Bell TA, Grayston JT, Krohn MA, et al. Pediatrics 1993; 92: 755–760.
17. Silver S, Phung LT and Silver G. J Ind Microbiol Biotechnol 2006; 33: 627–634.
18. Sibbald RG, Orsted H, Schultz GS, et al. Ostomy Wound Manage 2003; 49: 24–51.
19. Iqbal MA, Haque RA, Budagumpi S, et al. Inorg Chem 2013; 28: 64–69.
20. Sun D, Zhang N, Xu QJ, et al. J Mol Struct 2010; 969: 176–181.
21. Janiak C. J Chem Soc Dalton Trans 2000; 3885–3896.
22. Kissinger HE. Anal Chem 1957; 29: 1702–1706.
23. Ozawa T. Bull Chem Soc Jpn 1965; 38: 1881–1886.
24. Dai Y, Meng W, Feng XQ, et al. Chem Commun 2022; 58: 1558–1560.
25. Xun ZS, Lou YT and Chen L. Patent US 0300916A1 USA 2021, 2021.
26. Bruker. APEX2, SAINT and SADABS. Madison, WI: Bruker AXS, 2009.
27. Sheldrick GM. Acta Crystallogr A 2008; 64: 112–122.