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Structural Study of {[(H4fpytsc)$_2$]$^{2+}$[PF$_6$]$^-$[(NO$_3$]$^-$} from 4-Formylpyridine Thiosemicarbazone (4fpytsc) †

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Abstract: In the present research, bis(Pyridinium-4-carbaldehyde thiosemicarbazone) hexafluorophosphate, nitrate (**2**) have been prepared by protonation of 4-Formylpyridine thiosemicarbazone (4fpytsc, **1**). The compound **2** and the starting thiosemicarbazone have been well characterized and their molecular and crystal structures were studied by single-crystal X-ray diffraction. The supramolecular assembly of each crystal is also analyzed and discussed. The compounds were evaluated for their in vitro cytotoxicity against HeLa-229 cancer cell line.

Keywords: thiosemicarbazones; pyridinium salts; supramolecular architectures; hydrogen bond; cytotoxicity

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1. Introduction

Thiosemicarbazones can be considered one of the most versatile types of compounds in chemistry [1]. During decades of intense research, hundreds of thiosemicarbazones and derivatives have been obtained. This exhaustive research can be attributed to their use as intermediates in the preparation of many heterocyclic systems that are of great interest, both from the theoretical and practical point of view, and to play an important role in the design and discovery of new bioactive compounds [2]. Furthermore, many thiosemicarbazones have great versatility as ligands in coordination chemistry [3]. In particular, α-N-heterocyclic thiosemicarbazones represent an important class of NNS metal chelating agents [4]. With respect to their antitumor properties, they were originally developed as iron chelators. However, they are not only iron chelators, such as, for example, desferrioxamine which mainly sequesters extracellular iron due to its strong iron(III) binding capacity. In contrast, thiosemicarbazones are considered "iron-interacting" drugs influencing various iron-dependent biological pathways due to their ability to form highly stable complexes with iron(II) and iron(III) ions. [5] However, changing the attachment point of thiosemicarbazone moiety to positions β or γ on the heteroaromatic ring, often causes a decrease in activity presumably due to decreased coordination capacity [6]. Nevertheless, in the literature there are examples of biologically important bidentate thiosemicarbazones such as α-acetamidobenzaldehyde thiosemicarbazone, known as thiacetazone, which is used in the clinical treatment of tuberculosis [7].

On the other hand, in recent decades, pyridinium salts have been considered as scaffolds found in many natural and bioactive compounds [8]. Those pyridinium salts that are liquid at room temperature, the so-called pyridinium ionic liquids [9], such as 1-alkylpyridinium salts, are well known as potential solvents in synthesis and catalysis [10]. In organic chemistry, these pyridinium salts have unlimited synthetic value as key intermediates for the construction of certain species contained in some natural products such

as the pharmacologically active indole, piperidine, or dihydro and tetrahydropyridine [11]

As part of our studies on thiocarbonyl chemistry and in light of the above facts on thiosemicarbazones and derivatives of pyridinium salts, the combination of both types of compounds is likely to lead to interesting new bioactive agents. For that reason, we have undertaken the reaction between such bioactive substances. The structures of the synthesized materials were confirmed by both elemental and spectral (FT-IR, ^{1}H, ^{13}C NMR and mass) tools. The crystalline and molecular structure of the 2-(Pyridin-4-ylmethylene)hydrazinecarbothioamide (4fpytsc) and of bis(Pyridinium-4-carbaldehyde thiosemicarbazone) hexafluorophosphate, nitrate {$[(H4fpytsc)_2]^{2+}[PF_6]^-[NO_3]^-$}, obtained serendipitously, were determined by X-ray diffraction of single-crystals. In addition, the results of the cytotoxic activity of the thiosemicarbazone have been evaluated to determine in vitro antiproliferative activity against the HeLa-229 cell line (human cervical carcinoma).

2. Materials and Methods

All reagents and solvents were commercial products that were used as received, without further purification.

2-(Pyridin-4-ylmethylene)hydrazinecarbothioamide (4fpytsc) (**1**). To a solution of 1.05 g (0.01 mol) of Thiosemicarbazida in 40 mL of 96% ethyl alcohol, 1.05 mL (0.01 mol) of Pyridine-4-aldehyde was added. The final solution was heated under reflux for 2 h, cooled, and stirred for 24 h at room temperature. After this time, a colorless solid was separated by filtration, recrystallized from ethanol and dried on $CaCl_2$. Yield 96.4%, as colorless prismatic crystals.

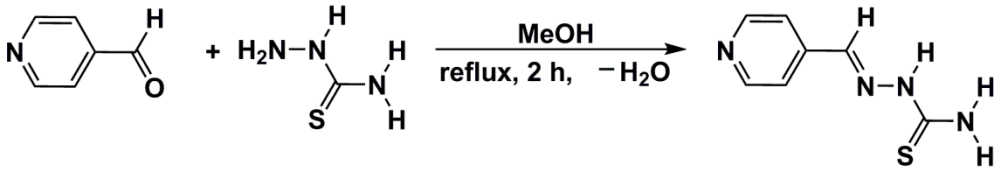
Elemental analysis. Found: C, 46.42; H, 4.69; N, 31.03; S, 17.94%. Calc. for $C_7H_8N_4S$ (180.23): C, 46.64; H, 4.47; N, 31.08; S, 17.79%. Mp 235 °C. IR(ν_{max}/cm^{-1}): 3424(s), 3265(s), 3157(s) ν(NH), 1620(s), 11,594(m), 1538(m) ν(CN) + ν(CC), 1417(m) ν(CN) + ν(CS), 996(w) ν(NN), 829(w) ν(C–S). EI MS, m/z, assignment: 180 [M], 163 [$C_7H_5N_3S$], 147 [$C_7H_7N_4$], 120 [$C_6H_6N_3$], 105 [$C_6H_5N_2$], 93 [C_6H_7N], 78 [C_5H_4N]. ^{1}H NMR (DMSO-d_6, ppm): 7.76 (1H, m, H11); 8.57 (1H, m, H12); 8.57 (1H, m, H13); 7.76 (1H, m, H14); 7.99 (1H, s, H16); 11.7 (1H, s, N13H); 8.20–8.38 (2H, s, N14H_2). ^{13}C RMN (DMSO-d6, ppm): 121.51 (C11); 150.36 (C12); 150.36 (C13); 121.51 (C14), 139.81 (C15), 141.82 (C16); 178.89 (C17).

Bis(Pyridinium-4-carbaldehyde thiosemicarbazone) hexafluorophosphate, nitrate {$[(H4fpytsc)_2]^{2+}[PF_6]^-[NO_3]^-$} (**2**). This compound was obtained serendipitously as follows. A solution of 4.7 mg (0.028 mmol) of silver nitrate in 5 mL of H_2O was added, slowly and with stirring, to a solution of 5 mg (0.028 mmol) of Pyridine-4-carbaldehyde thiosemicarbazone in 25 mL of methyl alcohol. Finally, 4.7 mg (0.028 mmol) of $NaPF_6$ were added. The resulting solution was kept stirring and in the absence of light for a week and after that time some yellow plate-shaped crystals were observed, which were separated by filtration.

Microanalyses (C, H and N) were carried out in a Carlo-Erba 1108 elemental analyzer. ESI-MS spectra were recorded on a VG Autospec opus mass spectrometer. FT-IR spectra were recorded from KBr pellets over the range 4000–400 cm^{-1} on a Bruker IFS-66v spectrometer). ^{1}H and ^{13}C NMR spectra in DMSO-d_6 were run on Bruker AMX 300 and WM 300 instruments, respectively, using TMS as internal reference. For X-ray analysis, intensity data were collected at room temperature on a Enraf Nonius MACH3 automatic diffractometer for **1** and Bruker X8 KappaAPEXII diffractometer for **2**. Structures were solved by direct methods followed by difference Fourier calculations and were refined by a full-matrix least-squares procedure using SHELXLTL. The structures of **1** and **2** were deposited at the Cambridge Crystallographic Data Centre with CCDC Nos. 2300610 and 2300611, respectively.

3. Results and Discussion

Pyridine-4-carbaldehyde thiosemicarbazone (1) was prepared by condensing the thiosemicarbazide with pyridine 4-carbaldehydes, according to a literature procedure, as shown in Scheme 1.



Scheme 1. Synthesis of pyridine-4-carbaldehyde thiosemicarbazone.

The synthesized compound was obtained in good yield and was satisfactorily characterized by elemental analysis, ESI-Mass, FT-IR, and (^{1}H, ^{13}C) nuclear magnetic resonance spectroscopy. The thiosemicarbazone is soluble in common organic solvents, such as dimethylformamide, ethyl alcohol, dimethyl sulfoxide, partially soluble in chloroform, methyl alcohol and acetonitrile and insoluble in water and dichloromethane. The mass and spectroscopic data agree with the proposed structure.

3.1. Structural

3.1.1. 2-(Pyridin-4-Ylmethylene)hydrazinecarbothioamide

Has been previously prepared and its molecular structure partially studied [6,12–14], including its monohydrate [15]. Compound **1** crystallizes in the $P2_1/n$ monoclinic space group and unit cell dimensions a = 7.241(5) Å, b = 13.962(5) Å, c = 8.417(5) Å, β = 90.91(3)°, and V = 850.8(8) Å^3. The asymmetric unit contains a single thiosemicarbazone molecule that is planar with a maximum deviation of its atoms from the least-squares plane of 0.057 Å (N13), a C16-N12-N13-C17 torsion angle of –179.1(3)°, and an angle between the plane of the pyridin ring and the thiosemicarbazone moiety of 18.31(9)°. An analysis of the distances and bond angles in each of the compounds showed that they do not differ significantly from the values found in other heterocyclic thiosemicarbazones [16]. The asymmetric unit for **1** is shown in Figure 1a.

The crystal packing of these cocrystals, involves the common supramolecular synthon thioamide-thioamide homodimer based on the $R_2^2(8)$ hydrogen-bonded ring motif (Figure 1b). These dimers are linked to four thiosemicarbazone molecules of the nearest neighbor dimers via two Nhy–H···Npy hydrogen bonds as donor, and other two Npy···H–Nhy ones as acceptor, thus forming corrugated layers in the "cb"-plane. The layers, which propagate perpendicularly to the crystallographic "a"-direction, contain holes of approximate dimensions 11.33 × 10.57 Å with a $R_6^6(44)$ graph-set motif (Figure 1b).

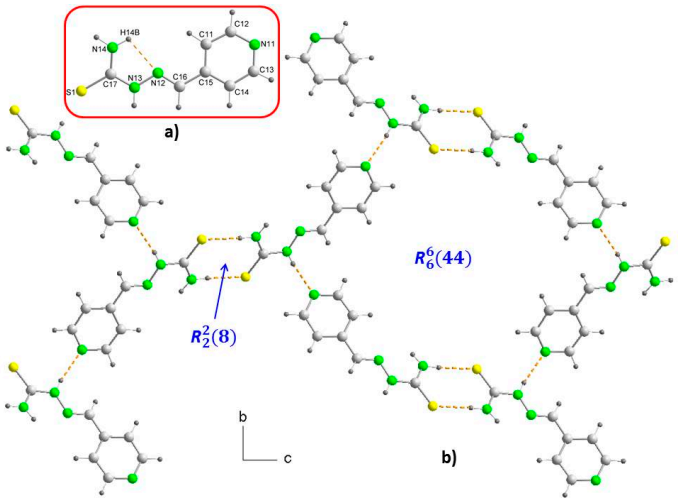


Figure 1. Perspective view of: (**a**) (4fpytsc, **1**), showing the asymmetrical unit, including intramolecular hydrogen bonding, and the atom-numbering scheme, and (**b**) A partial packing diagram for (**1**), showing one layer of the two-dimensional network, the intermolecular interactions, and the supramolecular synthons. Hydrogen bonds are shown as orange dashed lines.

3.1.2. Bis(Pyridinium-4-Carbaldehyde Thiosemicarbazone) Hexafluorophosphate, Nitrate

Compound **2**, was obtained serendipitously as an unexpected one-pot reaction where silver nitrate, pyridine-4-carbaldehyde thiosemicarbazone, sodium hexafluorophosphate and methanol (as solvent) are involved. Compound **2** crystallizes in the *C*2/*c* monoclinic space group and unit cell dimensions a = 45.102(3) Å, b = 13.9934(7) Å, c = 10.7524(6) Å, β = 99.999(2)°, and V = 6683.1(7) Å^3. The asymmetric unit for **2** is shown in Figure 2.

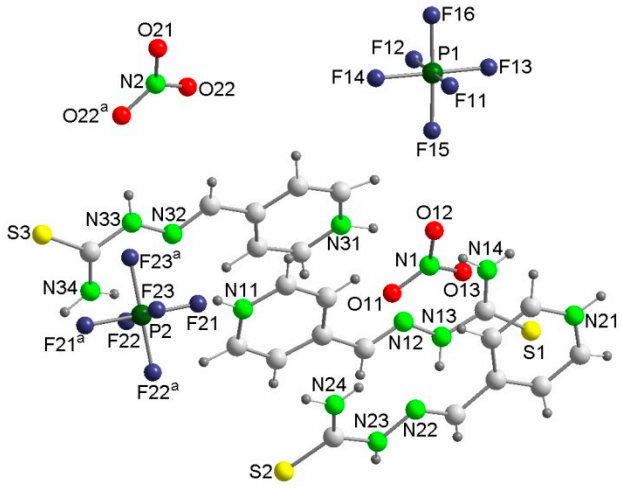


Figure 2. A perspective view showing the asymmetrical unit and the atom-numbering scheme, of **2.** Symmetry transformation; a = −x, y, ½ − z.

The asymmetric unit is made up of three molecules of 4-formyl-pyridinium thiosemicarbazone cations (I to III), (1 + 1/2) PF_6^- and (1 + 1/2) NO_3^-, where one cation each of PF_6^- and NO_3^- occupy special positions. The bond lengths and angles of the main cations agree with those observed in 4-formylpyridine thiosemicarbazone [6] and in 4-formylpyridinium thiosemicarbazone nitrate hydrate [17], or pyridine-4-aldehyde thiosemicarbazone perchlorate [18]. The pyridinium and thiosemicarbazone (side chain) fragments are almost planar; the dihedral angle between the plane of the pyridinium ring and the thiosemicarbazone moiety is 9.29(8)° for cation I, 13.26(3)° for cation II and 11.71(8)° for cation

III, and the torsion angle in the thiosemicarbazone moiety is −179.9° for C16-N12-N13-C17, −178.7° for C26-N22-N23-C27 and −176.6° for C36-N32-N33-C37.

Each of the cations has four hydrogen atoms bonded to the nitrogen atoms that can form hydrogen bonds as donors, against the oxygen and fluorine atoms of the anions NO_3^- and PF_6^-, as acceptors. These intra- and inter-molecular hydrogen bonds are characterized by average geometric parameters N–H···O (H···O, 2.16 Å; N···O, 2.94 Å; ∠N–H···O, 151.19°) and N–H···F (H···F, 2.29 Å; N···F, 3.04 Å; ∠N–H···F, 150.45°). Furthermore, in the crystal packing the 4-formylpyridinium thiosemicarbazone cations also assemble into dimers connected by weak hydrogen bonds involving the sulfur atoms of the nearest neighboring thiosemicarbazone (H···S, 2.58 Å; N···S, 3.40 Å; ∠N–H···S, 165.33°) (Figure 3). The crystal packing is also reinforced by supramolecular stacking interactions aromatic ring-aromatic ring, between the pyridinium rings of the molecule II and its nearest neighbor, with centroid-centroid distances (*Cg*2-*Cg*2) of 3.491(3) Å (α = 0.03°) and between the rings of molecules I and III [*Cg*1-*Cg*3, 3.473(4) Å, α = 4.36°], both parallel to the crystallographic axis "c".

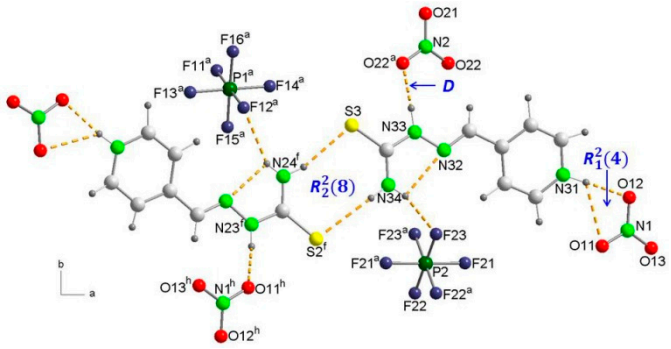


Figure 3. Partial packing diagram for (**2**), showing one layer of the two-dimensional network, the intermolecular interactions and the supramolecular synthons. Hydrogen bonds are shown as orange dashed lines. Symmetry transformations: a = −x, y, ½ − z; f = −x, y, 3/2-z; h = −x, −y, 1 − z.

3.2. Antiproliferative Activity

The in vitro anticancer activity of 1 was determined in human cervical cancer cells (HeLa-229) by the colorimetric MTT assay [(3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay] with an exposure time of 72 h. The percentage of inhibition of cell growth for the thiosemicarbazone is 10 demonstrating low antiproliferative activity.

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References

1. Kalinowski, D.S.; Quach, P.; Richardson, D.R. Thiosemicarbazones: The new wave in cancer treatment. *Future Med. Chem.* **2009**, *1*, 1143–1151.
2. Siddiqui, E.J.; Azad, I.; Khan, A.R.; Khan, T. Thiosemicarbazone Complexes as Versatile Medicinal Chemistry Agents: A Review. *J. Drug Delivery Ther.* **2019**, *9*, 689–703.
3. Beraldo, H.; Gambino, D. The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes. *Mini.-Rev. Med. Chem.* **2004**, *4*, 31–39.
4. Lobana, T.S.; Sharma, R.; Bawa, G.; Khanna, S. Bonding and structure trends of thiosemicarbazone derivatives of metals-An overview. *Coord. Chem. Rev.* **2009**, *253*, 977–1055.

5. Heffeter, P.; Pape, V.F.S.; Enyedy, E.A.; Keppler, B.K.; Szakacs, G.; Kowol, C.R. Anticancer thiosemicarbazones: Chemical properties, interaction with iron metabolism, and resistance development. *Antioxid. Redox Signal.* **2019**, *30*, 1062−1082.
6. Mendes, I.C.; Teixeira, L.R.; Lima, R.; Beraldo, H.; Speziali, N.; West, D.X. Structural and spectral studies of thiosemicarbazones derived from 3- and 4-formylpyridine and 3- and 4-acetylpyridine. *J. Mol. Struct.* **2001**, *559*, 355–360.
7. Belardinelli, J.M.; Morbidoni, H.R. Recycling and refurbishing old antitubercular drugs: The encouraging case of inhibitors of mycolic acid biosynthesis. *Expert Rev. Anti Infect. Ther.* **2013**, *11*, 429–440.
8. Pozharskii, A.F.; Soldatenkov, A.T.; Katritzky, A.R. *Heterocycles in Life and Society: An Introduction to Heterocyclic Chemistry, Biochemistry, and Applications*, 2nd ed.; John Wiley & Sons: Hoboken, NJ, USA, 2011; pp. 139−143.
9. Welton, T. Room-Temperature Ionic Liquids. Solvents for Synthesis and Catalysis. *Chem. Rev.* **1999**, *99*, 2071–2084.
10. Wasserscheid, P.; Welton, T. *Ionic Liquids in Synthesis*, 2nd ed.; Wiley-VCH: Weinheim, Germany, 2008.
11. Sowmiah, S.; Esperança, J.M.S.S.; Rebelo, L.P.N.; Afonso, C.A.M. Pyridinium salts: From synthesis to reactivity and Applications. *Org. Chem. Front.* **2018**, *5*, 453–493.
12. Restivo, R.; Palenik, G.J. The crystal and molecular structure of 4-formylpyridine thiosemicarbazone. *Acta Crystallogr. Sect. B Struct. Crystallogr. Cryst. Chem.* **1970**, *26*, 1397–1402.
13. Wang, H.; Hossain, A.M.S.; Zhang, Q.; Wu, J.; Tian, Y. 2-(Pyridin-4-ylmethylene) hydrazinecarbothioamide. *Inorg. Chim. Acta.* **2014**, *414*, 153–159.
14. Sahu, M.; Manna, A.K.; Rout, K.; Mondal, J.; Patra, G.K. 2-[(pyridin-4-yl)methylidene]hydrazine-1-carbothioamide. *Inorg. Chim. Acta.* **2020**, *508*, 119633.
15. Clegg, W.; Harrington, R.W. 2-[(pyridin-4-yl)methylidene]hydrazine-1-carbothioamide dihydrate, ROJJOS, CSD Communication, 2019.
16. Castiñeiras, A.; García-Santos, I.; Nogueiras, S.; Rodríguez-González, I.; Rodríguez-Riobó, R. Supramolecular interactions in biologically relevant compounds. 2-Pyrazineformamide thiosemicarbazones and some products of their cyclization. *J. Mol. Struct.* **2014**, *1074*, 1–18.
17. Zhang, Y. Crystal structure of 4-formylpyridine thiosemicarbazonium nitrate hydrate, $[C_7H_9N_4S][NO_3]$ H_2O. *Z. Kristallogr.-New Cryst. Struct.* **2009**, *224*, 289–289.
18. Smolentsev, A.I.; Lavrenova, L.G.; Elokhina, V.N.; Nakhmanovich, A.S.; Larina, L.I. Crystal structures of pyridine-4-aldehyde thiosemicarbazone perchlorate and trifluoromethane sulfonate. *J. Struct. Chem.* **2009**, *50*, 500–504.

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