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SYNTHESIS AND CHARACTERIZATION BY ^{1}H NMR AND ^{1}H, ^{15}N HSQC OF A SERIES OF MONOXIME THIOSEMICARBAZONE COMPOUNDS

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Abstract

This study reports the synthesis of a series of five monoxime thiosemicarbazone compounds: pyruvic aldehyde-1-oxime methylthiosemicarbazone (PAO-MTSC), pyruvic aldehyde-1-oxime ethylthiosemicarbazone (PAO-ETSC), pyruvic aldehyde-1-oxime tert-butylthiosemicarbazone (PAO-tBTSC), pyruvic aldehyde-1-oxime benzylthiosemicarbazone (PAO-MTSC), pyruvic aldehyde-1-oxime phenylthiosemicarbazone (PAO-PTSC). This series of compounds wascharacterized using ^{1}H NMR and ^{1}H^{15}N heteronuclear single quantum coherence (HSQC).

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Introduction

Thiosemicarbazones are known to exhibit the ability to function as ligands in numerous metal complexes with a variety of metals.[1] Most importantly, they are of interest in pharmacological research because some thiosemicarbazones and/or their metal complexes exhibit antibacterial, antiviral, and anticancer activities.[2] In order to explore their biological functions, the skeletal structure of thiosemicarbazones has been modified in order to find the best structures for cell membrane permeability, and this lab has been active in the synthesis of new thiosemicarbazone ligands using different substrates to form new metal complexes for biological function.[3-7] Oximes are also an important ligand type that have spawned research interest for many years forming many multidentate metal complexes.[8-10]

Prior work has been done to show the synthesis of butanedione-monoxime thiosemicarbazone compounds.[11,12] However, only one article in the literature in 1967 has shown successful work with pyruvic aldehyde-1-oxime (PAO) in creating thiosemicarbazones using thiosemicarbazide, methyl-thiosemicarbazide, and ethyl-thiosemicarbazide but the NMR analysis was very limited.[13] In order to further this research with more advanced NMR techniques, this lab has successfully synthesized and characterized by NMR the following series of monoxime thiosemicarbazone compounds shown in Figure 1. Furthermore, we have established in unreported work that these five compounds are excellent chelating tridentate ligands and are the focus of further work with several transition metals.

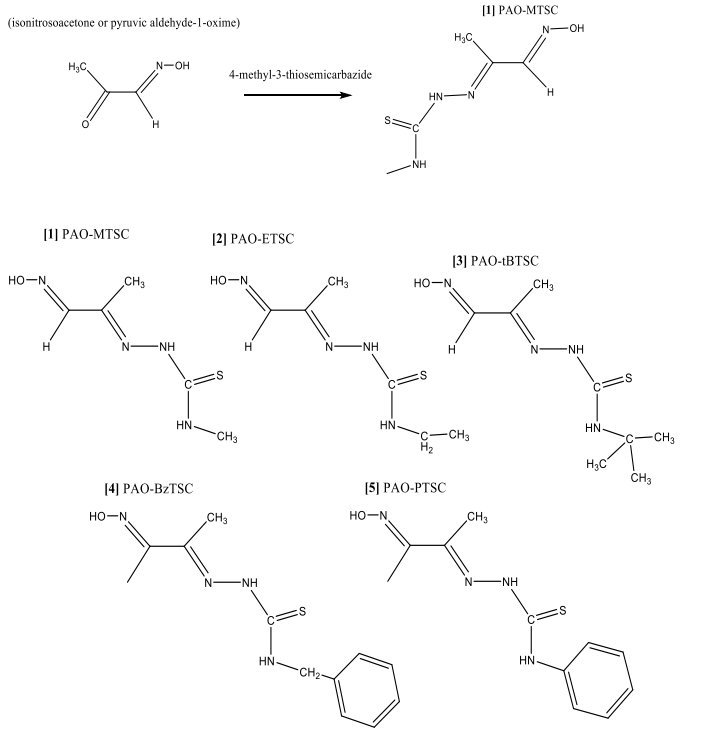

Figure 1. Reaction to yield monoxime thiosemicarbazones and structures of **[1]**-**[5]**.

Experimental

Chemicals were purchased from Sigma-Aldrich and Fischer chemical companies and used without further preparation unless otherwise noted. NMR spectroscopy was carried out at the Center for Structural Chemistry, Tennessee Technological University (USA). The spectra reported here were measured with a Bruker Avance III HD 500 spectrometer at 500.13 MHz (^{1}H) and 50.69 MHz (^{15}N) at 25 °C. For these measurements, the substances were dissolved in the appropriate deuterated solvent, and the chemical shifts were referenced to the solvent residual peak. Coupling constants (*J*) are given in hertz. The ^{1}H NMR experiments were acquired using Bruker's standard Proton (zg30) NMR pulse sequence with the following parameters: Relaxation delay, 1s; 90° pulse, 12.0 μs; spectral width, 10,000 Hz; number of data points, 32K; and digital resolution, 0.153 Hz/point. All of the monoxime thiosemicarbazones were synthesized by the same general procedure, an example is described by the following which is for **[3]** PAO-tBTSC:

[3] Pyruvic aldehyde-1-oxime tert-butylthiosemicarbazone (PAO-tBTSC)

2-[(2*E*)-2-(hydroxyimino)-1-methylethylidene]-*N*-tertbutyl-hydrazinecarbothioamide

In a 50 mL Erlenmeyer flask accompanied with a magnetic stir bar on a heat/stir plate containing 0.503g (5.78 x 10^{-3} mol) of pyruvic aldehyde-1-oxime in 15 mL of 1% aqueous acetic acid was added 0.804g (5.47 x 10^{-3} mol) 4-tertbutyl-3-thiosemicarbazide along with 15 mL of 1% aqueous acetic acid. The reaction

was run at room temperature. After thirty minutes of stirring the mixture, it was diluted with 10.0 mL of 1% aqueous acetic acid. The reaction was then left to run for 24 hours, at which time a white product was filtered and dried. Yield 0.814g (4.32 x 10^{-3} mol) 62.2% PAO-ETSC.
^{1}H NMR (500 MHz, DMSO) δ 11.60 (s, H), 10.24 (s,1H), 7.74 (s,1H), 7.71 (s,1H), 2.07 (s, 3H), 1.50 (s, 9H).

[1] Pyruvic aldehyde-1-oxime methylthiosemicarbazone (PAO-MTSC),
2-[(2*E*)-2-(hydroxyimino)-1-methylethylidene]-*N*-methyl-hydrazinecarbothioamide
Yield 78.6% PAO-ETSC.
^{1}H NMR (500 MHz, DMSO) δ 11.59 (s, 1H), 10.47 (s, 1H), 8.54 (q, *J* = 4.6 Hz, 1H), 7.66 (s, 1H), 2.98 (d, *J* = (4.6) Hz, 3H), 2.08 (s, 3H).

[2] Pyruvic aldehyde-1-oxime ethylthiosemicarbazone (PAO-ETSC),
2-[(2*E*)-2-(hydroxyimino)-1-methylethylidene]-*N*-ethyl-hydrazinecarbothioamide
Yield 75.5% PAO-ETSC.
^{1}H NMR (500 MHz, DMSO) δ 11.59 (s, 1H), 10.40 (s, 1H), 8.56 (t, *J* = 5.8 Hz, 1H), 7.70 (s, 1H), 3.56 (qd, *J* = (7.4, 5.8) Hz, 2H), 2.08 (d, *J* = 7.8 Hz, 3H), 1.13 (t, *J* = 7.1 Hz, 3H).

[4] Pyruvic aldehyde-1-oxime benzylthiosemicarbazone (PAO-BzTSC)
2-[(2*E*)-2-(hydroxyimino)-1-methylethylidene]-*N*-benzyl-hydrazinecarbothioamide
Yield 83.2% PAO-BzTSC.
^{1}H NMR (500 MHz, DMSO) δ 11.60 (s, H), 10.61 (s,1H), 9.08 (t, 7Hz, 2H), 7.69 (s, 1H), 7.38 – 7.03 (m, 2H), 7.03 (s, 1H), 4.80 (t, *J* = 7.2 Hz, 1H), 3.33 (s, 269H), 2.50 (dt, *J* = 3.6, 1.8 Hz, 131H), 2.09 (s, 1H).

[5] Pyruvic aldehyde-1-oxime phenylthiosemicarbazone (PAO-PTSC)
2-[(2*E*)-2-(hydroxyimino)-1-methylethylidene]-*N*-phenyl-hydrazinecarbothioamide
Yield 85.7% PAO-ETSC.
^{1}H NMR (500 MHz, DMSO) δ 11.65 (s, H), 10.87 (s,1H), 10.16 (s,1H), 7.81 (s,1H), 7.59 (d,1H), 7.36 (t,1H), 7.19 (d,1H), 2.14 (s, 3H).

Results and Discussion

In a previous article that we published in this Journal on another series of monoxime thiosemicarbazones based on butanedione, we synthesized an analogous set of compounds with the acronyms BDMO-MTSC to BMDO-PTSC.[12] However, those compounds were synthesized using isopropanol as a solvent instead of 1% acetic acid. We went back to that synthesis and tried instead 1% acetic acid as the solvent, and discovered that it was far superior to isopropanol, and gave much better yields and a cleaner product. Once we discovered that, we utilized the 1% acetic acid for this synthesis with great results. The compounds **[1]** - **[5]** were synthesized cleanly, as shown by NMR, and did not require recrystallization.

Assignments of proton resonances were correlated with structure, an example of which is shown in Figure 2. of compound **[2]** PAO-ETSC. The proton NMR analysis revealed (12-9 ppm) two sharp signals downfield that were assigned to the hydrazinic proton and the oxime proton generally, but the two peaks could not be distinctly differentiated. The thioamide proton could be seen at a more upfield resonance, broader with coupling in every case except the tertbutyl compound **[3]** and the phenyl compound **[5]** which are singlets. Ordinarily, the hydrazinic proton (NN-H) is the most downfield signal seen in the proton NMR, but the oxime proton (N-OH) is also observed in the same region. The ambiguity between the two signals is addressed by running a 2D NMR experiment called heteronuclear single quantum coherence (^{1}H, ^{15}N, HSQC) which revealed which protons were directly bound to nitrogen atoms. The results of one of these experiments, as illustrated in Figure 3, show a plot that is two dimensional with one axis for proton (^{1}H) and the other axis for a heteronucleus such as ^{15}N, utilized in this experiment. Figure 3 reveals that the most downfield resonance (11.55ppm) can now be unambiguously assigned to the oxime proton, which is not directly bound to nitrogen but is bound to an oxygen atom. The hydrazinic proton and the thioamide proton are assigned to resonances at 10.41 ppm and 8.56 ppm respectively and are directly bound to nitrogen as the correlation shows. These characteristic protons are all given in Table 1 for comparison. The resonances for the oxime protons are very consistent, as well as the hydrazinic protons resonances, but the thioamide protons vary most in position due to their proximity to the differing substituents on the various thiosemicarbazone arm. Figure 3 also reveals that the most upfield proton, in this

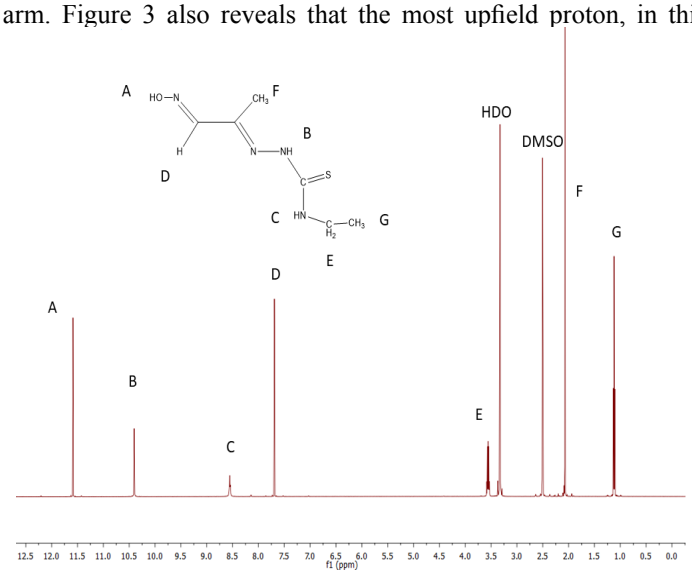

Figure 2. The ^{1}H NMR of oxime thiosemicarbazone [2] with structural assignments

Table 1. Downfield Proton NMR Resonances of Compounds [1] – [5] ppm.

Compound	Oxime	Hydrazinic	Thioamide
[1] PAO-MTSC	11.59 (s)	10.47 (s)	8.54 (q)
[2] PAO-ETSC	11.59 (s)	10.40 (s)	8.56 (t)
[3] PAO-tBTSC	11.60 (s)	10.25 (s)	7.74 (s)
[4] PAO-BzTSC	11.55 (s)	10.61 (s)	9.08 (t)
[5] PAO-PTSC	11.65 (s)	10.87 (s)	10.16 (s)

experiment, must be assigned to the backbone C-H pyruvic carbon.

Future work on these compounds will focus on their abilities to function as ligands to transition metals, such as Ni, Cu, Pd, and Pt in order to further our research into anti-cancer properties of designed metal complexes.[14-17]

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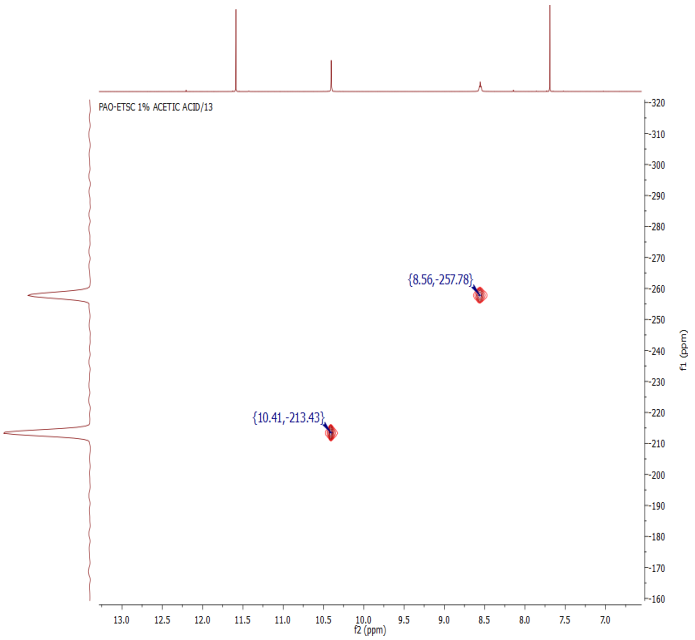

Figure 3. The ^{1}H, ^{15}N, 2D HSQC NMR of oxime thiosemicarbazone [2] showing the correlation and hydrazinic (10.41ppm) and thioamide protons (8.56ppm) bound to nitrogen.

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