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Synthesis and characterization of 2-pyridineformamide 3-pyrrolidinylthiosemicarbazone

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Abstract

Pyrrolidine-1-carbothiohydrazide was prepared by the transamination reaction of 4-methyl-4-phenyl-3-thiosemicarbazide with pyrrolidine in MeCN. 2-pyridineformamide 3-pyrrolidinyl-thiosemicarbazone (HAmPyrr) was synthesized by the reduction of cyanopyridine in the presence of pyrrolidine-1-carbothiohydrazide in MeOH with Na metal. The synthesized compound was characterized by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR spectroscopy and ESI mass spectrometry. The prominent (M+1) peak (m/z) of HAmPyrr in the electron spray ionization mass spectrum was found at 250.09 which correspond to the molecular ion plus H.

Keywords: *Pyrrolidine-1-carbothiohydrazide, Pyrrolidinylthiosemicarbazone, Reduction with sodium, Cyanopyridine.*

Introduction

Thiosemicarbazones (TSC) have been a focus of chemists and biologists because of their wide range of pharmacological effects. These compounds and their chemical relatives have been shown to have marked antibacterial, antiviral, antifungal, and most intriguingly, antineoplastic activity[1]. Heterocyclic thiosemicarbazones are believed to exercise their beneficial therapeutic properties in mammalian cells by inhibiting ribonucleotide reductase in the synthesis of DNA precursors[2]. The non-heme iron subunit has been shown to be inhibited/inactivated by thiosemicarbazones[3]. The activity of these compounds is strongly dependent on the nature of the heteroatomic ring and the position of attachment to the ring as well as the form of thiosemicarbazone moiety[4]. The structure-activity relationships study revealed that activity increases with the increase in size of the N(4) substituent[5-7].

In general, thiosemicarbazones are obtained by the condensation of corresponding thiosemicarbazide with aldehydes or ketones[8,9]. However, an alternative method of synthesis involves the use of nitrile as starting material[10]. In this case, the resulting thiosemicarbazone contains an additional amino group (Fig. 1), which gives improved water solubility. This in turn can improve the biological activity of these compounds[11]. The structures and antimicrobial, antineoplastic activities of a number of 2-pyridineformamde N(4) mono- and di-substituted thiosemicarbazones and their metal complexes have been studied[12-14].

This report includes synthesis and spectral characterization of the novel thiosemicarbazone, HAmPyrr. Two geometrical isomers about the imine double bond (*E* and Z) are possible for the thiosemicarbazone, of which the *E* isomer is stabilized by an intramolecular hydrogen bond between N(3)-H and the heterocyclic nitrogen. (Figure 1a)[15].

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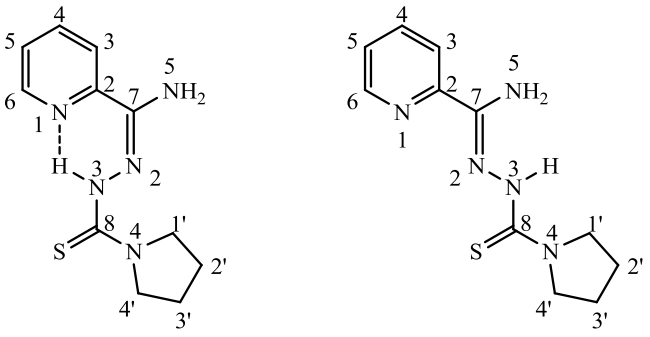

E-isomer (a) *Z*-isomer (b)

Fig. 1: *Isomers of HAmPyrr*

Experimental Methods

Measurements

Partial elemental analysis was performed on a CHN recorder MT-5 at IIT Madras, India. Melting point was determined by using Philip Harris melting point apparatus and was uncorrected. Infrared spectrum was recorded in 4000-500 cm^{-1} region on KBr pellets using Bruker Tensor FTIR spectrometer. NMR spectra were recorded in Varian Mercury 400 MHz spectrometer using $CDCl_3$ and DMSO-d_6 as the solvent. The ^1H and ^{13}C NMR chemical shifts in ppm were reported downfield from internal tetramethylsilane (TMS). ESI mass spectrum was recorded on Micromass Quattro LC triple quadrupole mass spectrometer. All the spectra were recorded at Wayne State University, USA.

Materials

2-Cyanopyridine was purchased from Aldrich and used as received and pyrrolidine-1-carbothiohydrazide was prepared as described by Scovill[16]. N-methyl aniline and sodium chloroacetate were purchased from Himedia, CS_2 from Merck, acetonitrile from Qualigens and were used as received. Methanol was purchased from Fisher scientific and used after distillation.

Synthesis of HAmPyrr

Following the literature procedure for the reduction of 2-cyanopyridine[10], sodium (0.092 g, 4.0 mmol) was added to MeOH (25 mL), which had been dried over Mg and I_2 [17], and the solution was stirred until complete dissolution was achieved. 2-Cyanopyridine (2.60 g, 24.9 mmol) was then added to the above solution and the mixture was stirred for 30 min. Pyrrolidine-1-carbothiohydrazide (3.62 g, 24.9 mmol) was added in small portions over a period of 1 h. A further quantity of MeOH (20 mL) was added and the mixture was heated under reflux for 4 h. Slow evaporation of the MeOH produced the yellowish crystals of HAmPyrr, Anal. Calc. for $C_{11}H_{15}N_5S$; C, 52.99; H, 6.06; N, 28.09; S, 12.86%. Found: C, 53.45; H, 5.69; N,27.72%.; m.p. 159-160°C, yield: 53.1 %; IR (KBr, cm^{-1}): 3388m, 3254m $\nu(NH_2)$; 3224m ν(NH); 1672s $\delta(NH_2)$; 1661s δ(NH); 1597s, 1579s, 1503s, 1486s ν(C=N) + (C=C); 1089m ν(N-N); 848m ν(C=S); 634m ρ(py). ^{1}H-NMR (400 MHZ, $CDCl_3$,δppm); δ_H 12.94 (1H N3H), 8.60 (1H H6), 8.00 (1H H4), 7.79 (1H H3) 7.39 (1H H5), 6.61 (2H NH_2), 3.78 (4H H1',H4'), 1.92 (4H H2',H3'); ^{13}C-NMR (DMSO-d_6, δ ppm)δ_C 176.48 (C=S), 150.29 (C2), 144.57 (C6), 143.80 (C7), 138.71 (C4), 126.79 (C3), 121.39 (C5), 48.57 (C1', C4'), 25.24 (C2', C3'). MS-ESI, m/z (r.i.): 250.09 (100) $[M+1]^+$, 233.05 (60) $[M - NH_2]^+$.

Results and Discussion

Infrared spectroscopy

The characteristic IR bands (cm^{-1}) (Fig.3) are in good agreement with the structure of the synthesized molecule (fig. 1). The thioamide IV band, which has a large ν(C=S) contribution, appeared at 848 cm^{-1} and the azomethine band ν(C=N) at 1597cm^{-1} [13]. The 1600-1400 cm^{-1} region of the spectra was complicated by the presence of thioamide bands and ring breathing vibrations of the pyridyl ring. However, in-plane deformation vibrations characteristic of pyridyl ring ρ(py) was observed at 634 cm^{-1}. The molecule has a proton adjacent to the thiocarbonyl group and consequently can exhibit in thione-thiol tautomerism. The IR spectra of HAmPyrr did not show any ν(SH) band in the region 2600-2200 cm^{-1}, indicating that the thiol tautomer was absent in the solid state[18] but exhibit ν(NH) band at 3224 cm^{-1}, indicating the existence of only the thione tautomer (Fig. 2(a)). The bands corresponding to $\nu(NH_2)$ appeared at 3388 and 3254 cm^{-1} [19]. Medium intensity band was observed at 1089 cm^{-1}, for the hydrazinic (N-N) bond[20].

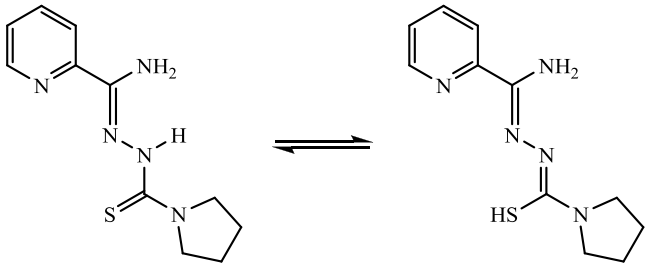

Fig. 2: *Tautomeric forms of HAmPyrr*

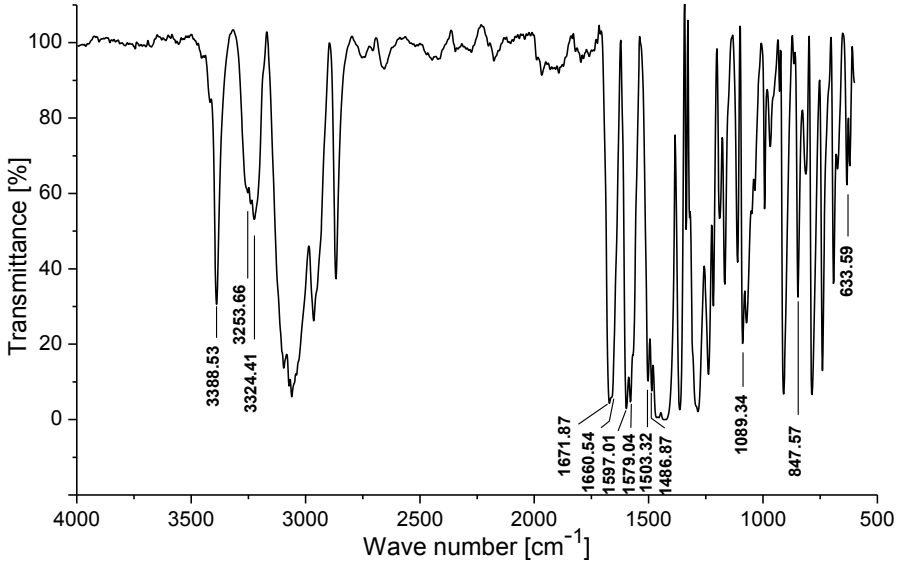

Fig. 3: *IR spectrum of HAmPyrr*

NMR spectroscopy

The numbering scheme used in the assignment of NMR signals is given in the Fig.1. The ^{1}H-NMR data were also consistent with the assigned structure. In ^{1}H-NMR spectra of the compound in $CDCl_3$ (Fig.4), the appearance of the signal of N(3)H at δ12.94 ppm indicates its involvement in the intramolecular hydrogen bonding with the pyridine ring nitrogen. In case of different isomers, if present, N(3)H proton should give more than one signals as has been observed with other pyridine thiosmicarbzones[13]. Further only two sets of signals were observed for methylene protons of pyrrolidine ring at 3.78 and 1.92 ppm. These observations indicate only one isomer dominates in $CDCl_3$. The pyridine C(6)H was observed deshielded (δ=8.60 ppm) compare to the other ring protons as it is close to the pyridine ring nitrogen. The amide hydrogens (NH_2) signal was observed at 6.61 ppm.

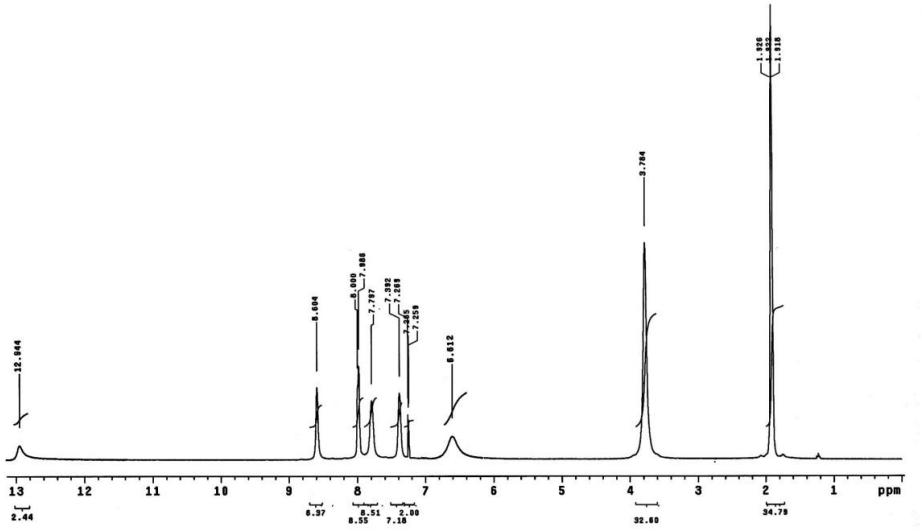

Fig. 4:*^{1}H-NMR spectrum of HAmPyrr*

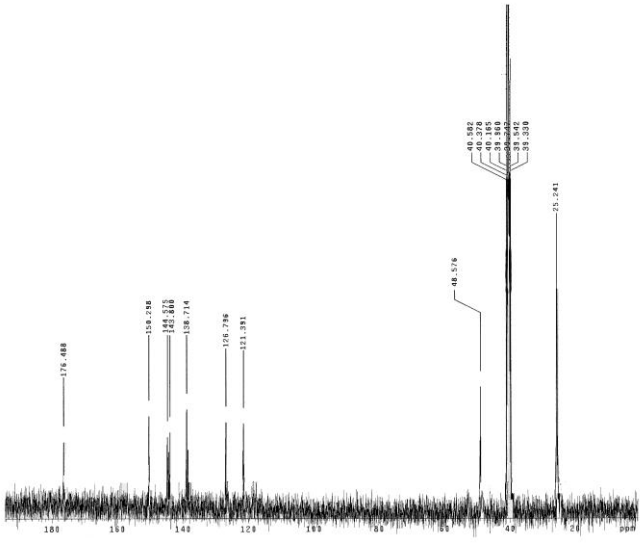

Fig 5: *^{13}C-NMR spectrum of HAmPyrr*

In ^{13}C-NMR spectra the signal due to thiosemicarbazone moiety's thione carbon (C=S) was found down field at 176.48 ppm. This carbon is most deshielded because of extensive electron delocalization along the conjugated framework of carbon skeleton, which reduces the electron density around the carbon atom[12]. The signals due to pyrrolidine ring carbons appeared at 48.57 and 25.24 ppm. The signal at 143.80 ppm was assigned to azomethine carbon (-CH=N-). The C(2) and C(6) carbons adjacent to pyridyl N were found to resonate at 150.29 ppm and 144.57 ppm respectively[19].

Mass spectrometry

The mass fragmentation data are compatible with the proposed molecular formula. The ESI mass spectrum of HAmPyrr recorded in the positive ion mode showed a peak at m/z 250.09 for molecular ion plus H; $[M+1]^+$. Loss of NH_2 fragment is in agreement with a peak at m/z 233.05 [21].

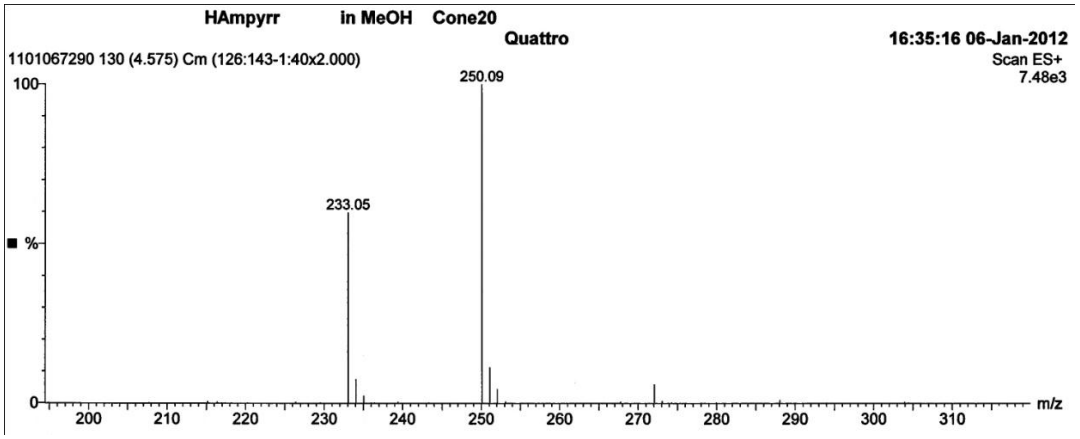

Fig.6: *ESI MS spectrum of HAmPyrr*

Conclusions

2-pyridineformamide 3-pyrrolidinylthiosemicarbazone (HAmPyrr) was synthesized by the reduction of cyanopyridine in the presence of pyrrolidine-1-carbothiohydrazide with Na in methanol. The elemental analysis result of the synthesized compound was in good agreement with the calculated values for the expected molecule. Selected diagnostic IR bands as well as the ^{1}H-NMR and ^{13}C-NMR spectra correlate with the proposed structure of the synthesized compound. The single peak due to N(3)H proton establish the predominance of only one isomer in the $CDCl_3$ solution. The ESI mass spectral data further confirmed the structure of the synthesized compound as indicated by the $[M+1]^+$ peak corresponding to its molecular ion plus H.

Acknowledgments

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