การสังเคราะห์และศึกษาโครงสร้างของลิแกนด์ที่เป็นอนุพันธ์ของเอโซพิริดีน

Synthesis and Structural Characterization of Ligand Containing Azopyrimidine Derivative

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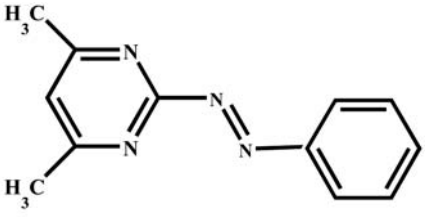
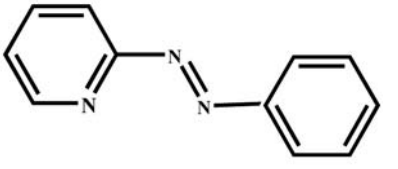
บทคัดย่อ

งานวิจัยนี้เป็นการสังเคราะห์และศึกษาโครงสร้างของลิแกนด์ 4,6-dimethyl-2-(phenylazo) pyrimidine (dmazpym) ซึ่งเป็นลิแกนด์ไบเดนเทตชนิดใหม่ซึ่งมีโครงสร้างคล้ายคลึงกับลิแกนด์ 2-(phenylazo) pyridine (azpy) ซึ่งสังเคราะห์โดยใช้ปฏิกิริยาการควบแน่นระหว่าง 2-amino-4,6-dimethylpyrimidine กับ nitrosobenzene เมื่อมีโซเดียมไฮดรอกไซด์และใช้เบนซีนเป็นตัวทำละลาย ผลการศึกษาโดยเทคนิคอินฟราเรดสเปกโทรสโกปี พบว่าแถบการยืดของ N=N ในลิแกนด์ dmazpym ปรากฏที่เลขคลื่นต่ำกว่าลิแกนด์ azpy นอกจากนี้ได้ศึกษาโครงสร้างของลิแกนด์โดยเทคนิคนิวเคลียร์แมกเนติก เรโซแนนซ์สเปกโทรสโกปีด้วย



4,6-dimethyl-2-(phenylazo)pyrimidine
(dmazpym)



2-(phenylazo)pyridine
(azpy)

คำสำคัญ: ลิแกนด์, ไบเดนเทต, เอโซพิริมิดีน, สเปกโทรสโกปี

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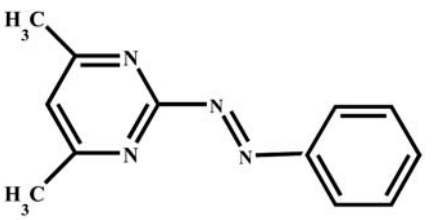
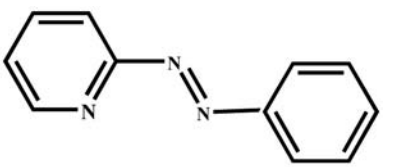
ABSTRACT

The aim of this research is to synthesize and characterize a new bidentate ligand, 4,6-dimethyl-2-(phenylazo)pyrimidine (dmazpym) ligand. Its structure is similar to 2-(phenylazo)pyridine (azpy). The dmazpym ligand was synthesized by condensing 2-amino-4,6-dimethylpyrimidine with nitrosobenzene in the presence of sodium hydroxide and benzene was used as solvent. The results from infrared spectroscopy showed that the N=N stretching mode of dmazpym was observed at lower frequency than that of azpy. Nuclear magnetic resonance spectra of the ligand were also studied.



4,6-dimethyl-2-(phenylazo)pyrimidine
(dmazpym)



2-(phenylazo)pyridine
(azpy)

Key words: ligand, bidentate, azopyrimidine, spectroscopy

INTRODUCTION

Azo compounds have been used for a long time as dyes and pigments in industry as well as optical storage technology (A°strand *et al*., 2000). Moreover, azo dye - metal complexes are increasingly important as analytical agent (Fan and Zhu, 1997) and show interesting property in biological chemistry (Velders *et al*., 2000; Hotze *et al*., 2004; Hotze *et al*., 2003; Chen *et al*., 2006). In the recent years, there has been a great deal of interest in the synthesis and characterization of azo compound as azoimidazole, azopyrazole and azopyrimidine because of their π-acidity of azoimine moiety, -N=N-C=N- which stabilize low oxidation state of metal ions. Such azo compounds with the moiety 2-(phenylazo)pyridine or azpy act as a potent chelating ligand towards some metal ions (Krause and Krause, 1980; Velders *et al*., 2004; Hotze *et al*., 2004) such as $[Ru(azpy)_2Cl_2]$. The isomeric of this compound was found to be reactive as anticancer agents in many cell lines such as MCF-7 (breast cancer), IGROV (ovarian cancer) and H226 (nonsmall cell lung cancer) (Velders *et al*., 2000).

In this work, azopyrimidine was chosen to synthesize because pyrimidine is an active compound and its derivatives are component of the biological important nucleic acid. Compounds

containing nitrogen and sulphur as donor atoms are anticancer and antiviral agents (Masoud *et al*., 2007). In addition, pyrimidine has a better π-acidity property than pyridine (Santra *et al*., 1999). An azopyrimidine derivative with two methyl groups has not been found. Therefore, it is our interest to synthesize a new azo compound, 4,6-dimethyl-2-(phenylazo)pyrimidine (dmazym) and characterize its structure by elemental analysis and some spectroscopic techniques.

MATERIALS AND METHODS

Materials

4,6-dimethyl-2-(phenylazo)pyrimidine ligand (dmazpym) was prepared according to the modified method (Krause and Krause, 1980).

Instrumentation

Elemental compositions of the ligand (C, H, N) were collected using CHNS-O Analyzer, CE Instruments Flash EA 1112 Series, Thermo Quest. Mass spectra were recorded on MAT 95 XL Thermo Finnigan Mass spectrometer. Infrared spectra were obtained from EQUINOX 55 Bruker Fourier Transform Infrared Spectrometer. UV-Visible spectra were recorded on a U-1800 spectrophotometer. 1D and 2D NMR spectra in $CDCl_3$ were collected on a Varian UNITY INOVA 500MHz Fourier Transform NMR spectrometer. Tetramethylsilane ($SiMe_4$) was used as an internal reference.

Procedures

Synthesis of the 4,6-dimethyl-2-(phenylazo) pyrimidine ligand (dmazpym)

The 4,6-dimethyl-2-(phenylazo)pyrimidine ligand was prepared by condensation of 2-amino-4,6-dimethypyrimidine (250 mg, 2.00 mmol) and nitrosobenzene (230 mg, 2.20 mmol) in the presence of sodium hydroxide in benzene. The mixture was refluxed with stirring continued for 15 h. The light-green solution gradually turned to orange-brown. The product was extracted with benzene and purified by column chromatography on a silica gel. The orange band was collected and the solution was evaporated to dryness. The orange solid was obtained (melting point 70.0-70.3^{o}C). The yield was 90 mg (21%). All other chemicals were of reagent grade and were used without further purification. Anal. Calc. for $C_{12}H_{12}N_4$: C, 60.91, N, 26.39: H, 5.70. Found: C, 60.47, N, 26.09: H, 5.67. IR (KBr); ν(N=N), 1330 cm^{-1}, ν(C=C, C=N), 150, 1484, 1592 cm^{-1}

RESULTS AND DISCUSSION

Synthesis

4,6-dimethyl-2-(phenylazo)pyrimidine (dmazpym) was prepared by condensing 2-amino-4,6-dimethylpyrimidine with nitrosobenzene in 1 :1 molar ratio in basic solution under refluxing conditions for 15 h. Purification was carried out by chromatography. The ligand has unsymmetric bidentate with azoimine functional moiety, -N= N-C=N-. The results of elemental analysis and mass spectroscopic data of dmazpym are in good agreement with those required by the proposed formulae. From the Figure 1, the most intense peak at *m/z* 182.9 which give 100% relative

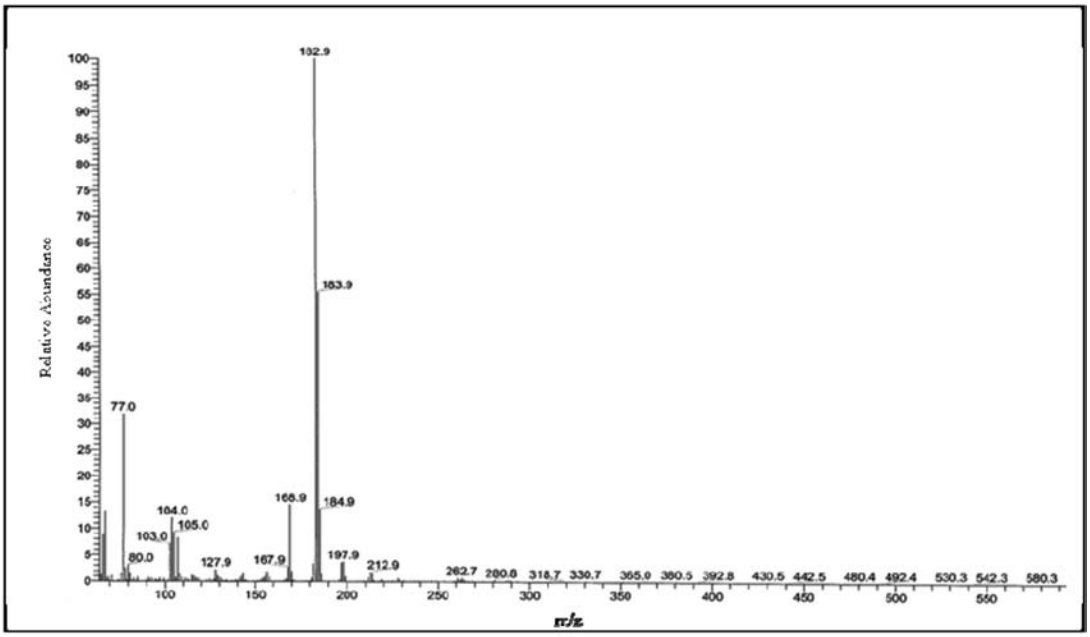


Figure 1 EI-MS spectrum of the 4,6-dimethyl-2-(phenylazo)pyrimidine (dmazpym) ligand

abundance is assigned to the molecular weight of dmazpym with loosing two methyl groups and one protonation. Thus, the measured molecular weight was consistent with expected value.

Spectral characterization

IR spectrum of dmazpym ligand displayed many characteristic frequencies in the range 4000-400 cm^{-1}. The important functional groups C=N, C=C and N=N stretching modes were observed in this range. From IR data, the dmazpym ligand showed the N=N stretching vibration at 1330 cm^{-1}, that of azpy ligand occurred at higher frequency, 1421cm^{-1} (Krause and Krause, 1980). The decrease of N=N stretching frequency in dmazpym ligand due to the effect of methyl substituent which is electron donating group.

Electronic spectra of dmazpym were recorded in UV and visible region in ten different solvents; hexane, chloroform($CHCl_3$), dichloro-methane (CH_2Cl_2), ethyl acetate (EtOAc), Acetone, acetonitrile (CH_3CN), *N,N*-dimethylformamide (DMF), *N,N*-dimethyl sulfoxide (DMSO), ethanol (EtOH), methanol (MeOH) and all data are summarized in Table 1. In addition, the absorption spectrum of dmazpym in CH_2Cl_2 is shown in Figure 2.

The dmazpym ligand exhibits two absorption bands in the range 282-306 nm ($\varepsilon \sim$ 12400 - 10100 $M^{-1} cm^{-1}$) and 430-442 nm ($\varepsilon \sim$ 500-200 $M^{-1}cm^{-1}$). These are assigned to 11 $\pi \rightarrow \pi^*$ in UV region and $n \rightarrow \pi^*$ transitions in visible region, respectively.

The NMR (500 MHz) spectra of dmazpym were recorded in $CDCl_3$ and tetramethylsilane (TMS) was used as an internal reference. The proton numbering pattern of dmazpym is shown in Figure 3.

Table 1 The electronic absorption spectral data of dmazpym in different solvents.

solvents	λ_{max}, nm (ε^a x 10^{-4} $M^{-1}cm^{-1}$)
Hexane	282 (1.20), 434 (0.02)
$CHCl_3$	306 (0.85), 438 (0.03)
CH_2Cl_2	301 (1.14), 434 (0.04)
EtOAc	289 (1.14), 433 (0.03)
Acetone	443 (0.04)
CH_3CN	298 (1.24), 430 (0.05)
DMF	292 (1.01), 433 (0.03)
DMSO	298 (1.13), 442 (0.04)
EtOH	304 (1.12), 437 (0.03)
MeOH	305 (1.10), 436 (0.04)

[a] Molar extinction coefficient

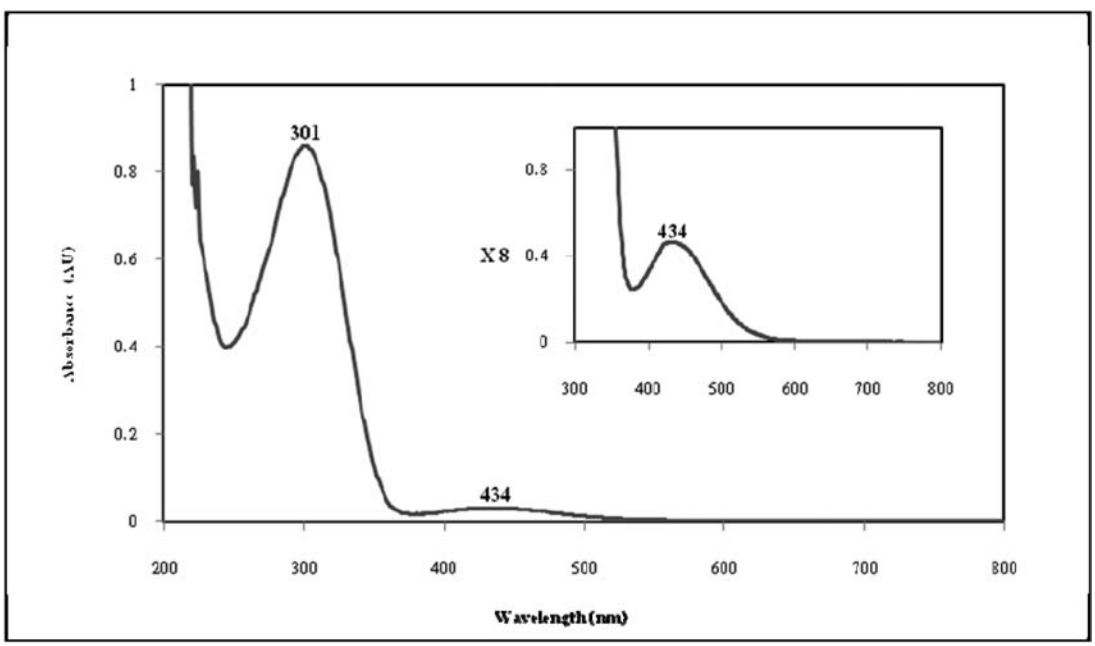


Figure 2 UV-Visible absorption spectrum of the 4,6-dimethyl-2-(phenylazo)pyrimidine (dmazpym ligand in CH_2Cl_2.

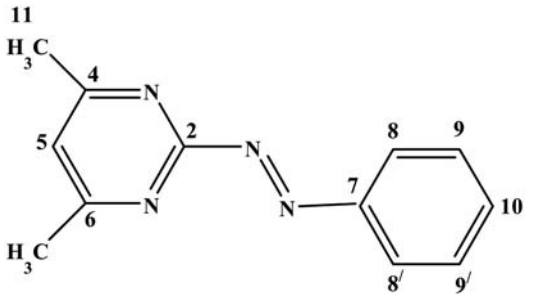


Figure 3 The structure and atom numbering scheme of dmazpym.

The structure of dmazpym was determined by using 1D and 2D NMR spectroscopic techniques; ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY NMR, DEPT NMR, ^{1}H-^{13}C HMQC NMR. The chemical shift and *J*-coupling constant data of dmazpy are listed in Table 2 and NMR spectra are shown in Figure 4 to Figure 8.

The ^{1}H NMR spectrum of dmazpym is shown in Figure 4. There are two types of protons, aliphatic and aromatic proton. The ^{1}H NMR spectrum exhibited four signals for twelve protons. The details of each signal could be explained below.

The proton 8, 8′ positions were equivalent and appeared at 8.08 ppm as doublet of doublet (dd) (*J* = 7.5, 1.5 Hz). These protons showed the most downfield position due to the located next to the azo nitrogen. The proton 9, 9′ were equivalent which located next to proton 8, 8′. The resonance showed multiplet (m) peak at 7.45 ppm. The proton 10 was located next to proton 9, 9′. The splitting pattern was multiplet (m) at the same position of proton 9 (7.45 ppm). The proton 5 resonance appeared at 7.08 ppm as singlet (s). It is due to the effect of two methyl groups on pyrimidine ring to proton H5 which was increase electron density. So, this proton appeared more higher field than that of H8, 8′ H9, 9′ and H10. In addition, the signal of two methyl group appeared at 2.6 ppm corresponded to six protons at the most high field.

Moreover, the peak assignments were confirmed by using ^{1}H-^{1}H COSY NMR spectrum as Figure 5. They showed the correlation of ^{1}H-^{1}H coupling. The results from ^{13}C NMR spectrum as Figure 6 corresponded to the result of DEPT NMR spectrum as Figure 7, which showed both of methane and methyl carbon signals. The ^{13}C NMR spectrum of dmazpym ligand showed 8 signals for 12 carbons. The signal of quaternary carbon C4 and C6 which located near nitrogen of

Table 2 ^{1}H and ^{13}C NMR assignments of dmazpym [500 MHz,in $CDCl_3$].

position	δ_H (ppm), Multiplicity, *J* in Hz	δ_C (ppm)
H8, 8′	8.08, 2H, dd, 7.5, 1.5 Hz	124.00
H9, 9′	7.45, 3H, m	128.97
H10		132.77
H5	7.08, 1H, s	120.07
H11	2.60, 6H, s	24.00

s = singlet, dd = doublet of doublet, m = multiplet

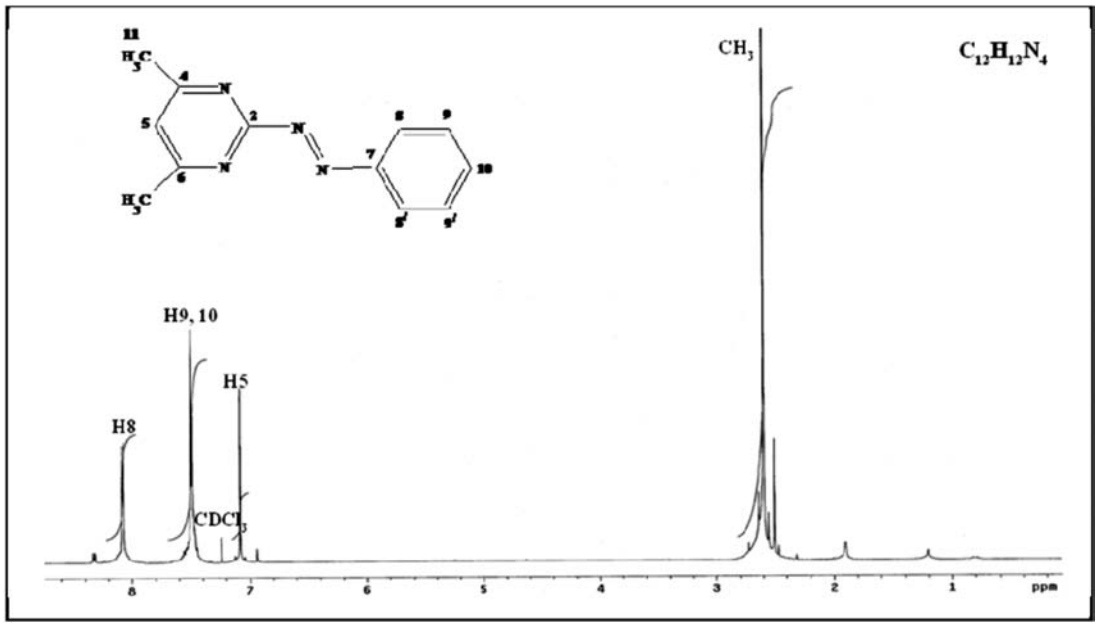


Figure 4 ^{1}H NMR spectrum of the dmazpym ligand in $CDCl_3$.

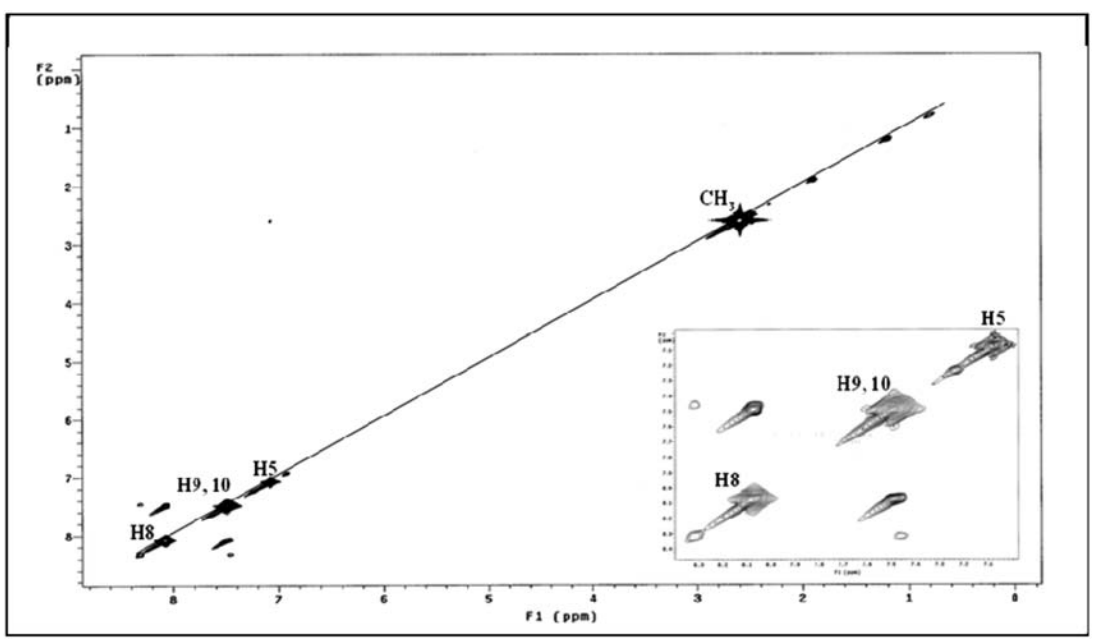


Figure 5 ^{1}H-^{1}H COSY NMR spectrum of the dmazpym ligand in $CDCl_3$.

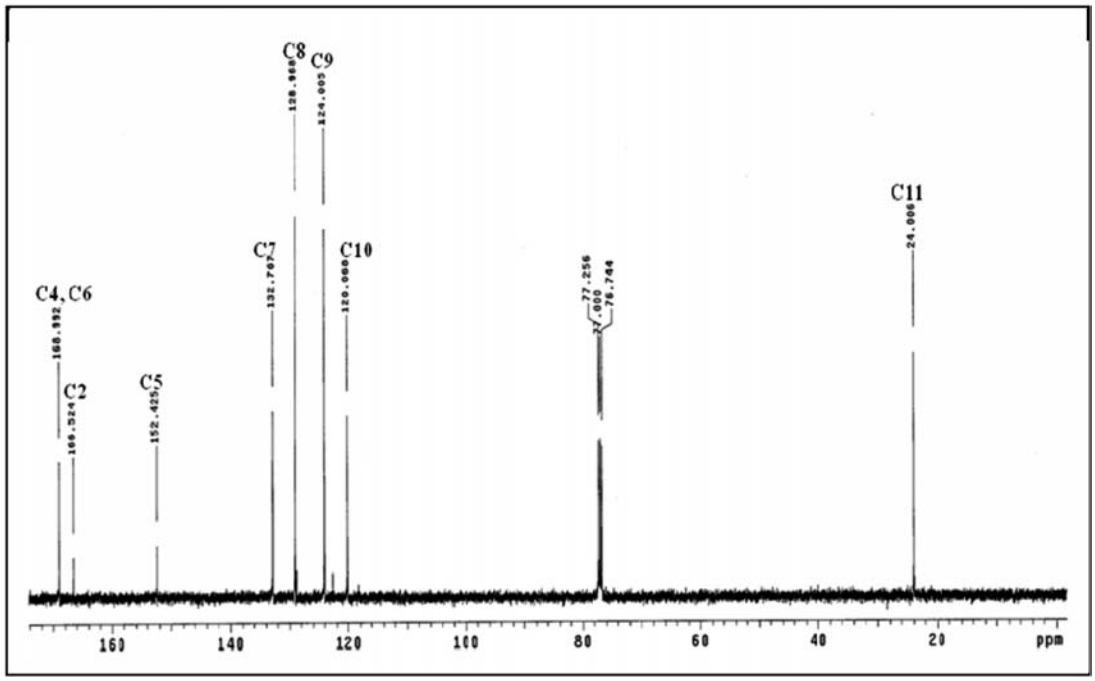


Figure 6 ^{13}C NMR spectrum of the dmazpym ligand in $CDCl_3$.

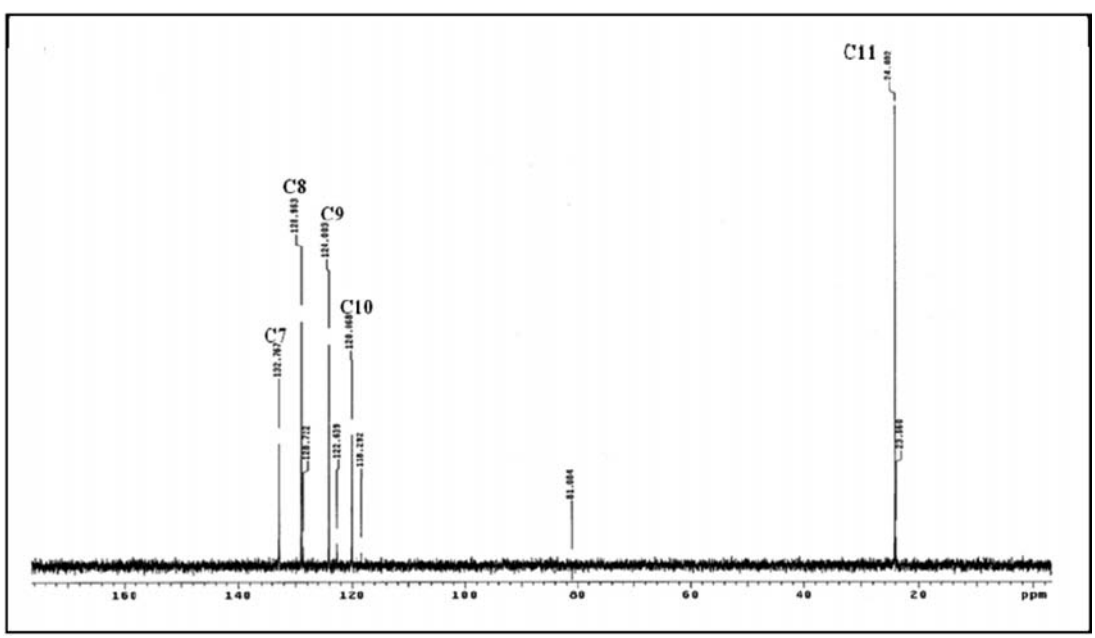


Figure 7 DEPT NMR spectrum of the dmazpym ligand in $CDCl_3$.

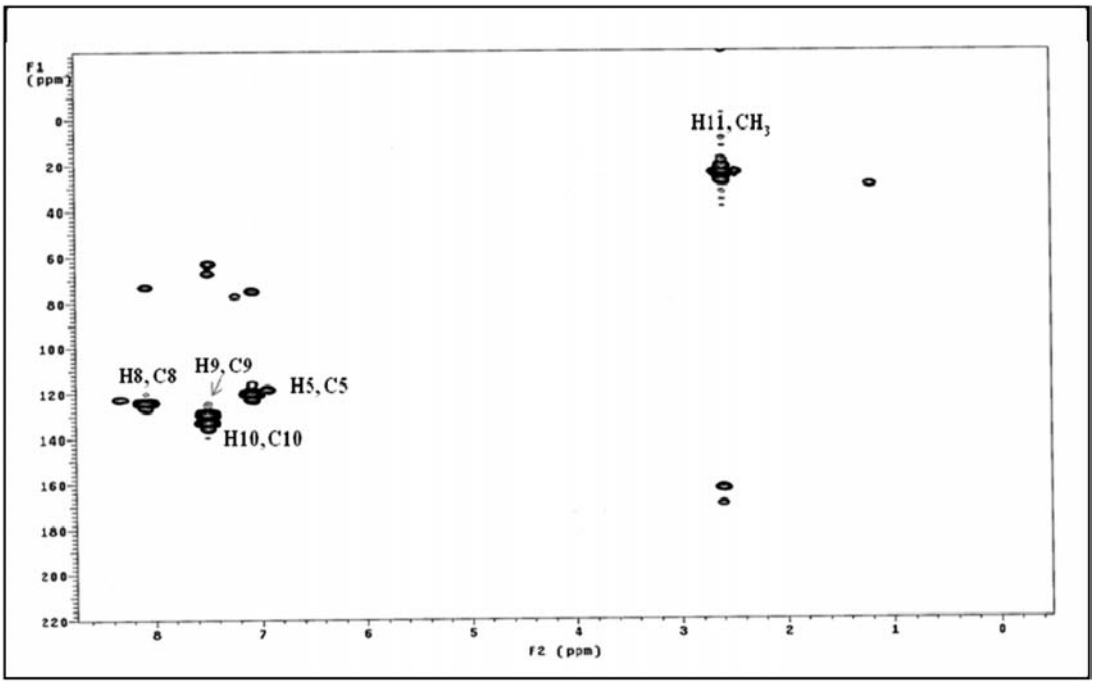


Figure 8 ^{1}H-^{13}C HMQC NMR spectrum of the dmazpym ligand in $CDCl_3$.

pyrimidine ring appeared at the most downfield (168.99 ppm). The signals at 166.52 and 152.42 ppm belonged to quaternary carbon C2 and C7, respectively. The signal of C5 located between methyl group appeared at 120.07 ppm. The signal at 124.00 and 128.97 ppm were assigned to two equivalent carbon of carbon C8 and C9, respectively. The carbon C10 signal on phenyl ring occurred at 132.77 ppm. Moreover, the ^{13}C assignments were supported by the HMQC NMR spectrum as in Figure 8, which showed the correlation between ^{1}H and ^{13}C NMR spectra.

Therefore, the results of 1D and 2D NMR spectra were helpful to us to assign all signals corresponded to the correctly expected structure of dmazpym ligand.

CONCLUSION

4,6-dimethyl-2-(phenylazo)pyrimidine (dmazpym) was synthesized as a new bidentate ligand having azoimine (-N=N-C=N-) moiety like azpy. The molecular structure was confirmed by 1D and 2D NMR spectroscopy. For the further work, antimicrobial activity of this compound is planned to be investigated by testing with microorganism.

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