中國醫藥大學藥學院
藥物化學研究所碩士論文

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化學選擇性合成

1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines 和

pyrazolyl-2-azadienes 新方法暨抗癌活性之探討

Chemoselective synthesis, antiproliferative activities and

SAR study of 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines and

pyrazolyl-2-azadienes

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謝辭

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Contents

Chart List

Abbreviation List

Cesium carbonate ($CsCO_3$)

Dimethylaminopyrium (DMAP)

N,*N*-Dimethylformamide (DMF)

Ethyl alcohol (EtOH)

Hydrogen chloride (HCl)

Methyl alcohol (MeOH)

Phosphoryl chloride ($POCl_3$)

Potassium carbonate (K_2CO_3)

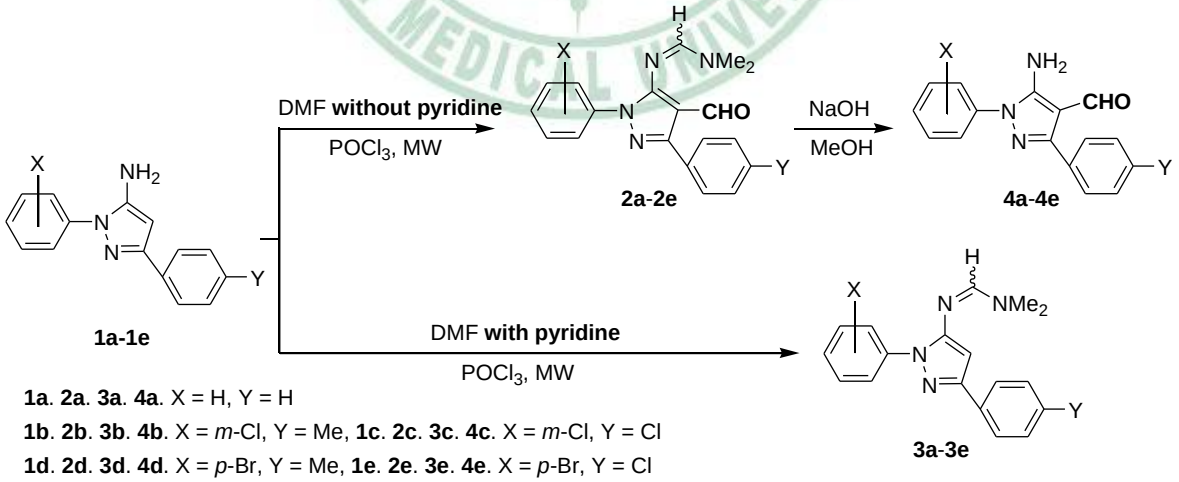
Sodium hydroxide (NaOH)

Triethylamine (NEt_3)

中文摘要

本論文是利用微波加速化學反應及化學選擇性控制方法，合成出 1*H*-pyrazol-5-yl-*N*,*N*-dimethyl-formamidine 和 pyrazolyl-2-azadiene 的兩類化合物，將 5-amino-1,3-diphenyl pyrazole，1*H*-pyrazol-5-yl-*N*,*N*-dimethyl-formamidines，pyrazolyl-2-azadiene，5-amino-4-formylpyrazoles 四類具構效關係的化合物經由藥理活性篩選 (NCI-H661，NPC-TW01，Jurkat)。

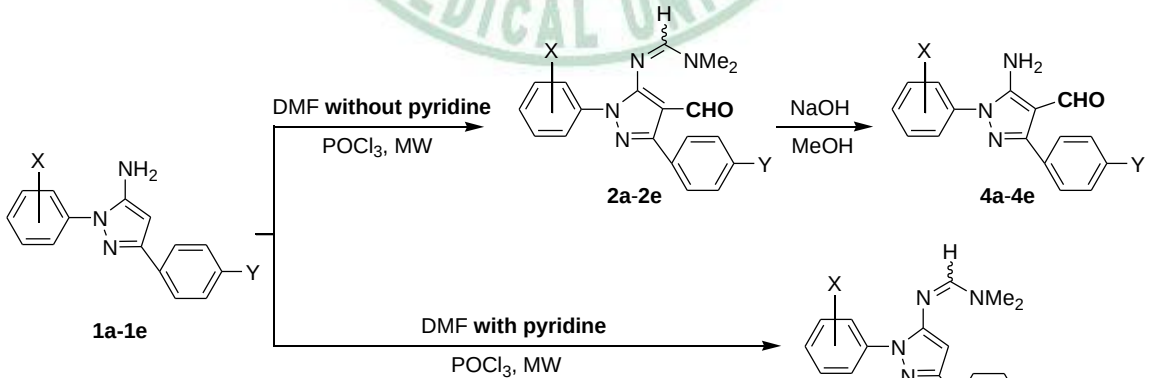
其結果顯示 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines 衍生物 **2b**，**2c**，**2d** 具較佳的藥理活性 (**IC_{50}: 6.0~9.2μM**)，從藥理活性也指出在 pyrazole derivatives 須同時擁有 amidinyl 與 formyl 官能基才能增強藥理活性。



Abstract

Chemoselective microwave-assisted amidination was successfully developed to alternatively synthesize 1*H*-pyrazol-5-yl-*N*,*N*-dimethyl-formamidine and pyrazolyl-2-azadiene two classes compounds. All of the starting materials and resulting products were tested against NCI-H226, NPC-TW01, and Jurkat cancer cell lines to evaluate their difference in antiproliferative activities for realizing the structure activity relationship study.

Following the SAR result, 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidine compounds **2b**, **2c** and **2d** possessed the best potent with IC_{50} values in low micromolar range. On the other hand, we found that the formyl group at C-4 position and the grafted amidinyl group in the main core of pyrazolic molecule were necessary for the inhibitory activity

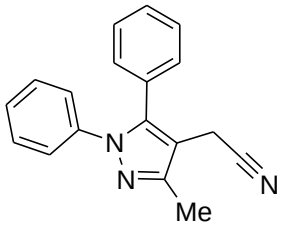


Chapter 1 Introduction

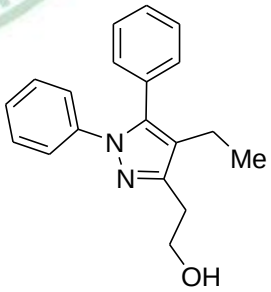
Pyrazoles attract attentions due to their wide range of pharmacological properties; such as anti-asthmatic,[1] antibacterial,[2] anti-inflammatory,[3] antifungal,[4] anticancer,[5] antiviral,[6] anticonvulsant,[7] and antimicrobial.[8]

The bioactivities of functionalized *N*-arylpyrazoles have been extensively studied[9] and the C-5 substituted pyrazoles are also explored in the design of pharmaceuticals and agrochemical agents.[10]

Michael J. Genin[11] in 2000 reported that novel 1,5-diphenylpyrazole nonnucleoside HIV-1 Reverse Transcriptase inhibitors, PNU-32945 and 1,5-Diphenyl-3-(2-hydroxyethyl)-4-ethylpyrazole (Figure 1), were found to have excellent activity versus delavirdine-resistant P236L[12] (IC_{50} = 1.1 μM) reverse transcriptase (RT) for inhibition of viral replication in cell cultures.



PUN-32945



1,5-Diphenyl-3-(2-hydroxyethyl)-4-ethylpyrazole

Figure 1. PNU-32945 and derivatives

Recently, the studies of pyrazole derivatives focus on antibacterial, antifungal, and anticancer as their key utilization .

Section 1.1 Pyrazoles derivatives as Antibacterial Lead Compound

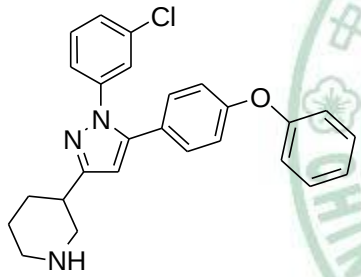
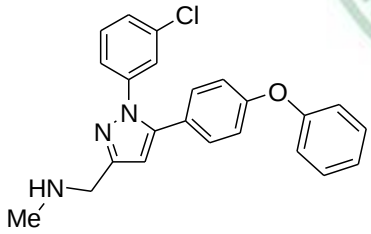
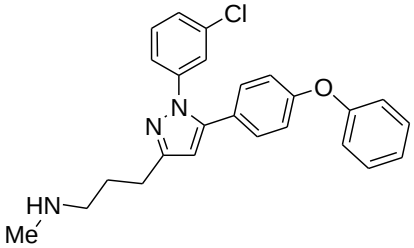
Akihiko Tanitame[2a] used a new screening system for the specific inhibitors of chromosome partitioning in *Escherichia coli*,[13] and had previously reported that 4-piperidyl moiety in pyrazole ring and 1-(3-chlorophenyl)-5-(4-phenoxyphenyl)-3-(4-piperidyl)pyrazole (Figure 2) having a piperidine ring represents a series of bacterial DNA gyrase inhibitors that have effective antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis*.[14] Akihiko Tanitame have also demonstrated that 1-(3-chlorophenyl)-5-(4-phenoxyphenyl)-3-(4-piperidyl) pyrazole (Figure 2), shows improved DNA gyrase inhibition and target-related antibacterial activity.[13a] Moreover, 4-piperidyl moiety in pyrazole ring and 1-(3-chlorophenyl)-5-(4-phenoxyphenyl)-3-(4-piperidyl)pyrazole had the similar inhibitory values against clinically isolated multidrug resistant Gram-positive bacteria with a minimal concentration.

5-(4-phenoxyphenyl)-3-(4-piperidyl)pyrazole 1-(3-chlorophenyl)-5-(4-phenoxyphenyl)-3-(4-piperidyl)pyrazole

Figure 2. Pyrazoles derivatives as antibacterial agent

Akihiko Tanitame transformed piperidyl functional group of 1-(3-chlorophenyl)-5-(4-phenoxyphenyl)-3-(4-piperidyl)pyrazole to other functional groups and evaluated their antibacterial activity in vitro (Table 1).

Table 1. Antibacterial activity of the pyrazole derivatives

Compound (MIC: μg/mL)	*Staphylococcus aureus*		*Enterococcus faecalis.*	
	FDA 209P	KMP9	NIHJ JC-2	W3110
	2μg/mL	2μg/mL	16μg/mL	2μg/mL
	4μg/mL	4μg/mL	16μg/mL	2μg/mL
	2μg/mL	2μg/mL	8μg/mL	2μg/mL
Novobiocin	0.25μg/mL	0.25μg/mL	64μg/mL	0.5μg/mL

*MIC: Minimum inhibitory concentration

The emergence of bacterial strains with resistance to currently marketed antibacterial agents has promoted interest in the discovery of noval antibacterial agents with novel modes of action.[15] One set of potential new targets are the family of bacterial amino acyl-tRNA synthetases.[16] These enzymes are necessary for bacterial growth and have been validated as drug targets by the reserch and development of pseudomonic acid, whose mode of action is the inhibition of bacterial isoleucinyl tRNA synthetase.[17]

Some of a broad program to discover bacterial tRNA synthetase inhibitors,[18] 1*H*-3-carboxylic acid-5-(2',4'-dichloro[1,1'-biphenyl]-4-yl)-1-(2,4-dichlorophenyl)-Pyrazole (Figure 3) was determined as an inhibitor of methionyl-tRNA synthetase (MetRS) by high-throughput screening.[19] It is a modest micromolar inhibitor of the bacterial MetRS enzyme from two important Gram positive bacterials, *Staphylococcus aureus* and *Enterococci faecalis* (SaMetRS and EfMetRS) but at same time it also inhibits human MetRS (hMetRS) at similar concentrations. As a result, in 2003, John Finn report that noval compounds with improved potency on bacterial MetRS and selectivity versus hMetRS. [2b]

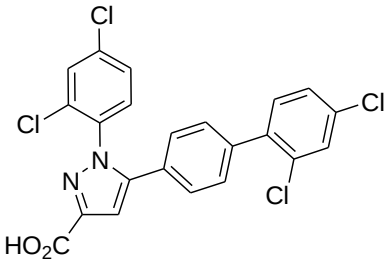
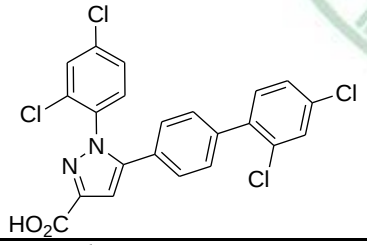
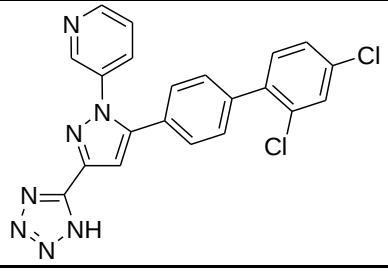


Figure 3. Pyrazoles derivative as antibacterial agent

John Finn[2b] synthesized a series of pyrazoles with significantly improved potency on reducing bacterial methionyl-tRNA synthetase and had less effect on human methionyl-tRNA synthetase (Table 2).

Optimization of a micromolar pyrazole enchance it's selective antibacterial factor and further provided a set of submicromolar 1*H*-3-tetrazole-5-(2',4'-dichloro[1,1'-biphenyl]-4-yl)-1-(2,4-dichlorophenyl)-Pyrazole. This compounds have significantly enchanced selectivity for the bacterial MetRS enzyme as compare the human MetRS enzyme. The advances in potency and selectivity for the pyrazole series suggests that MetRS may be a potential and selectivity target for discovering other series of inhibitors.

Table 2. Antibacterial activity of the pyrazole derivatives

Compound	SaMetRS	EfMetRS	human MetRS
	4.88μM	8.99μM	11.9μM
	0.13μM	7.0μM	10.6μM

*MetRS: methionyl-tRNA synthetase,
SaMetRS: *Staphylococcus aureus* methionyl-tRNA synthetase
EfMetRS: *Enterococci faecalis* methionyl-tRNA synthetase

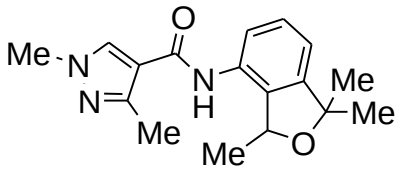
Section 1.2 Pyrazoles derivatives as Antifungal Lead Compounds

Pyrazophos,[4a] (Figure 4) the first fungicide of this class to be commercialized, was marketed by Hoechst AG in 1974 to control powdery mildew in vegetables, and many pyrazole derivatives are now commercially available. The advantages, such as a new mode of action, wide spectrum, low toxicity toward mammalian cells, and favorable profiles to humans, have prompted chemists to design and synthesize novel pyrazole derivatives.

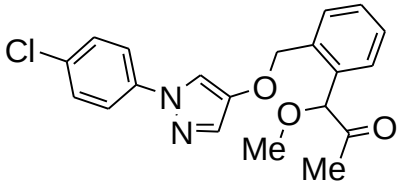
pyrazophos

Figure 4. Pyrazophos

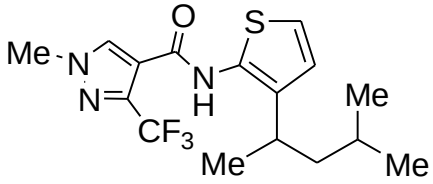
Recently, pyrazole compounds, such as Furametpur, Penthiopyrad and Pyraclostrobin (Figure 5), have been found to have potential antifungal activities for the control of some plant diseases. With growing applications on their synthesis and bioactivity, chemists and biologists in recent years have paid more attention to the research of pyrazole derivatives[20] (Figure 5).



Furametpyr



Pyraclostrobin



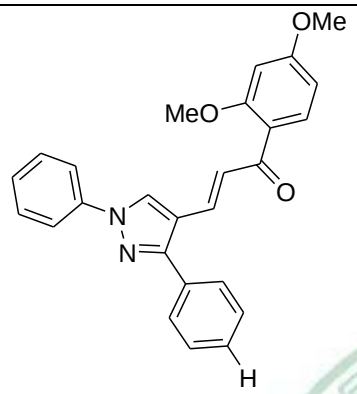
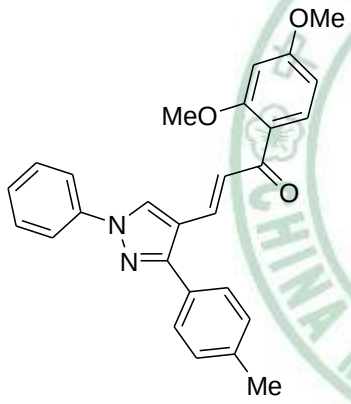
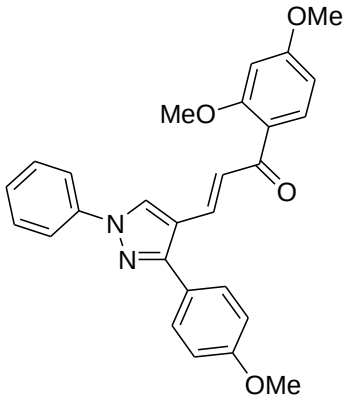
Penthiopyrad

Figure 5. Furametpur, Penthiopyrad and Pyraclostrobin

Synthesis of flavonols having C-2 position moiety in pyrazole was recently reported as potent antifungal and antibacterial agents.[21,21b] Furthermore, the presence of enone function in chalcone moiety with pyrazole ring also increased the biological activity.[22]

Babasaheb P. Bandgar[4b] reported the synthesis and biological activity of pyrazole chalcones as antifungal agents. The toxicity of the compounds was evaluated theoretically and experimentally have been defined their potential as safe leading compounds for bioavailability (Table 3).

Table 3. Antifungal activity of pyrazole chalcone derivatives

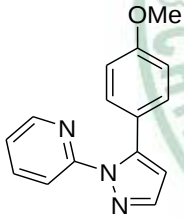
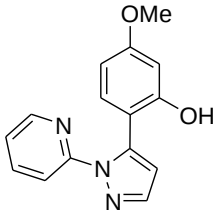
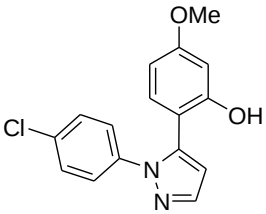
Compound	Fungi (MIC at 250μM)	
	Trichoderma viridae (MTCC 167)	Aspergillus flavus (MTCC 2501)
	14	15
	15	12
	16	14
Nystatin	12	11

*MIC: Minimum inhibitory concentration

Section 1.3 Pyrazoles derivatives as Anticancer Lead Compounds

In 2011, Alessandro Balbi[5a] studied novel pyrazole derivatives on their antiproliferative activity in human ovarian adenocarcinoma A2780 cells and murine P388 leukemia cells (Table 4). In particular, three compounds were active on human ovarian adenocarcinoma A2780 ($p < 0.001$) and murine leukemia P388 cells ($p < 0.001$) (Table 4).

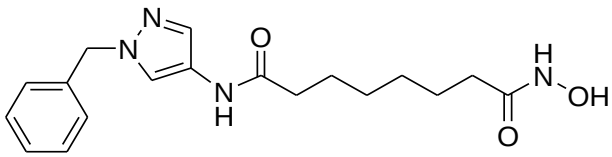
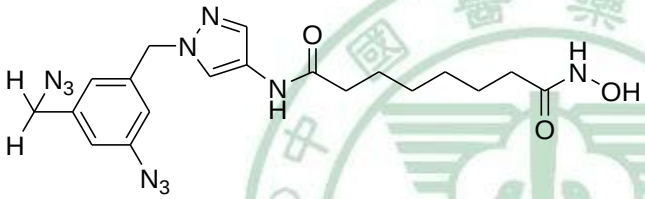
Table 4. IC_{50} as calculated by the MTT assay.

Compound	Ovary (A2780)	Leukemia (P388)
	2.89μM	5.38μM
	1.22μM	1.56μM
	2.35μM	7.51μM

Histone deacetylases (HDACs)[23] are widely established enzymatic targets for multiple therapeutic applications.[24] It is well accepted that therapeutic application of HDAC inhibitors depends on their isoform selectivity profile[25] and their HDAC class, making isoform selective inhibitors an important issue in the design and development of novel HDAC-based therapeutics.

In 2011, Pavel A. Petukhov,[5b] reported that diazide inhibitors for the two class I isoforms HDAC8 and HDAC3 may have particular value for the treatment of neuroblastoma, leukemia,[26] and gastric, prostate, and colorectal cancer.[27] And he found that novel diazide-containing pyrazole-based Histone deacetylase inhibitors have low nanomolar inhibitory activity against HDAC3 and HDAC8. The pyrazole-based inhibitor, *Octanedioic Acid [1-(3-Azido-5-azidomethylbenzyl)-1H-pyrazol-4-yl]amide Hydroxyamide*, exhibit one of the most active HDAC8 inhibitors reported in the literature with an IC_{50} of 17 nM (Table 5).

Table 5. HDAC3 and HDAC8 Isoform Inhibitory Activity (IC_{50}, nM) of Pyrazole-Based Compounds

Compound	IC_{50} ± SD (nM)	
	HDAC3	HDAC8
	44 ± 5.8	76 ± 5.0
	128 ± 9.8	17 ± 3.0

Section 1.4 Improved bioactivities by Amidinyl Group

Amidine analogue of melphalan and AB4 (Figure 6) have been found to have anticancer activity by decreasing the number of viable cells in both estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) breast cancer cells. Although the cytotoxicity was concentration-dependent to both cell lines, it was more pronounced to MDA-MB-231 than in MCF-7. Anticancer activity of AB4 was shown to be more potent than melphalan in both MDA-MB-231 and MCF-7, with IC_{50} values of 45 ± 2 and 62 ± 2 μM, respectively. Comparative to AB4, melphalan[28] required higher concentration 130 ± 2 and 125 ± 2 μM for MDA-MB-231 and MCF-7.

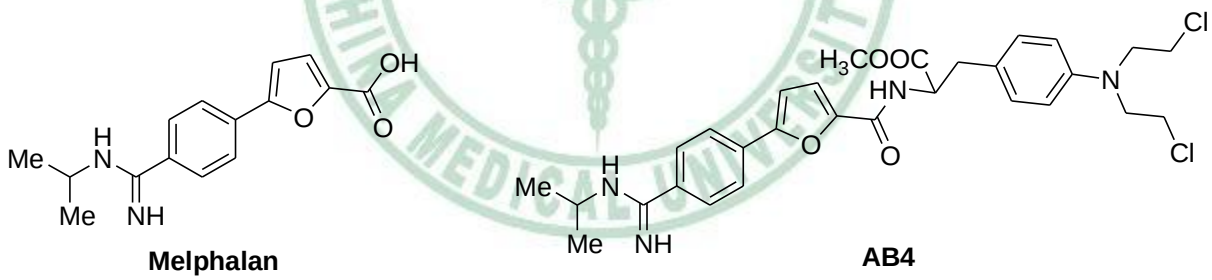


Figure 6. Melphalan and AB4

Introducing an amidino group into the core molecule to enhance its antibacterial activity[29] are still the subject of interest, such as penicillin. Moreover, antibiotic such as anthracycline (Figure 7) was shown to decrease toxicity and enhance anticancer activity.[30] Similarly, amidine group in mecillinam (Figure 7) inhibited

Escherichia coli, Klebsiella spp., Enterobacter spp., Citrobacter spp., Shigella spp., and *Salmonella spp*. with a mean minimum inhibitory concentration of 16/μg/mL.[31]

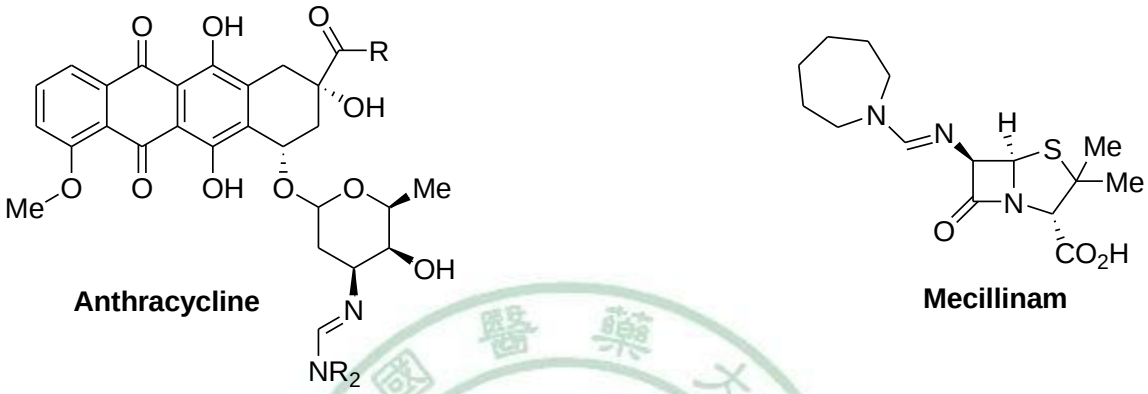


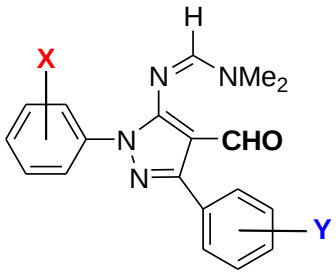
Figure 7. Anthracycline and mecillinam

Section 1.5 Antiproliferative of Formylated Amidinyl Pyrazole Derivatives

Our laboratory had previous developed a new microwave-assisted amidination method by treating various primary amines including aromatic amines and pyrazol-5-amines with amide solvents and $POCl_3$ coupling agent. Based on our experimental data, the yielding formylation amidinyl pyrazole products seemed to be determinated by the dissociation of the substituting amides.

Based on the growth inhibitory activity data, compounds with *m*-Cl-Ph and *p*-Br-Ph groups at *N*-1 position and compounds with *p*-Me-Ph and *p*-Cl-Ph groups at C-3 position in pyrazolic ring possessed the most potent activity. In addition, the formyl group at C-4 position in the core pyrazolic ring is indispensable for the inhibitory activity (Table 6).[32]

Table 6. Bioactivity of Formylated Amidinyl Pyrazole Derivatives



		GI_{50} (μM) for Antiproliferative activity		
X	Y	NCI-H661	NPC-TW01	Jurkat
m-Cl	H	6.9	6.4	8.3
p-Br	H	6.7	7.4	7.3
H	*p*-Me	11.9	9.7	9.5
H	*p*-Cl	8.6	8.1	7.9

Chapter 2 Research Approach

In 2010, our laboratory developed a newly microwave-assisted amidination method to prepare methnimidamide compounds by using 5-amino-1,3-disubstituted pyrazoles, amide solvents, and $POCl_3$.[33] Accroding to the growth inhibitory activitive data, compounds with *m*-Cl-Ph and *p*-Br-Ph groups at *N*-1 position and compounds with *p*-Me-Ph and *p*-Cl-Ph groups at C-3 position in pyrazolic ring showed the most potent activity.

Herein, we reported a new chemoselective microwave-assisted amidination method to synthesize 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines (Figure 8) and pyrazolyl-2-azadienes (Figure 9) by using the suitable amount of basic pyridine as the basic catalyst. The reactivity and bioactivity for the different skeletal of methnimidamides (Figure 10) and starting material 5-amino-1,3-disubstituted pyrazole (Figure 11) were also explored.

Figure 8. 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidine derivatives

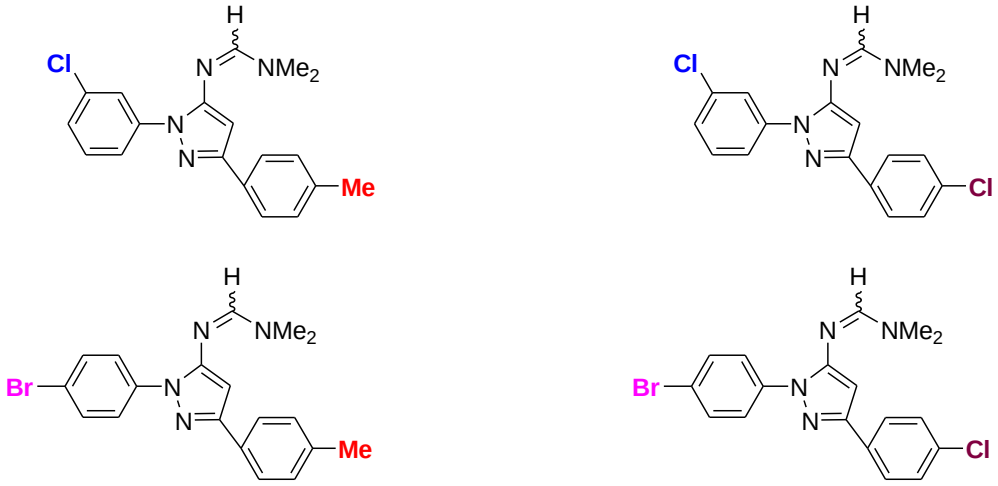


Figure 9. Pyrazolyl-2-azadiene derivatives

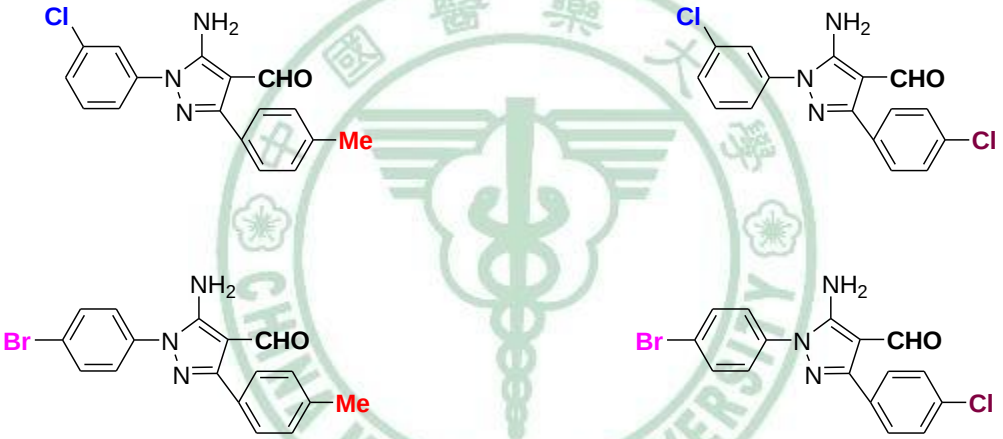


Figure 10. Methnimidamide derivatives

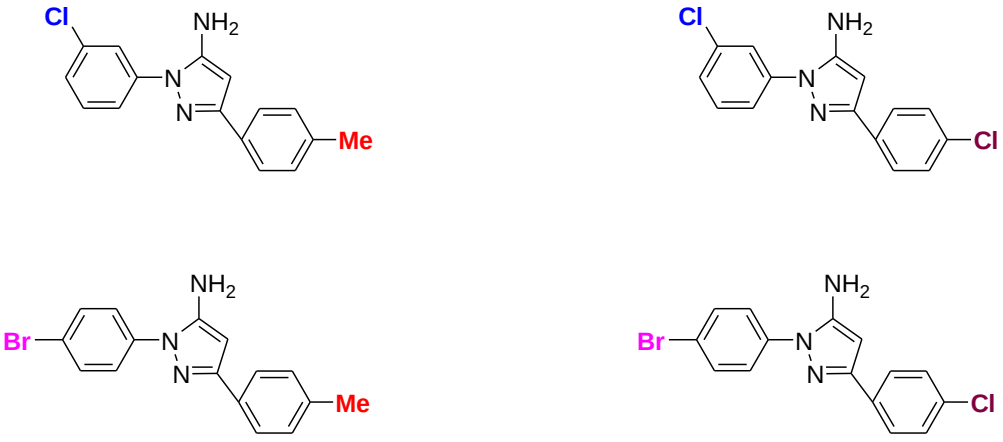


Figure 11. 5-amino-1,3-disubstituted pyrazole derivatives

Chapter 3 Result and Discussion

Results of this study showed that four series compounds including 5-amino-1,3-diphenyl pyrazole (**1a–1e**), 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines (**2a–2e**), Pyrazolyl-2-azadienes (**3a–3e**), and methnimidamide (**4a–4e**) was successfully synthesized. All of compounds were tested for structure activity relationship and biological activity.

Section 3.1 Chemistry

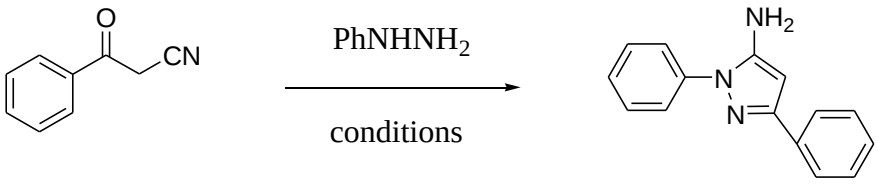
Our laboratory reported that if benzoylacetonitrile was allowed to react with phenylhydrazine in neat condition at reflux for 2.0 h, it will result in the synthesis of 5-amino-1,3-diphenyl pyrazole (**1a–1e**). A model procedure involved the treatment of 5-amino-1,3-disubstituted pyrazoles **1a–1e** with $POCl_3$ (~1.2 equivalent) in DMF at 30–40°C with 100 W of microwave energy within 15–20min was developed to synthesize 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines (**2a–2e**). The newly chemoselective methodology is applicable to transform compounds **1a–1e** to the corresponding pyrazolyl-2-azadiene products **3a–3e** without formyl group. A newly basic condition by using NaOH in MeOH solution was used to synthesize the de-amidination of methnimidamide (**4a–4e**).

Section 3.1.1 Synthesis of 5-amino-1,3-diphenyl pyrazole (1a–1e)

The traditional Synthetic method for the synthesis of 5-amino-1,3-diphenyl pyrazole was to react benzoylacetonitrile with phenylhydrazine under distinctive conditions. Distinctive conditions include: (a) heated in EtOH,[34] (b) microwave irradiation,[35] and (c) heated in acetic acid (Scheme 1).

The first method was refluxing benzoylacetonitrile with the same equivalent of phenylhydrazine in EtOH for >8.0 h (see Scheme 1, path a).[34] Product 5-amino-1,3 diphenyl pyrazole was generated in only 41% yield via tandem condensation and thermal cyclization. Another method was the use of microwave irradiation of benzoylacetonitrile with hydrazine in EtOH solution for >4.0 h to provide 5 amino-1,3-diphenyl pyrazole in 58% yield (see Scheme 1, path b).[35] These first two methods did not produce desired yield of 5-amino-1,3-diphenyl pyrazole. The third method was used acetic acid as the solvent could provide 75–85% yield of 5-amino-1,3-diphenyl-pyrazole (see Scheme 1, path c).

Benzoylacetonitrile was allowed to react with phenylhydrazine in neat condition at reflux for 2.0 h (see Scheme 1, path d). Our laboratory carried out the reaction in such neat condition that we successfully generated 94% yield of 5-amino-pyrazole.



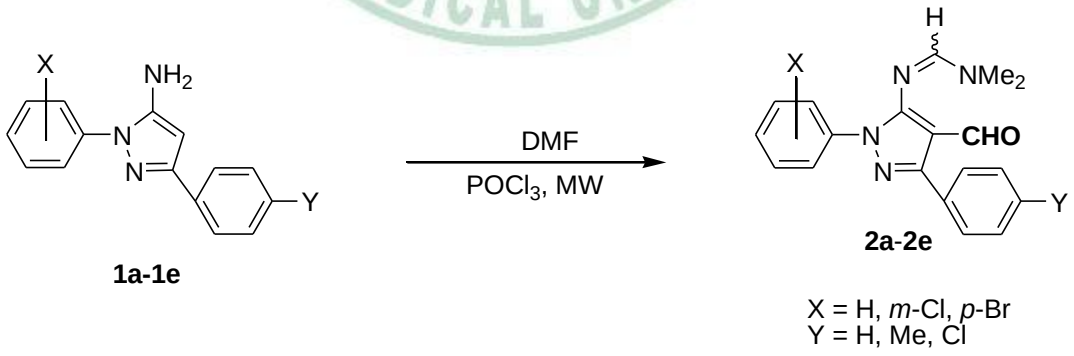
1a-1e

Conditions
a. EtOH,reflux, >8.0 h, 41%
b. EtOH, microwave, >4.0 h, 58%
c. AcOH, temp., 75-85%
d. neat, reflux, 2.0 h, 94%

Scheme 1

Section 3.1.2 Synthesis of 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines (2a–2e)

Scheme 2 illustrates the amidination of 5-amino-1,3-diphenyl pyrazole to the corresponding and the optimization of the reaction. A model procedure involved the treatment of 5-amino-1,3-disubstituted pyrazoles **1a–1e** with $POCl_3$ (~1.2 equivalent) in DMF at 30–40°C with 100 W of microwave energy within 15–20min. After work-up and purification by column chromatography on silica gel, the corresponding 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines **2a–2e** were readily obtained in 77–97% yields (see Table 7; Scheme 2). In addition to grafting the amidinyl group on the main core of 5-amino pyrazole, the formyl group was also introduced at the C-4 position of pyrazolic ring. Compounds **2a–2e** were fully characterized by spectroscopic methods.[34]



Scheme 2

Table 7. Result of Synthesis of Formylated Amidinyl Pyrazoles **(2a-2e)**

5-Amino-1,3-*N*,*N*-disubstituted pyrazoles			Methnimidamide **(2a–2e)**	
S.M. **(1a–1e)**	X	Y	Products	Yields (%)
1a	H	H	**2a**	94
1b	*m*-Cl	Me	**2b**	82
1c	*m*-Cl	Cl	**2c**	81
1d	*p*-Br	Me	**2d**	90
1e	*p*-Br	Cl	**2e**	92

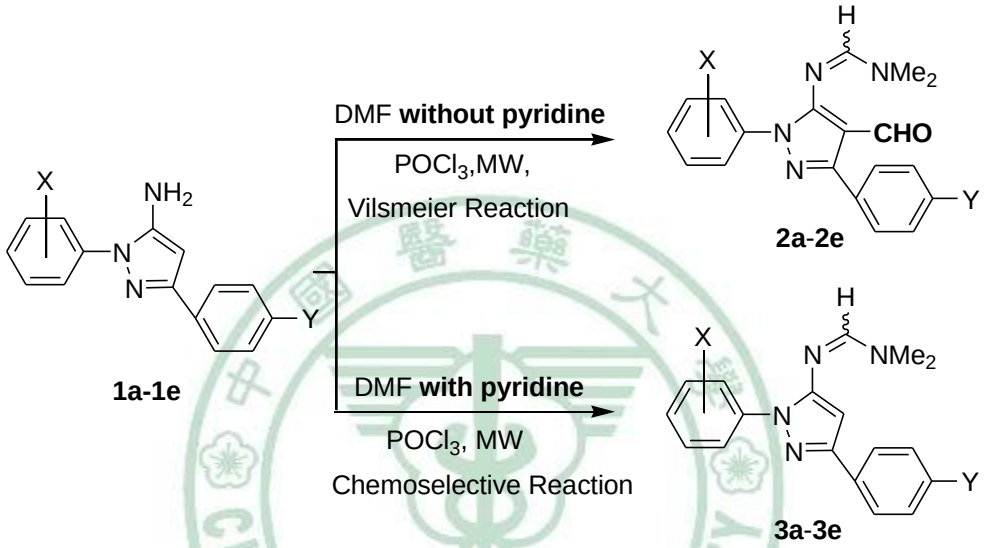
Section 3.1.3 Synthesis of Pyrazolyl-2-azadienes (3a–3e)

For the investigation of the effect of formyl group on activity, we evaluated the chemoselective microwave-assisted amidination methodology to prepare a series of pyrazolyl-2-azadienes **3a–3e** without introducing a formyl group at C-4 position on pyrazolic ring as the comparing model. Initially, we chose 5-amino-1-3-diphenylpyrazole **1a** as modeling case and treated **1a** with different inorganic or organic basic agents to quench the excess amount of active imineniun species or neutralize hydrochloride for diminishing the formation of the formylated methnimidamide product 2. The bases included sodium hydroxide (NaOH), potassium carbonate (K_2CO_3), cesium carbonate ($CsCO_3$), triethylamine (NEt_3), dimethylaminopyrium (DMAP), and pyridine.

Firstly, the amidination reaction was performed on 5-amino-1-3-diphenylpyrazole **1a** with-out basic catalytic agent as the blank study. The reaction only provided the formylated methnimidamide **2a** in 94% yield.

When the reaction was treated with 2.0 equivalent of inorganic base including sodium hydroxide (NaOH), potassium carbonate (K_2CO_3), and cesium carbonate ($CsCO_3$), the methnimidamide product **3a** without the formyl group was provided in poor yields, except for using cesium carbonate (see entries 2–4 of Table 8). For cesium carbonate, the methnimidamide product **3a** was obtained in 78% isolated

yield with the recovery of a small amount of the starting materials **1a**. On the other hand, the starting materials **1a** and the small amount of the formylated methnimidamide compound **2a** were simultaneously obtained in NaOH as basic catalytic agent.



Scheme 3

When the same condition was applied to the commercially available organic bases including NEt_3, DMAP, or pyridine, the methnimidamide product **3a** without formyl group was obtained in 34–82% yields as the major product (see entries 5–7 of Table 8). Particularly, the best chemoselective result was achieved by using pyridine as the basic catalyst. The use of various equivalent of pyridine was also studied from 1.0 equiv to 4.0 equiv. We found that using 3.0 equivalent of pyridine can give pyrazolyl-2-azadiene product **3a** in the best yield (97% yield, see entry 9 of Table 8).

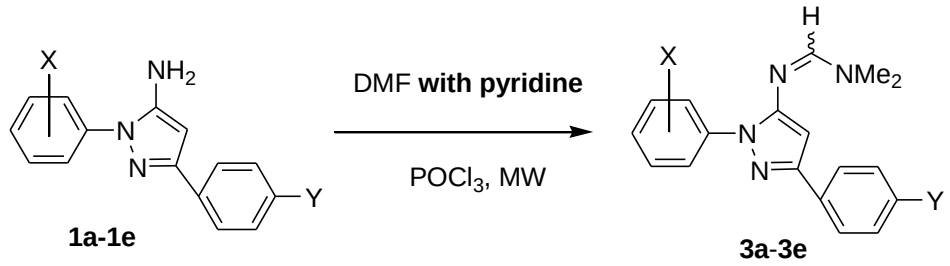
Furthermore, the newly chemoselective methodology can be applicable to compounds **1a–1e** to provide the corresponding pyrazolyl-2-azadiene products **3a–3e** without formyl group in 78–98% yields (see Table 9). The reliable chemoselective procedure involved the treatment of 5-amino-1,3-disubstituted pyrazoles **1a–1e** with ~1.2 equivalent of $POCl_3$ and 3.0 equivalent of pyridine in DMF at 30–40°C with 100 W of microwave energy within 15–20 min. After work-up and purification were performed, the desired pyrazolyl-2-azadiene products **3a–3e** without formyl group were obtained in 78–98% isolated yields (see Table 9; Scheme 3). Following the aforementioned studies, the chemoselective amidination reaction seemed determinate to the suitable amount of pyridine basic agent.

Table 8. The basic catalyzed study for preparation of pyrazolyl-2-azadienes **3a** without formyl group in the chemoselective microwave-assisted amidination

Entry	Basic Agents		Yield (%)	
	Catalyst	Equiv[a]	Formylated methnimidamides (**2a**)	Pyrazolyl-2-azadienes (**3a**)
1	Without catalyst	-	94	-[b]
2	NaOH	2	27	-[b,c]
3	K_2CO_3	2	-[b,c]	4
4	$CsCO_3$	2	-[b,c]	78
5	Triethylamine (NEt_3)	2	-[b,c]	34
6	Dimethylaminopyridine (DMAP)	2	-[b,c]	50
7	Pyridine	2	18	82
8	Pyridine	1	15	75
9	Pyridine	3	-[b]	97
10	Pyridine	4	44	53

[a]based on the weight of 5-amino-1-3-diphenylpyrazole (**1a**).
[b]not detectable.
[c]Starting material **1a** was recovery.

Table 9. The results of chemoseletive amidination reaction for preparation of pyrazolyl-2-azadiene products **3a**–**3e**

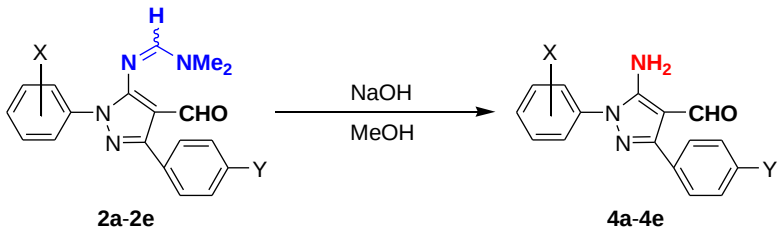


5-Amino-1,3-*N*,*N*-disubstituted pyrazoles			Pyrazolyl-2-azadienes (**3a**–**3e**)	
S.M. (**1a**–**1e**)	X	Y	Products	Yields (%)
1a	H	H	**3a**	97
1b	*m*-Cl	Me	**3b**	91
1c	*m*-Cl	Cl	**3c**	98
1d	*p*-Br	Me	**3d**	93
1e	*p*-Br	Cl	**3e**	78

Section 3.1.4 Synthesis of the de-amidination of methnimidamide (4a–4e)

To identify the essentiality of amidinyl group for the inhibitory activity study, a series of de-amidination compounds **4a–4e** were sequentially prepared as the comparison model for the further structure–activity relationship study. When we searched the previous reported literature about de-amidination, only one method was found by using HCl aqueous solution.[35] However, the purification procedure was troublesome, especially in neutralization procedure.

Consequently we investigated a newly basic condition by using NaOH in MeOH solution. The reliable procedure involved the treatment of methnimidamide **2a–2e** with two equivalent of NaOH at reflux in MeOH solution within 2–3 h. After the extraction work-up and simple purification through the short column chromatography on silica gel, the corresponding de-amidination 5-amino-4-formylpyrazole products **4a–4e** were obtained in 83–96% yields (see Table 10; Scheme 4).



Scheme 4

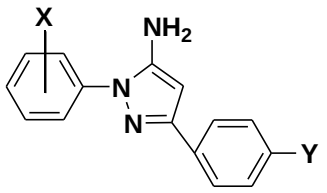
Table 10. Result of Synthesis of 5-Amino-4-formylpyrazoles **(4a–4e)**

Methnimidamide (**2a**–**2e**)			5-Amino-4-formylpyrazoles (**4a**–**4e**)	
S.M. (**2a**–**2e**)	X	Y	Products	Yields (%)
2a	H	H	**4a**	92
2b	*m*-Cl	Me	**4b**	85
2c	*m*-Cl	Cl	**4c**	87
2d	*p*-Br	Me	**4d**	83
2e	*p*-Br	Cl	**4e**	96

Section 3.2 Biological evaluations

The growth inhibitory activity of all amidine compounds is evaluated against a panel of human cancer cell lines, including lung carcinoma (NCI-H226), nasopharyngeal (NPC-TW01), and T-cell leukemia (Jurkat) cells. The GI_{50} value is the concentration that results in a 50% decrease in the cell growth relative to an untreated control. All of starting materials **1a**–**1e** were selected and used as the comparison model for the inhibitory activity study. Among of starting substrates, only compound **1d** possessed the negligible inhibitory activity against three cell lines [the GI_{50} values of **1d** are 54.3 μM (NCI-H226), 80.2 μM (NPC-TW01), and 45.0 μM (Jurkat), see Table 11].

Table 11. Antiproliferative activity of 5-amino-1,3-diphenyl pyrazole (**1a–1e**)



Compounds	Prozoles (**1a**–**1e**)		GI_{50} (μM) [a,b]		
	X (*N*-1)	Y (C-3)	NCI-H226	NPC-TW01	Jurkat
1a	H	H	72.2	>100	83.0
1b	*m*-Cl	Me	63.5	>100	56.6
1c	*m*-Cl	Cl	75.1	>100	>100
1d	*p*-Br	Me	54.3	80.2	45.0
1e	*p*-Br	Cl	58.7	64.4	61.3

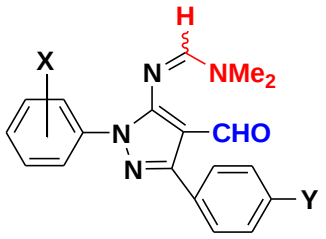
[a]NCI-H226: human lung carcinoma; NPC-TW01: human nasopharyngeal carcinoma; Jurkat: human T-cell leukemia

[b]All tested compounds were dissolved in 100% DMSO at a concentration of 20 mM as the stock solution. Cells were cultured without or in the presence of the methnimidamide derivatives at different concentrations for 72 h. Cell survival was determined by MTT assay. Drug molar concentration causing 50% cell growth inhibition (GI_{50}) was calculated. Each value represents the mean ± SD of three independent experiments.

Formylated methnimidamide **2a** was also used as the comparison model for other analogs **2b**–**2e** against the cancer cell lines. Compounds **2b** and **2c** containing the same *m*-Cl-Ph substituted group on *N*-1 position and either *p*-Cl-Ph or *p*-Me-Ph groups on C-3 position in pyrazolic ring displayed the better inhibitory activity against the three cancer cell lines with GI_{50} values between 7.2 μM and 9.2 μM (see Table 12). The results also showed that they were more active against NPC-TW01 and Jurkat than NCI-H226. For compounds **2d** and **2e** with *p*-Br-Ph on *N*-1 position and either *p*-Cl-Ph or *p*-Me-Ph groups at C-3 position on pyrazolic ring, compound **2d** showed the better inhibitory activity against the three cancer cell lines with GI_{50} values between 6.0 μM and 8.2 μM. Due to the bulky *p*-Br-Ph group and *p*-Cl-Ph groups on the *N*-1 and C-3 position of pyrazole not favoring to reach the blocking side, the poor result of bioactivity was observed in compound **2e**.

Following the structure activity relationship study results, compounds **2b**–**2d** possessed the better activity than **2a** and **2e**. On the other hand, the antiproliferative activity data was consistent with our design approach and compound **2b**–**2d** can be considered as the potency lead drugs.

Table 12. Antiproliferative activity of 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines (**2a–2e**)



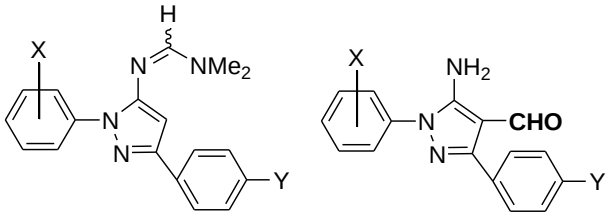
Compounds	Prozoles (**2a**–**2e**)		GI_{50} (μM)		
	X (*N*-1)	Y (C-3)	NCI-H226	NPC-TW01	Jurkat
2a	H	H	31.4	9.3	23.5
2b	*m*-Cl	Me	**8.9**	**7.2**	**7.8**
2c	*m*-Cl	Cl	**9.2**	**7.4**	**7.7**
2d	*p*-Br	Me	**8.2**	**6.0**	**6.7**
2e	*p*-Br	Cl	62.9	>100	38.9

[a]NCI-H226: human lung carcinoma; NPC-TW01: human nasopharyngeal carcinoma; Jurkat: human T-cell leukemia

[b]All tested compounds were dissolved in 100% DMSO at a concentration of 20 mM as the stock solution. Cells were cultured without or in the presence of the methnimidamide derivatives at different concentrations for 72 h. Cell survival was determined by MTT assay. Drug molar concentration causing 50% cell growth inhibition (GI_{50}) was calculated. Each value represents the mean ± SD of three independent experiments.

For the further the structure–activity relationship investigation, pyrapzolyl-2-azadienes **3a**–**3e** and de-amidination compounds **4a**–**4e** were evaluated against three cancer cell lines as the comparison study. Following the antiproliferative activity result, the data indicated that compounds **3a**–**3e** [GI_{50}: > 59.8 μM (NCI-H226), > 60.7 μM (NPC-TW01), and > 74.5 μM (Jurkat)] and **4a**–**4e** [GI_{50}: > 8.5 μM (NCI-H226), > 28.2 μM (NPC-TW01), and > 34.4 μM (Jurkat)] were less potent than compounds **2a**–**2d**. The experimental result in Table 13 demonstrated the formyl group at C-4 position and grating the amidinyl group toward amino moiety at C-5 in pyrazolic ring are essential for the promotion of inhibitory activity. Furthermore, the data indicated that tendency for sensitivity is nasopharyngeal (NPC-TW01) > T-cell leukemia (Jurkat) cell > lung carcinoma (NCI-H266) for methnimidamide compounds **2a**–**2e**.

Table 13. Antiproliferative activity of Pyrazolyl-2-azadienes (**3a–3e**) and the de-amidination of methnimidamide (**4a–4e**)



3a-3e **4a-4e**

Compounds	Prozoles (**3a**–**3e**, and **4a**–**4e**)		GI_{50} (μM)[a,b]		
	X (*N*-1)	Y (C-3)	NCI-H226	NPC-TW01	Jurkat
3a	H	H	>100	>100	>100
3b	*m*-Cl	Me	80.9	>100	>100
3c	*m*-Cl	Cl	75.6	92.7	74.5
3d	*p*-Br	Me	73.9	>100	>100
3e	*p*-Br	Cl	59.8	60.7	84.9
4a	H	H	>100	>100	87.3
4b	*m*-Cl	Me	71.8	>100	86.3
4c	*m*-Cl	Cl	79.7	>100	78.9
4d	*p*-Br	Me	8.5	28.2	34.4
4e	*p*-Br	Cl	49.1	59.7	90.7

[a]NCI-H226: human lung carcinoma; NPC-TW01: human nasopharyngeal carcinoma; Jurkat: human T-cell leukemia

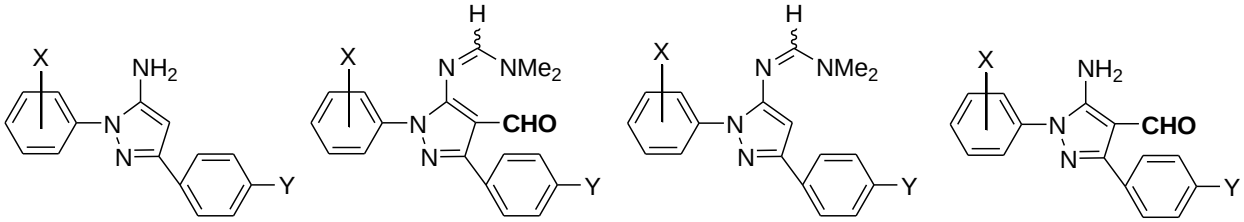
[b]All tested compounds were dissolved in 100% DMSO at a concentration of 20 mM as the stock solution. Cells were cultured without or in the presence of the methnimidamide derivatives at different concentrations for 72 h. Cell survival was determined by MTT assay. Drug molar concentration causing 50% cell growth inhibition (GI_{50}) was calculated. Each value represents the mean ± SD of three independent experiments.

Chapter 4 Conclusion

We have successfully developed a new chemoselective microwave-assisted amidination method to prepare 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines **2a**–**2e** with the formyl group and pyrazolyl-2-azadienes **3a**–**3e** without formylation by using pyridine as the basic agent. Furthermore, we have also evaluated the new de-amidination methodology to prepare the 5-amino-4-formylpyrazoles **4a**–**4e** as the compared study (Figure 12).

Based on the growth inhibitory activity data, compounds **2b**, **2c**, and **2d** with *m*-Cl-Ph and *p*-Br-Ph groups at *N*-1 position and *p*-Me-Ph and *p*-Cl-Ph groups at C-3 position in pyrazolic ring possessed the most potent activity.

Following the structure activity relationship study, we have demonstrated that introducing formyl group at C-4 position and grafting amidinyl group in the pyrazole core molecule are necessary for the improved bioactivity.



X = H, *m*-Cl, *p*-Br
Y = H, Me, Cl

Figure 12. Pyrazole compounds

Chapter 5 Experimental Section

Section 5.1 General Procedure

All chemicals were reagent grade and use as purchased. All reactions were carried out under argon or nitrogen atmosphere and monitored by Analytical thin-layer chromatography (TLC). Flash column chromatography was carried out on silica gel (230–400 mesh). Ethyl acetate and hexanes, purchased from Mallinckrodt Chemical Co., were dried and distilled from CaH_2. Toluene (reagent grade, from Merck Chemical Co.) was dried by distillation from CaH_2 under nitrogen. 4-Methylbenzoylacetonitrile, phenylhydrazine was purchased from Acros Chemical Co. 4-Bromophenylhydrazine hydrochloride, 4-chlorobenzoylacetonitrile, 3-chlorophenylhydrazine hydrochloride was purchased from Alfa Aesar Chemical Company. Benzoylacetonitrile were purchased from TCI. *N*,*N*-Dimethylformamide, pyridine were purchased from Scharlau Chemical Co. Phosphorylchloride were purchased from FERAK Chemical Co.

TLC was performed on precoated plates (silica gel 60 F-254) purchased from Merck Inc. Mixtures of ethyl acetate and hexanes were used as eluants. Infrared (IR) spectra were measured on a Bomem Michelson Series FT-IR spectrometer. The wavenumbers reported are referenced to the polystyrene absorption at 1601

cm^{-1}. Absorption intensities are recorded by the following abbreviations: s, strong; m, medium; w, weak. Proton NMR spectra were obtained on a Bruker (200 MHz or 400 MHz) spectrometer by use of $CDCl_3$ as solvent. Carbon-13 NMR spectra were obtained on a Bruker (75 MHz or 100 MHz) spectrometer by used of $CDCl_3$ as solvent. Carbon-13 chemical shifts are referenced to the center of the $CDCl_3$ triplet (δ 77.0 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (Hz). Microwave irradiation instrument was purchased from CEM Discover. The microwave irradiation condition was set in 100 W at 30–40 °C within 10–20 min. ESI-MS spectra were obtained from an Applied Biosystems API 300 mass spectrometer. High-resolution mass spectra were obtained by means of a JEOL JMS-HX110 mass spectrometer. Elemental analyses were carried out on a Heraeus CHN–O RAPID element analyzer.

A solution of pyrazol-5-amine derivatives (1a–1e, 1.0 equiv) and $POCl_3$ (1.2 equiv) in DMF solution (3 mL) at 30–40 °C was treated with 100 W of microwave energy within 10–20 min. When the reaction was completed, the reaction mixture was concentrated, added to water (10 mL) and extracted with CH_2Cl_2 (4 × 20 mL). The organic extracts were washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue solution was

purified by column chromatography on silica gel to give the corresponding methnimidamide products (2a–2e) in 81–94% yields.

Section 5.2 Spectrum

Standard Procedure for the Synthesis of Methnimidamide Compounds (2a–2e)

N'-[4-Formyl-1,3-diphenyl-1*H*-pyrazol-5-yl-*N*,*N*-dimethyl-methanimidamide ***(2a)***

mp (purified by column chromatography on silica gel) 120–122 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.01 (s, 3 H, CH_3), 3.12 (s, 3 H, CH_3), 7.26–7.47 (m, 6 H, ArH), 7.65–7.70 (m, 2 H, ArH), 7.84–7.89 (m, 2 H, ArH), 8.68 (s, 1H, N=C–H), 9.68 (s, 1H, aldehyde); ^{13}C NMR (50MHz, $CDCl_3$) δ 34.3 (CH_3), 40.7 (CH_3), 108.4, 124.3 (2 × CH), 126.8, 128.3 (2 × CH), 128.4 (2 × CH), 128.7, 129.4 (2 × CH), 132.3, 139.2, 154.1, 155.8, 159.0, 185.2; IR (KBr) 3059 (m), 2920 (m), 2800 (w), 2742 (w), 1670 (s), 1597 (m), 1508 (m), 1381 (m), 1257 (m), 1134 (m), 1095 (m), 975 (m), 767 (m), 694 (m) cm^{-1}; EIMS *m/z* (relative intensity) 318 (100), 317 (M^+, 42), 303 (17), 289 (9), 274 (19), 248 (8), 186 (14), 159 (7), 77 (24), 51 (5); Anal. Calcd for $C_{19}H_{18}N_4O$; C: 71.68; H: 5.70; N: 17.60, Found: C: 71.72; H: 5.71; N: 17.58.

*N'-[1-(2-chlorophenyl)-4-formyl-3-(4-methylphenyl)-1H-pyrazol-5-yl]-N,N-dimethyl-methanimidamide (**2b**)*

mp (purified by column chromatography on silica gel) 166–168 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.40 (s, 3 H, CH_3), 3.01 (s, 3 H, CH_3), 3.11 (s, 3 H, CH_3), 7.17–7.34 (m, 4 H, ArH), 7.51–7.55 (m, 2 H, ArH), 7.79–7.85 (m, 2 H, ArH),

8.01–8.03 (m, 2 H, ArH), 8.69 (s, 1 H, N=C–H), 9.64 (s, 1H, aldehyde); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.4 (CH_3), 34.4 (CH_3), 40.8 (CH_3), 108.5, 122.0, 124.2, 126.5, 128.7 (3 × CH), 129.2 (3 × CH), 133.9, 138.9, 140.3, 154.2, 156.1, 159.1, 185.2; IR (KBr) 2920 (m), 1666 (s), 1627 (m), 1589 (m), 1489 (m), 1384 (m), 1261 (m), 1134 (m), 1099 (m), 1072 (m), 987 (m), 825 (m), 825 (m), 781 (m), 740 (m) , 682 (m) cm^{-1}; EIMS *m/z* (relative intensity) 368 (M^{+2}, 31), 366 (100), 365 (M^+, 20), 337 (8), 322 (14), 220 (11), 185 (7), 111 (11), 91 (7), 83 (7), 75 (4); Anal. Calcd for $C_{20}H_{19}ClN_4O$; C: 65.48; H: 5.22; N: 15.27, Found: C: 65.50; H: 5.19; N: 15.23.

*N'-[4-formyl-1-(2-chlorophenyl)-3-(4-chlorophenyl)-1H-pyrazol-5-yl]-N,N-di-methyl-methanimidamide (**2c**)*

mp (purified by column chromatography on silica gel) 162–164 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.03 (s, 3 H, CH_3), 3.13 (s, 3 H, CH_3), 7.23–7.43 (m, 4 H, ArH), 7.58–7.64 (m, 2 H, ArH), 7.77–7.82 (m, 1 H, ArH), 7.99–8.01 (m, 1 H, ArH), 8.63 (s , 1 H, N=C–H), 9.60 (s, 1 H, aldehyde); ^{13}C NMR (50 MHz, $CDCl_3$) δ 34.5 (CH_3), 40.8 (CH_3), 108.5, 121.9, 124.1, 126.6, 128.7 (2 × CH), 129.4, 130.5 (3 × CH), 134.0, 135.0, 140.1, 154.5, 154.6, 158.9, 184.4; IR (KBr) 2924 (m), 2360 (m), 1666 (s), 1624 (m), 1585 (m), 1481 (m), 1384 (m), 1095 (m), 837 (m), 783 (m), 736 (m) cm^{-1}; EIMS *m/z* (relative intensity) 388 (M^{+2}, 65), 387 (M^{+1}, 21), 386 (100), 385 (M^+, 19), 371 (16), 357 (9), 342 (14), 330 (9), 316 (8), 220 (16),

111 (18), 83 (9); Anal. Calcd for $C_{19}H_{16}Cl_2N_4O$; C: 58.93; H: 4.16; N: 14.47, Found: C: 58.89; H: 4.17; N: 14.46.

*N'-[1-(4-bromophenyl)-4-formyl-3-(4-methylphenyl)-1H-pyrazol-5-yl]-N,N-di-methyl-methanimidamide (**2d**)*

mp (purified by column chromatography on silica gel) 198–200 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.36 (s, 3 H, CH_3), 2.98 (s, 3 H, CH_3), 3.09 (s, 3 H, CH_3), 7.21–7.25 (m, 2 H, ArH), 7.47–7.55 (m, 4 H, ArH), 7.77–7.81 (m, 2 H, ArH), 8.68 (s , 1 H, N=C–H), 9.64 (s, 1 H, aldehyde); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.4 (CH_3), 34.4 (CH_3), 40.7 (CH_3), 108.5, 120.1, 125.6 (2 × CH), 129.21 (5 × CH), 131.4 (2 × CH), 138.3, 138.9, 154.1, 156.0, 159.1, 185.1; IR (KBr) 2920 (m), 1662 (s), 1624 (m), 1489 (s), 1381 (m), 1265 (m), 1134 (m), 1091 (m), 1010 (m), 975(m), 829 (m), 740 (m), 501 (m) cm^{-1}; EIMS *m/z* (relative intensity) 412 (M^{+2}, 99), 410 (100), 409 (M^+, 26), 395 (12), 366 (15), 266 (10), 185 (10), 155 (6), 83 (7), 58 (5); Anal. Calcd for $C_{20}H_{19}BrN_4O$; C: 58.40; H: 4.66; N: 13.62, Found: C: 58.44; H: 4.69; N: 13.58.

*N'-[1-(4-bromophenyl)-4-formyl-3-(4-chlorophenyl)-1H-pyrazol-5-yl]-N,N-di-methyl-methanimidamide (**2e**)*

mp (purified by column chromatography on silica gel) 195–197 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.01 (s, 3 H, CH_3), 3.13 (s, 3H, CH_3), 7.38–7.63 (m, 6 H, ArH), 7.73–7.79 (m, 2 H, ArH), 8.63 (s , 1 H, N=C–H), 9.61 (s, 1 H, aldehyde);

^{13}C NMR (50 MHz, $CDCl_3$) δ 34.5 (CH_3), 40.8 (CH_3), 108.5, 120.3, 125.6 (2 × CH), 128.7 (2 × CH), 130.5 (2 × CH), 131.5 (2 × CH), 135.0 (2 × CH), 138.1 (2 × CH), 154.5, 158.9, 184.4; IR (KBr) 2364 (m), 2333 (m), 1666 (s), 1624 (m), 1516 (m), 1485 (m), 1381 (m), 1261 (m), 1138 (m), 1076 (m), 1010 (m), 813 (m), 740 (m), 578 (m), 547 (m), 505 (m) cm^{-1}; EIMS *m/z* (relative intensity) 432 (M^{+2}, 100), 430 (73), 429 (M^+, 20), 388 (13), 374 (8), 266 (14), 232 (8), 205 (9), 155 (11), 111 (4), 83 (10); Anal. Calcd for $C_{19}H_{16}ClN_4O$; C: 52.86; H: 3.74; N: 12.98, Found: C: 52.88; H: 3.71; N: 13.01.

Standard Procedure for the Synthesis of Pyrazolyl-2-azadiene Compounds (3a–3e)

A solution of pyrazol-5-amine derivatives (**1a–1b**, 1.0 equiv), $POCl_3$ (1.2 equiv) and pyridine (3.0 equiv) in DMF solution (3 mL) at 30–40 °C was treated with 100 W of microwave energy within 10–20 min. When the reaction was completed, the reaction mixture was concentrated, added to water (10 mL) and extracted with CH_2Cl_2 (4 × 20 mL). The organic extracts were washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue solution was purified by column chromatography on silica gel to give the corresponding methnimidamide products (**3a–3e**) in 78–98% yields.

*N'-(4-formyl-1,3-diphenyl-1H-pyrazol-5-yl)-N,N-dimethyl-methanimidamid-e (**3a**)*

mp (purified by column chromatography on silica gel) 113–115 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.29 (s, 3 H, CH_3), 3.01 (s, 3 H, CH_3), 6.15 (s, 1 H), 7.17–7.43 (m, 6 H, ArH), 7.78 (s, 1 H), 7.83–7.97 (m, 3 H, ArH); ^{13}C NMR (50MHz, $CDCl_3$) δ 34.5 (CH_3), 40.2 (CH_3), 88.4, 123.5 (2 × CH), 125.5 (2 × CH), 125.6, 127.6, 128.3 (2 × CH), 128.5 (2 × CH), 134.0, 140.3, 150.8, 152.6, 154.4; IR (KBr) 3059 (m), 2920 (m), 1635 (s), 1593 (m), 1543 (m), 1496 (m), 1392 (m), 1361 (m), 1257 (m), 1103 (m), 948 (m), 759 (m), 694 (m) cm^{-1}; EIMS *m/z* (relative intensity) 290 (100), 298 (M^+, 10), 246 (29), 219 (7), 198 (8), 186 (14), 171 (15), 145 (10),

83 (9), 77 (20); Anal. Calcd for $C_{18}H_{18}N_4$; C: 74.46; H: 6.25; N: 19.30, Found: C: 74.43; H: 6.28; N: 19.27

*N'-[1-(2-chlorophenyl)-4-formyl-3-(4-methylphenyl)-1H-pyrazol-5-yl]-N,N-di-methyl-methanimidamide (**3b**)*

mp (purified by column chromatography on silica gel) 109–115 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.38 (s, 3 H, CH_3), 2.94 (s, 3 H, CH_3), 2.95 (s, 3 H, CH_3), 6.10 (s, 1 H), 7.15–7.35 (m, 4 H, ArH), 7.70 (s 1 H, ArH), 7.75–7.80 (m, 2 H, ArH), 7.94–8.00 (m, 1 H, ArH), 8.18–8.20 (m, 1 H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.1 (CH_3), 34.3 (CH_3), 40.0 (CH_3), 88.2, 120.6, 122.7, 124.9, 125.3 (2 × CH), 129.0 (2 × CH), 130.8, 133.6, 137.4, 141.4, 151.0, 152.8, 154.2; IR (KBr) 3109 (m), 2920 (s), 2808 (m), 1647 (s), 1585 (m), 1546 (m), 1523 (m), 1489 (m), 1388 (m), 1354 (m), 1261 (m), 1149 (m), 1103 (s), 1072 (m), 1037 (m), 948 (m), 875 (m), 825 (s), 783 (m), 756 (m), 678 (m), 513 (m) cm^{-1}; EIMS *m/z* (relative intensity) 340.2 (M+2, 54), 338 (100), 317 (M^+, 10), 294 (15), 279 (6), 220 (10), 185 (18), 151 (3), 111 (7), 91 (4), 83 (9); Anal. Calcd for $C_{19}H_{19}ClN_4$; C: 67.35; H: 5.65; N: 16.54, Found: C: 67.36; H: 5.62; N:16.51.

*N'-[4-formyl-1-(2-chlorophenyl)-3-(4-chlorophenyl)-1H-pyrazol-5-yl]-N,N-di-methyl-methanimidamide (**3c**)*

mp (purified by column chromatography on silica gel) 108–110 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.93 (s, 6 H, CH_3), 6.03 (s, 1 H), 7.18–7.35 (m, 3 H, ArH),

7.65 (s, 1 H), 7.73–7.79 (m, 2 H, ArH), 7.91–7.95 (m, 1 H, ArH), 8.13–8.15 (m, 1 H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 34.5 (CH_3), 40.2 (CH_3), 88.3, 120.8, 122.9, 125.3, 126.8 (2 × CH), 128.6 (2 × CH), 129.3, 132.3, 133.3, 133.8, 141.3, 149.9, 153.1, 154.4; IR (KBr) 2920 (m), 1643 (s), 1585 (m), 1543 (m), 1504 (m), 1485 (m), 1354 (m), 1261 (m), 1153 (m), 1107 (m), 1014 (m), 948 (m), 879 (m), 837 (s), 783 (m), 756 (m), 678 (m) cm^{-1}; EIMS *m/z* (relative intensity) 362 (M+4, 14), 360 (M+2, 80), 358 (100), 357 (M^+, 7), 316 (15), 299 (6), 220 (12), 205 (13), 179 (7), 111 (12), 96 (2), 83 (10); Anal. Calcd for $C_{18}H_{16}Cl_2N_4$; C: 60.18; H: 4.49; N: 15.60, Found: C: 60.21; H: 4.53; N: 15.57.

*N'-[1-(4-bromophenyl)-4-formyl-3-(4-methylphenyl)-1H-pyrazol-5-yl]-N,N-di-methyl-methanimidamide (**3d**)*

mp (purified by column chromatography on silica gel) 115–117 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.35 (s, 3 H, CH_3), 2.97 (s, 3 H, CH_3), 3.02 (s, 3 H, CH_3), 6.11 (s, 1 H), 7.16–7.24 (m, 2 H, ArH), 7.46–7.50 (m, 2 H, ArH), 7.70–7.90 (m, 5 H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.4 (CH_3), 34.5 (CH_3), 40.3 (CH_3), 88.3, 118.7, 124.7 (2 × CH), 125.4 (2 × CH), 129.2 (2 × CH), 130.9, 131.3 (2 × CH), 137.5, 139.4, 151.2, 152.7, 154.5; IR (KBr) 2920 (m), 1631 (s), 1489(m), 1388 (m), 1103 (m), 829 (m), 759 (m), 497 (m) cm^{-1}; EIMS *m/z* (relative intensity) 384 (M+2, 100), 382 (99), 381 (M^+, 5), 340 (17), 326 (5), 259 (10), 185 (21), 155 (4), 115 (7),

91 (4), 83 (7); Anal. Calcd for $C_{18}H_{19}BrN_4$; C: 59.54; H: 5.00; N: 14.62, Found: C: 59.57; H: 5.02; N: 14.58.

*N'-[1-(4-bromophenyl)-4-formyl-3-(4-chlorophenyl)-1H-pyrazol-5-yl]-N,N-di-methyl-methanimidamide (**3e**)*

mp (purified by column chromatography on silica gel) 152–154 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.87 (s, 3 H, CH_3), 2.92 (s, 3H, CH_3), 6.04 (s, 1 H), 7.30–7.34 (m, 2 H, ArH), 7.45–7.53 (m, 2 H, ArH), 7.65 (s , 1 H), 7.72–7.77 (m, 2 H, ArH), 7.85–7.92 (m, 2 H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 34.3 (CH_3), 40.1 (CH_3), 88.1, 118.7, 124.4 (2 × CH), 126.6 (2 × CH), 128.4 (2 × CH), 131.1 (2 × CH), 132.2, 133.1, 139.2, 149.7, 152.8, 154.3; IR (KBr) 2920 (m), 1635 (s), 1539 (m), 1489 (m), 1357 (m), 1099 (m), 1010 (m), 948 (m), 829 (m), 759 (m), 497 (m) cm^{-1}; EIMS *m/z* (relative intensity) 404 (M+2, 100), 402 (89), 401 (M^+, 4), 360 (18), 279 (9), 266 (11), 205 (15), 155 (7), 115 (4), 83 (9), 57 (4); Anal. Calcd for $C_{18}H_{16}BrClN_4$; C: 53.55; H: 3.99; N: 13.88, Found: C: 53.51; H: 4.02; N: 13.91.

Standard Procedure for the Synthesis of 5-Amino-4-formylpyrazoles (4a–4e)

A solution of methnimidamide derivatives (**2a–2e**, 1.0 equiv) and NaOH (2.0 equiv) in MeOH solution (15 mL) at reflux within 2–3 h. When the reaction was completed, the reaction mixture was concentrated to remove solvent, added to water (10 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic extracts were washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue solution was purified by short column chromatography on silica gel to give the corresponding 5-amino-4-formylpyrazole products (**4a**–**4e**) in 83–96% yields.

*5-Amino-1,3-diphenyl-1H-pyrazole-4-carbaldehyde (**4a**)*

mp (purified by column chromatography on silica gel) 154–155 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.13 (s, 2 H, NH_2), 7.40–7.72 (m, 10 H, ArH), 9.81 (s, 1 H, CHO); ^{13}C NMR (50MHz, $CDCl_3$) δ 104.7, 124.0 (2 × CH), 128.4, 128.6 (2 × CH), 128.8 (2 × CH), 129.2, 129.9 (2 × CH), 131.6, 136.9, 150.1, 153.4, 185.4 (CHO); IR (KBr) 3425 (m), 3309 (m), 2827 (m), 2353 (m), 1647 (s), 1508 (m), 1253 (m), 1165 (m), 979 (m), 914 (m), 844 (m), 755 (m) cm^{-1}; EIMS *m/z* (relative intensity) 263 (M^+, 100); Anal. Calcd for $C_{16}H_{13}N_3O$; C: 72.99; H: 4.98; N: 15.96, Found: C: 73.02; H: 5.01; N: 15.93

*5-Amino-1-(2-chlorophenyl)-3-(4-methylphenyl)-1H-pyrazole-4-carbaldehyd-e (**4b**)*

mp (purified by column chromatography on silica gel) 147–148 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.40 (s, 3 H, CH_3), 6.03 (s, 2 H, NH_2), 7.25–7.27 (m, 2 H, ArH), 7.37–7.39 (m, 1 H, ArH), 7.43–7.48 (m, 2 H, ArH), 7.57–7.58 (m, 2 H, ArH), 7.64 (s, 1 H, ArH), 9.84 (s, 1 H, CHO); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.4 (CH_3), 104.9, 121.6, 124.2, 128.5, 129.5 (4 × CH), 130.9 (2 × CH), 135.8, 138.1, 139.3, 150.0, 153.9, 185.7 (CHO); IR (KBr) 3406 (m), 3298 (m), 2368 (m), 1624 (s), 1512 (m), 1226 (m), 1168 (m), 1087 (m), 1033 (m), 829 (m), 744 (m) cm^{-1}; EIMS *m/z* (relative intensity) 313 (M + 2, 32), 311 (M^+, 100); Anal. Calcd for $C_{17}H_{14}ClN_3O$; C: 65.49; H: 4.53; N: 13.48, Found: C: 45.47; H: 4.56; N:13.47.

*5-Amino-1-(4-chlorophenyl)-3-(4-chlorophenyl)-1H-pyrazole-4-carbaldehyde (**4c**)*

mp (purified by column chromatography on silica gel) 144–145 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.06 (s, 2 H, NH_2), 7.37–7.48 (m, 5 H, ArH), 7.61–7.62 (m, 3 H, ArH), 9.80 (s, 1 H, CHO); ^{13}C NMR (50 MHz, $CDCl_3$) δ 104.7, 121.6, 124.2 128.6, 129.0 (2 × CH), 129.7 (2 × CH), 129.8, 130.9, 135.4, 135.8, 137.9, 150.1, 152.5, 185.0 (CHO); IR (KBr) 3406 (m), 3298 (m), 2924 (m), 2850 (m), 2368 (m), 1624 (s), 1512 (m), 1359 (m), 1222 (m), 1168 (m), 1095 (m), 829 (m), 744 (m) cm^{-1}; EIMS *m/z* (relative intensity) 333 (M + 2, 65), 331 (M^+, 100); Anal. Calcd for $C_{16}H_{11}Cl_2N_3O$; C: 57.85; H: 3.34; N: 12.65, Found: C: 57.88; H: 3.32; N: 12.69.

*5-Amino-1-(4-bromophenyl)-3-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde (**4d**)*

mp (purified by column chromatography on silica gel) 87–88 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.38 (s, 3 H, CH_3), 6.10 (s, 2 H, NH_2), 7.24–7.26 (m, 2 H, ArH), 7.41–7.43 (m, 2 H, ArH), 7.53–7.57 (m, 4 H, ArH), 9.75 (s, 1 H, CHO); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.2 (CH_3), 104.7, 121.7, 125.1 (2 × CH), 128.3 (3 × CH), 129.4 (2 × CH), 132.8 (2 × CH), 135.9, 139.1, 149.9, 153.6, 185.3 (CHO); IR (KBr) 3290 (m), 2924 (m), 2850 (m), 2368 (m), 1643 (s), 1519 (m), 1373 (m), 1249 (m), 1165 (m), 1072 (m), 983 (m), 825 (m), 740 (m) cm^{-1}; EIMS *m/z* (relative intensity) 357 (M + 2, 99), 355 (M^+, 100); Anal. Calcd for $C_{17}H_{14}BrN_3O$; C: 57.32; H: 3.96; N: 11.80, Found: C: 57.28; H: 3.94; N: 11.81.

*5-Amino-1-(4-bromophenyl)-3-(4-chlorophenyl)-1H-pyrazole-4-carbaldehyde (**4e**)*

mp (purified by column chromatography on silica gel) 191–192 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.97 (s, 2 H, NH_2), 7.42–7.48 (m, 4 H, ArH), 7.61–7.67 (m, 4 H, ArH), 9.82 (s, 1 H, CHO); ^{13}C NMR (50 MHz, $CDCl_3$) δ 104.8, 122.3, 125.4 (2 × CH), 129.1 (2 × CH), 128.7 (2 × CH), 129.9, 133.2 (2 × CH), 135.4, 135.8, 150.0, 152.5, 185.0 (CHO); IR (KBr) 3302 (m), 2920 (m), 2850 (m), 2368 (m), 1639 (m), 1492 (m), 1261 (m), 1153 (m), 1010 (m), 829 (m), 736 (m), 578 (m) cm^{-1}; EIMS *m/z* (relative intensity) 379 (M+2, 25), 377 (100), 376 (M^+, 52), 348 (6), 221 (3), 162 (10), 97 (13), 75 (13), 71 (20), 57 (29); Anal. Calcd for $C_{16}H_{11}BrClN_3O$; C: 51.02; H: 2.94; N: 11.16, Found: C: 51.03; H: 2.91; N: 11.12.

Section 5.3 Cell lines

Human non-small cell lung carcinoma (NCI-H661) was purchased from American Type Culture Collection (ATCC; Rockville, MD). T-cell leukemia (Jurkat) was obtained from Japanese Collection of Research Bioresources (JCRB) and nasopharyngeal carcinoma (NPC-TW01) was purchased from Bioresource Collection and Research Center (BCRC, Taiwan). All the tumor cell lines were maintained in either RPMI-1640 or Modified essential medium (MEM) supplied with 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2/95% air in the present of penicillin and streptomycin.

Section 5.4 Growth inhibition assay

Logarithmic phase cells were seeded in a 96-well plate and incubated overnight prior to addition of the designated compounds. After incubation with different concentrations of the tested compounds for 72 h, cells were incubated with MEM containing 0.5 mg/mL MTT for 2 h. The conversion of MTT to formazan by metabolically viable cells was measured by the absorbance at 570 nm in a 96-well microtiter plate reader. The percentage conversion by mock-treated control cells was used to evaluate the effect of the chemicals on cell growth and to determine the concentration that inhibited 50% of growth (GI_{50}).

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Addendum

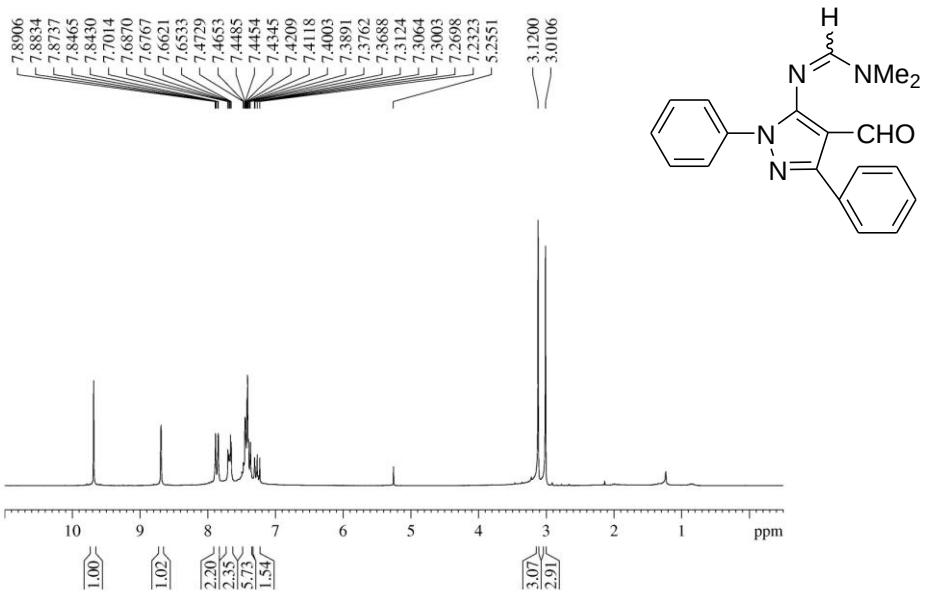

Figure 13 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **2a**

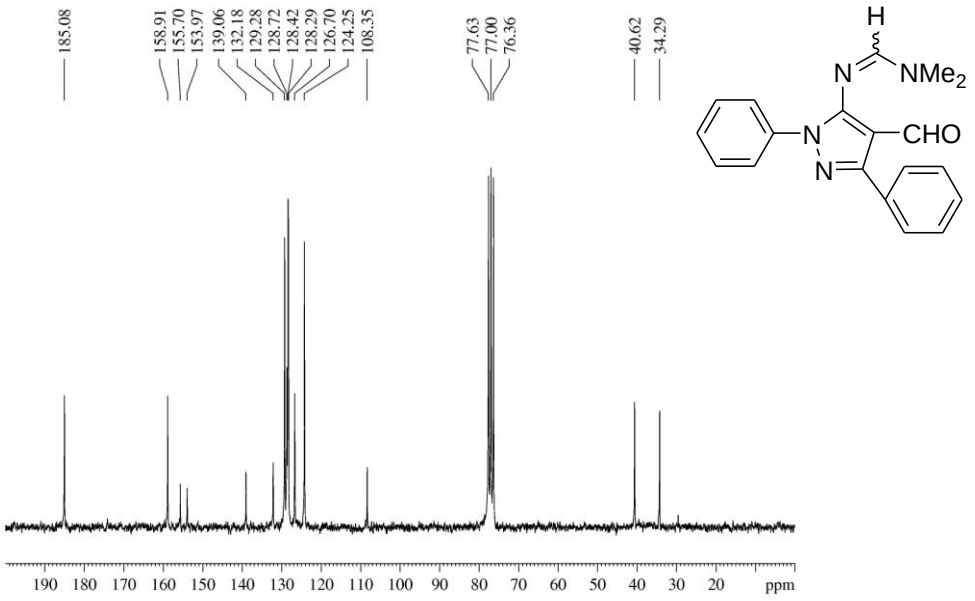

Figure 14 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **2a**

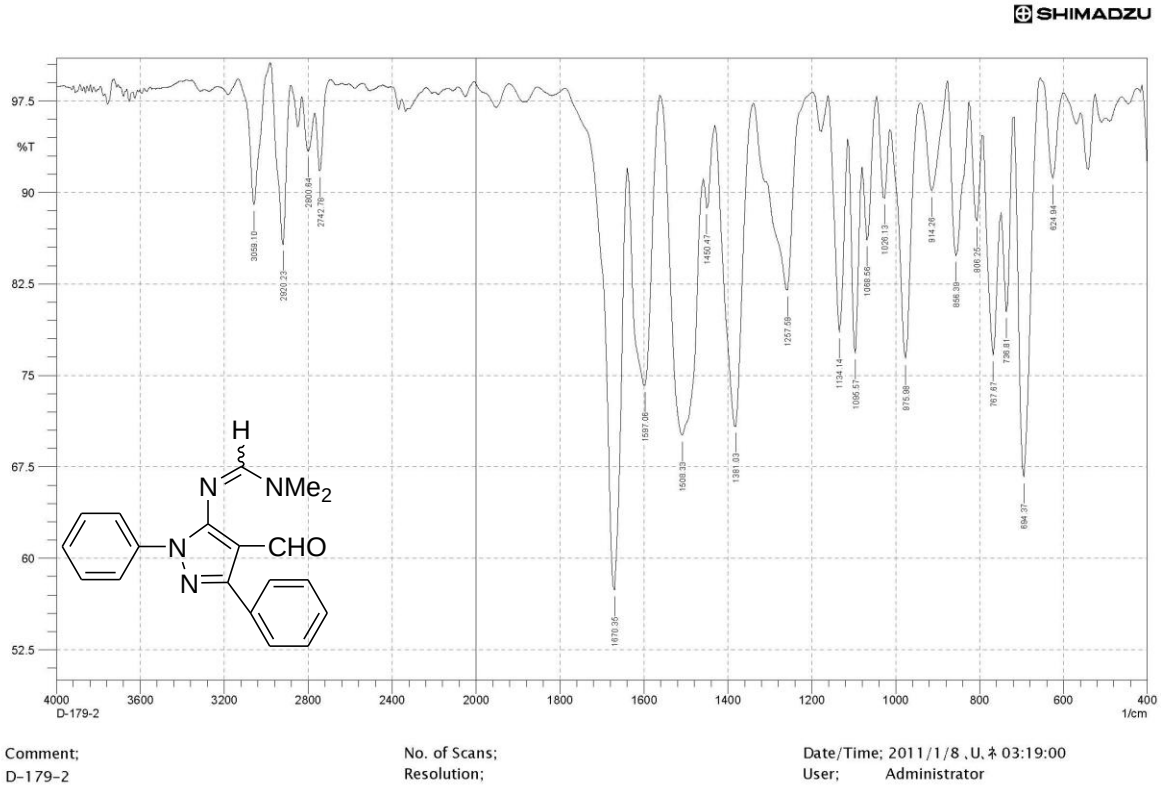

Figure 15 IR spectrum of compound **2a**

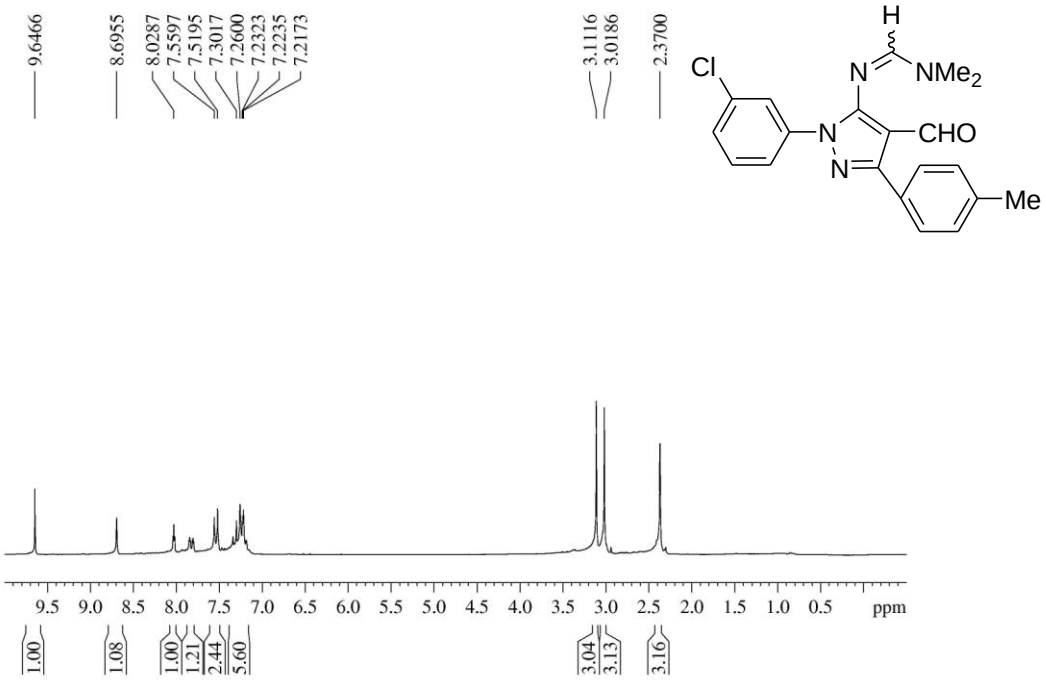

Figure 16 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **2b**

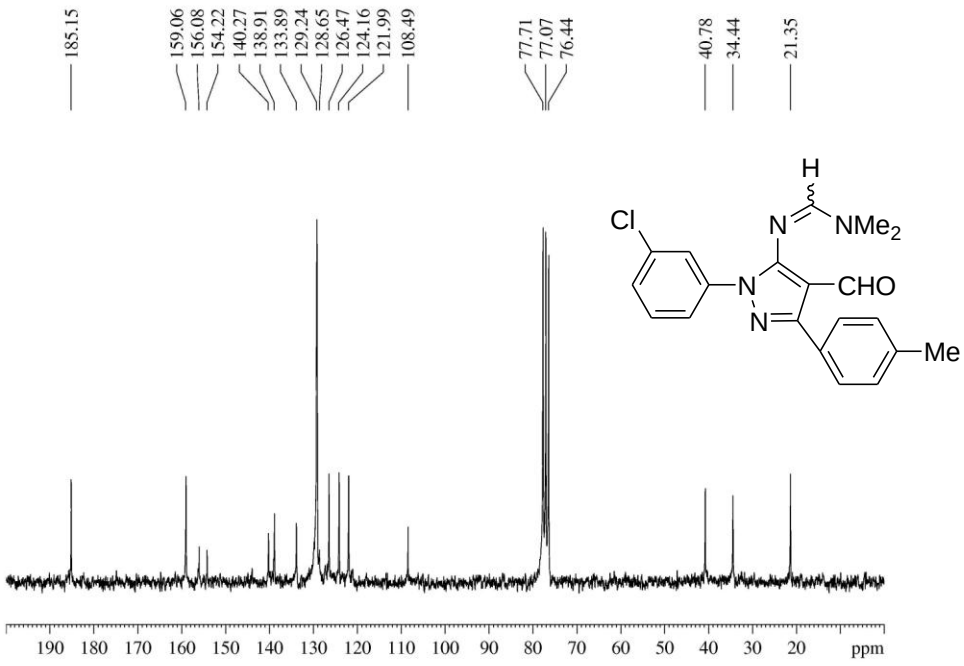

Figure 17 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **2b**

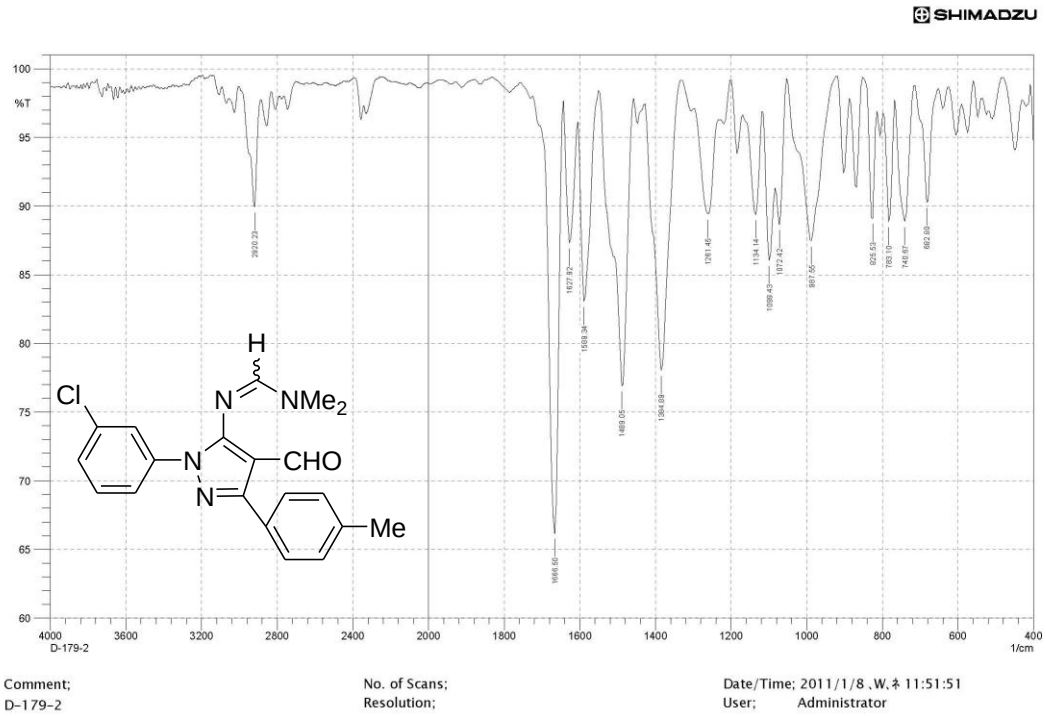

Figure 18 IR spectrum of compound **2b**

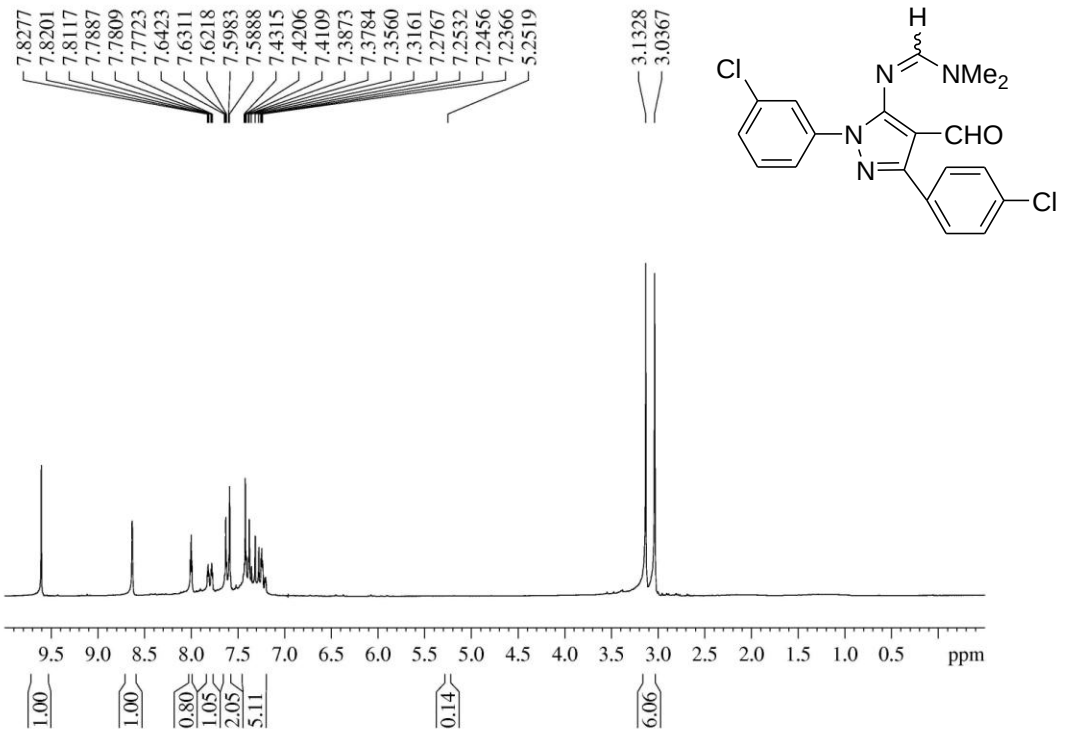

Figure 19 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **2c**

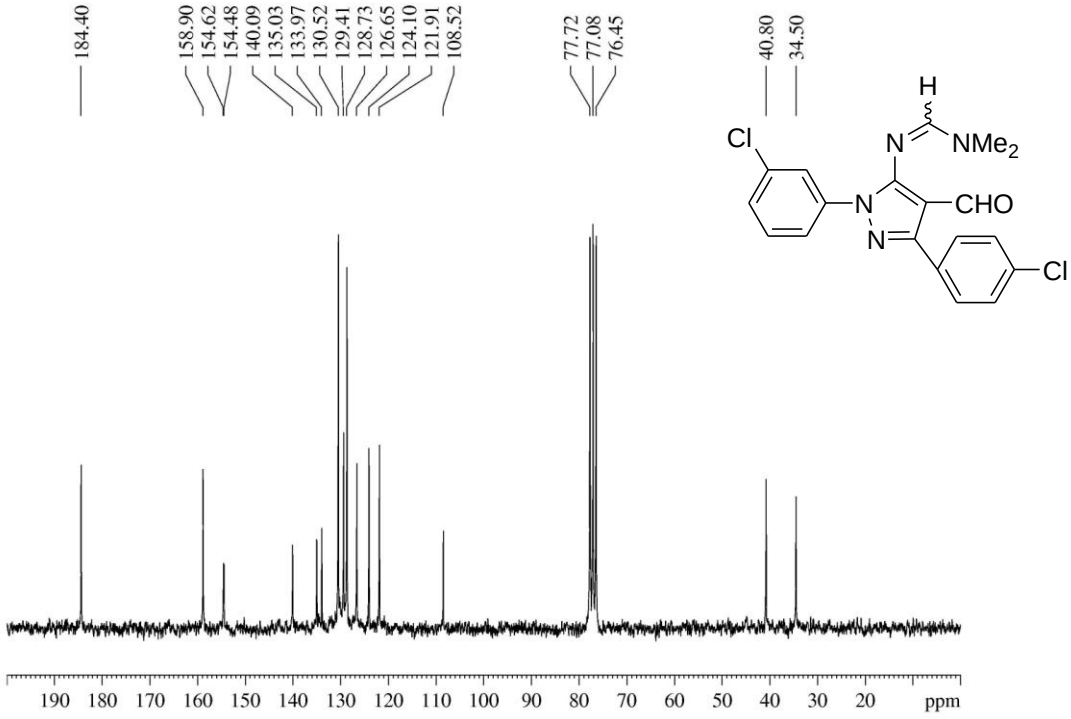

Figure 20 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **2c**

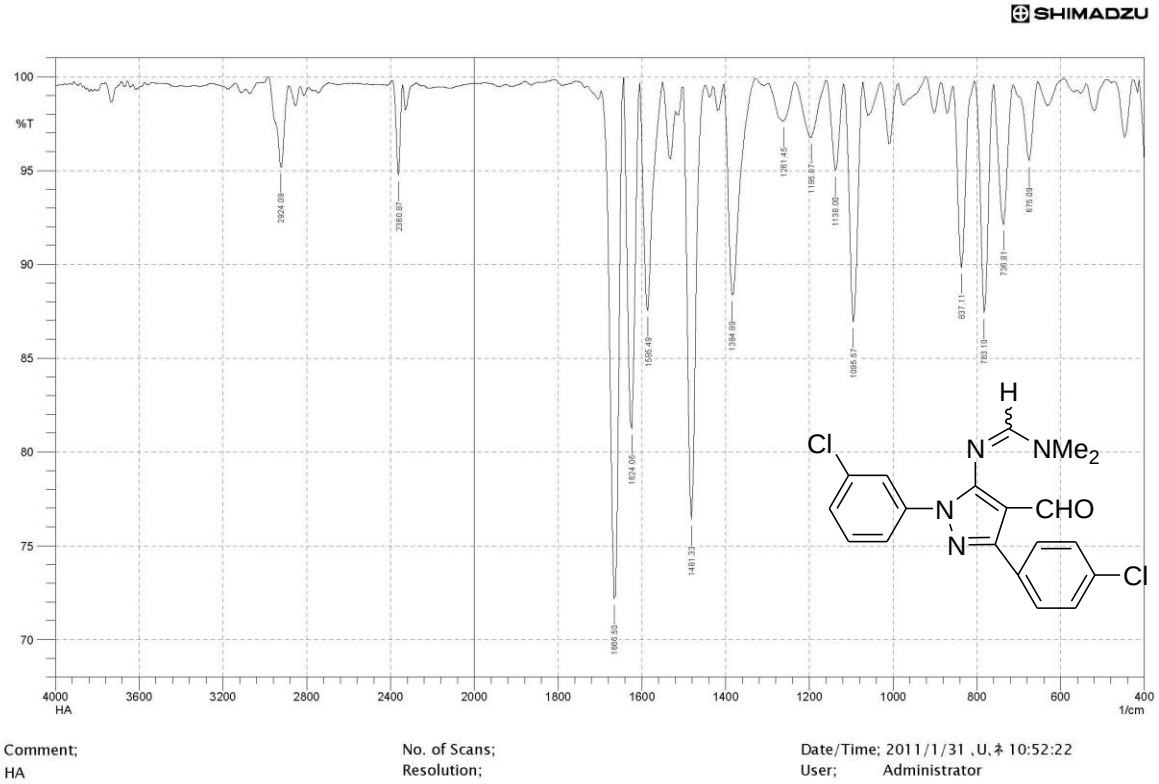

Figure 21 IR spectrum of compound **2c**

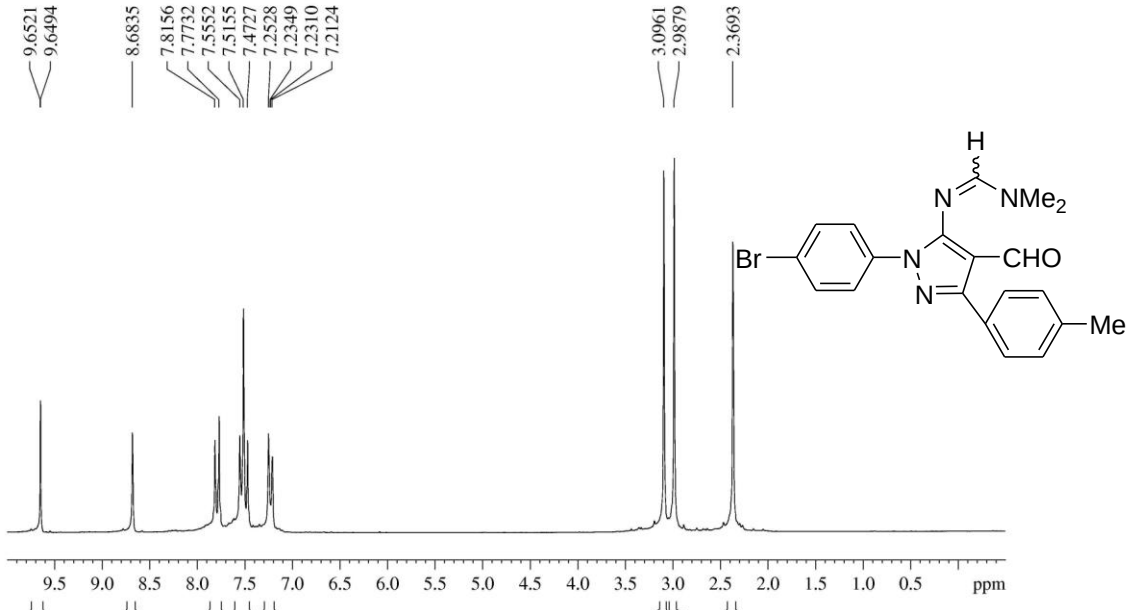

Figure 22 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **2d**

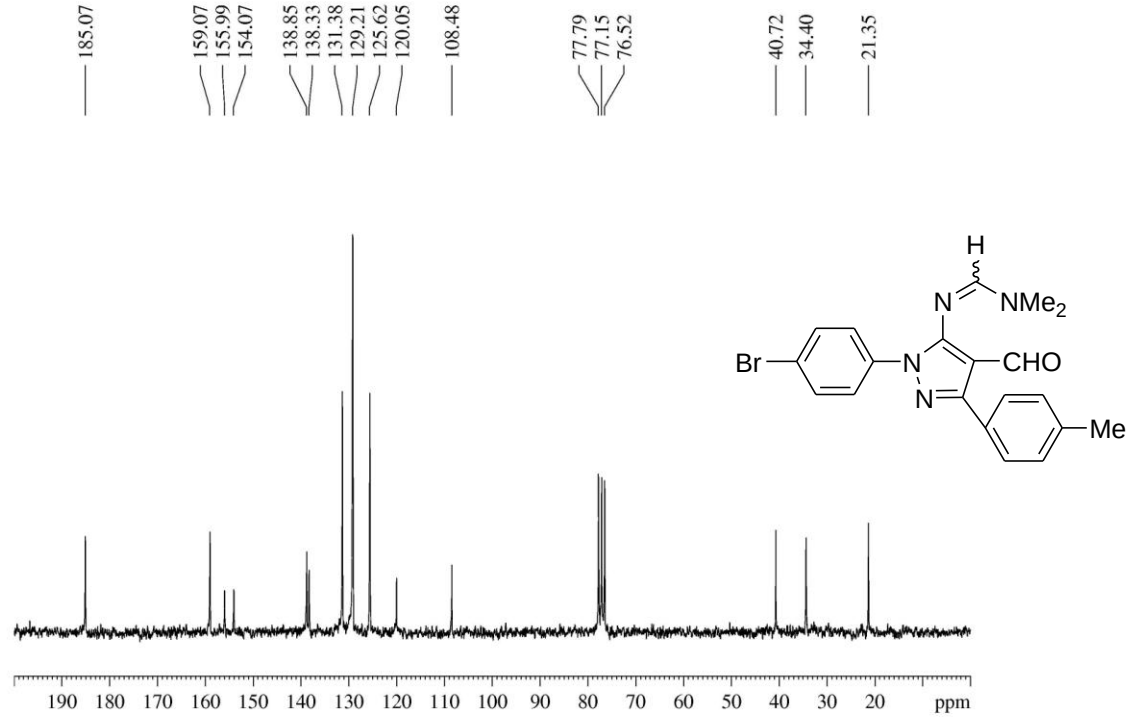

Figure 23 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **2d**

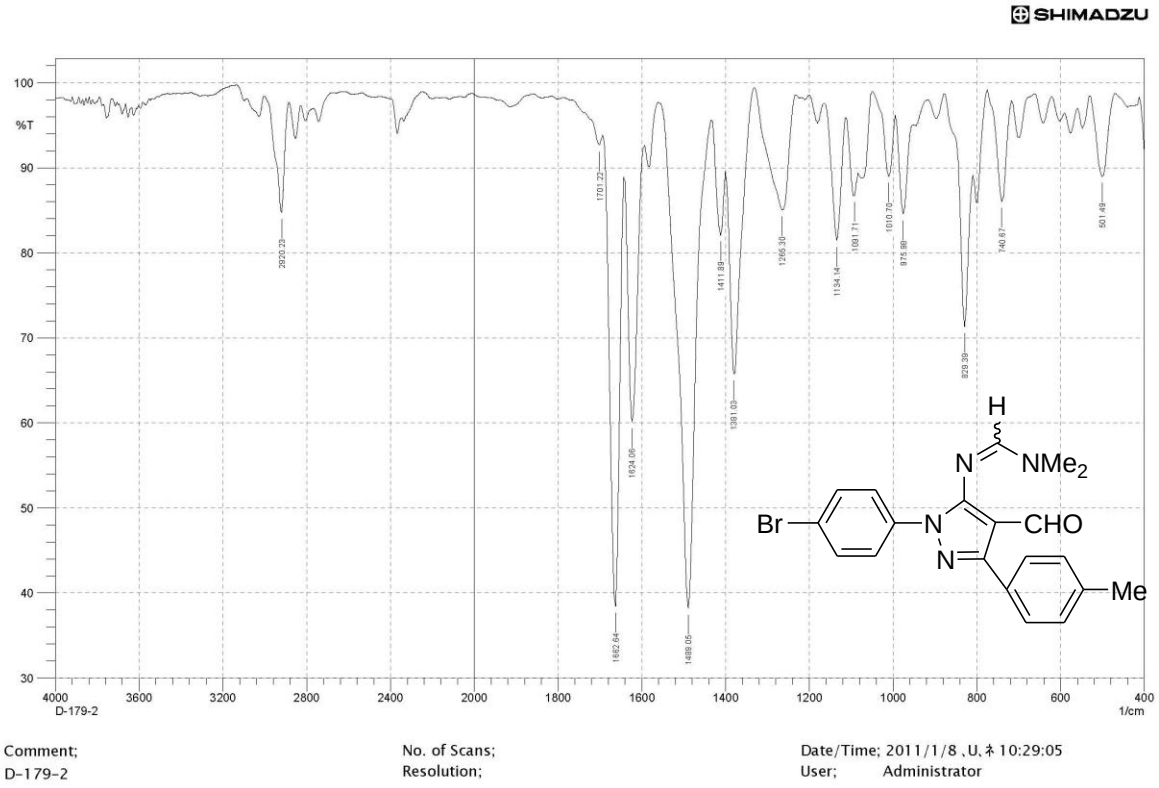

Figure 24 IR spectrum of compound **2d**

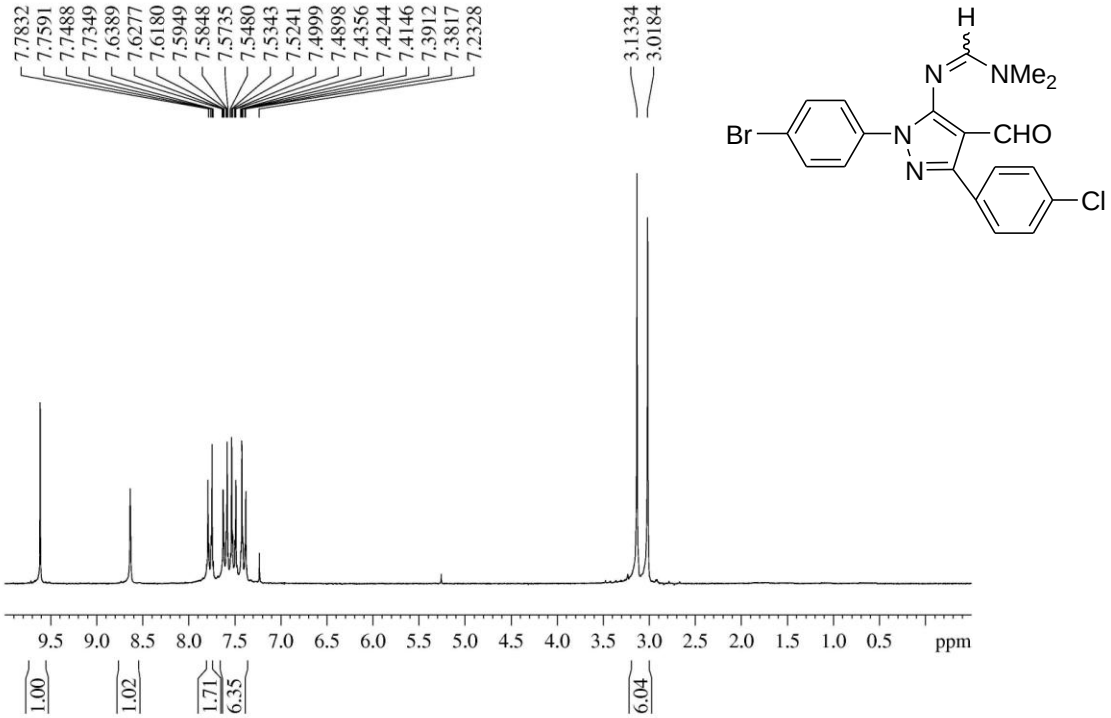

Figure 25 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **2e**

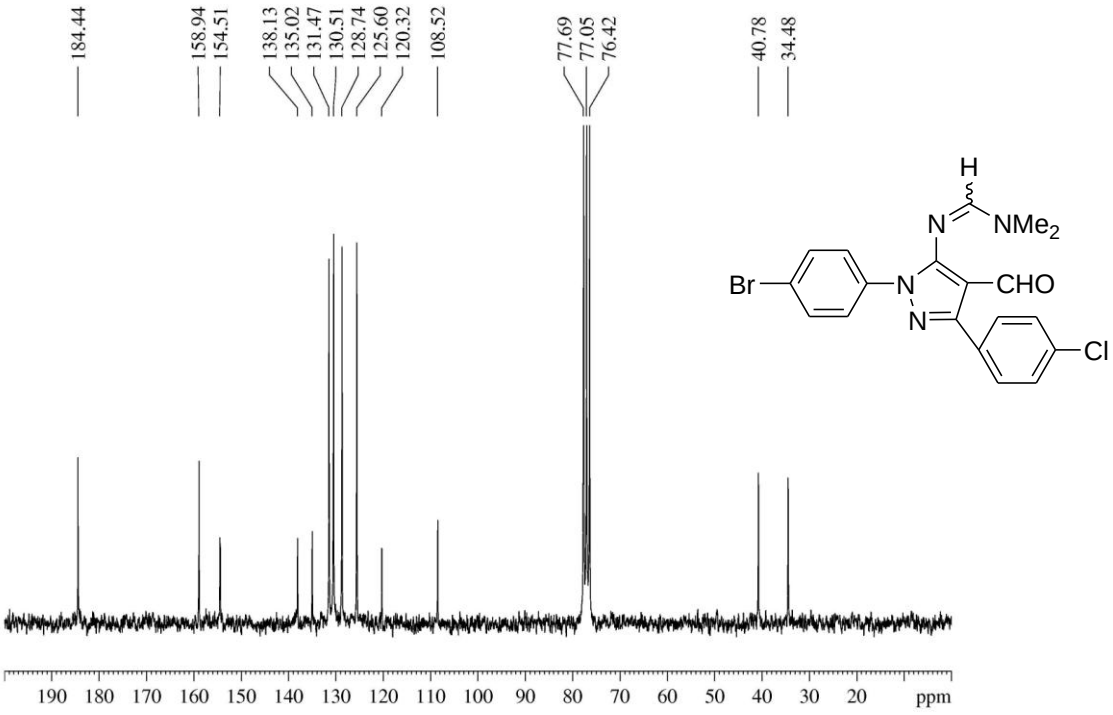

Figure 26 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **2e**

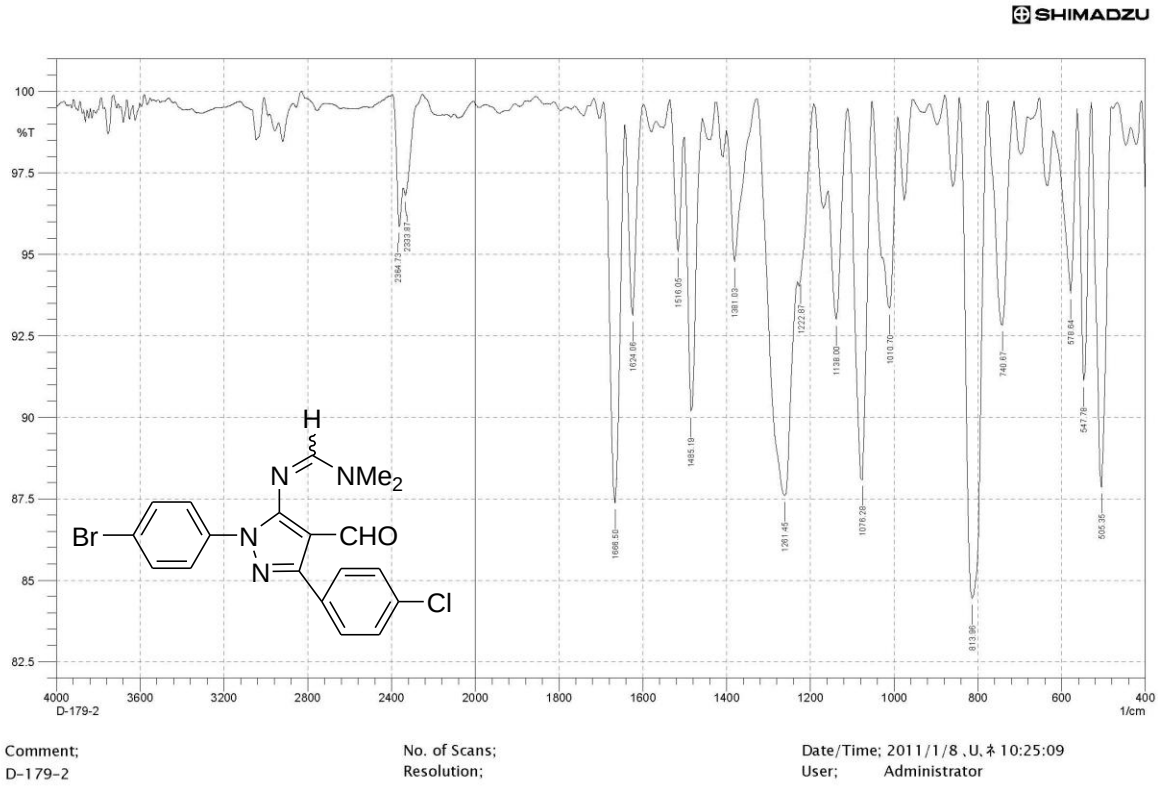

Figure 27 IR spectrum of compound **2e**

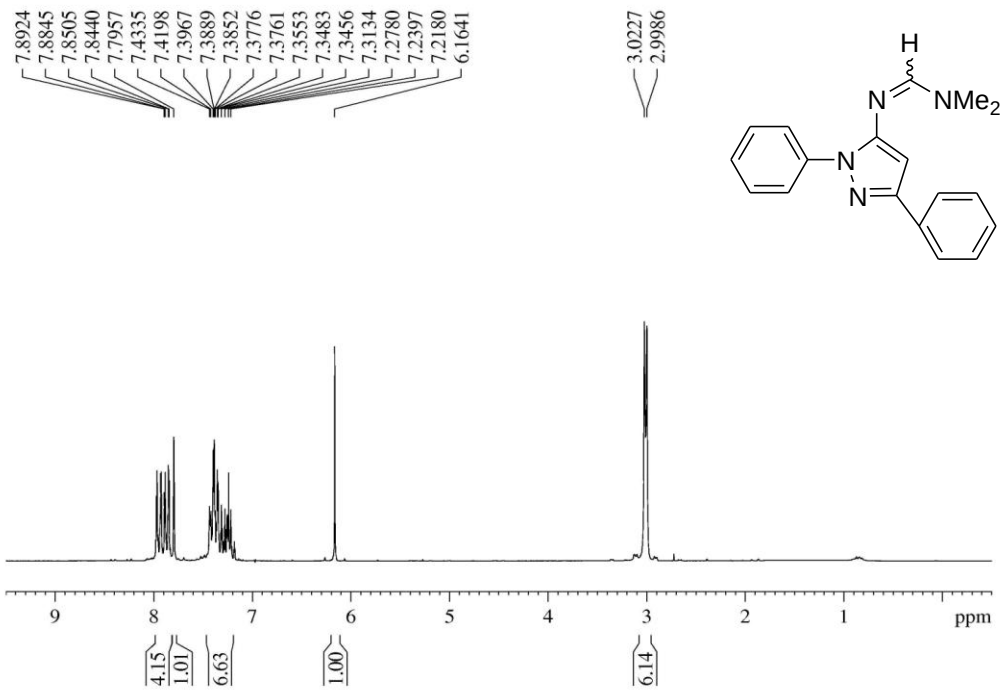

Figure 28 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **3a**

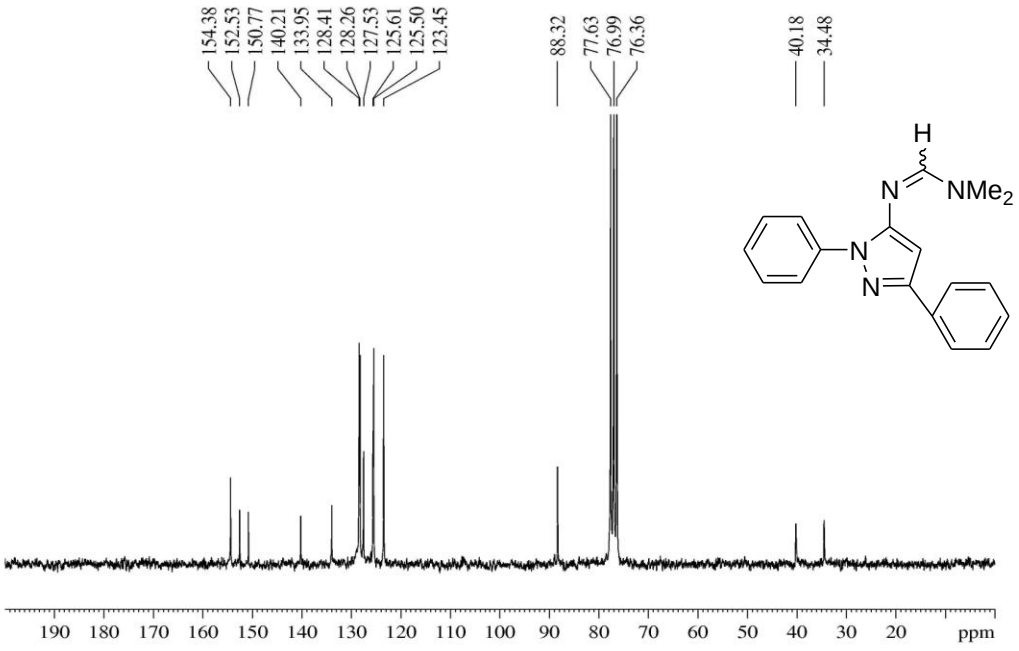

Figure 29 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **3a**

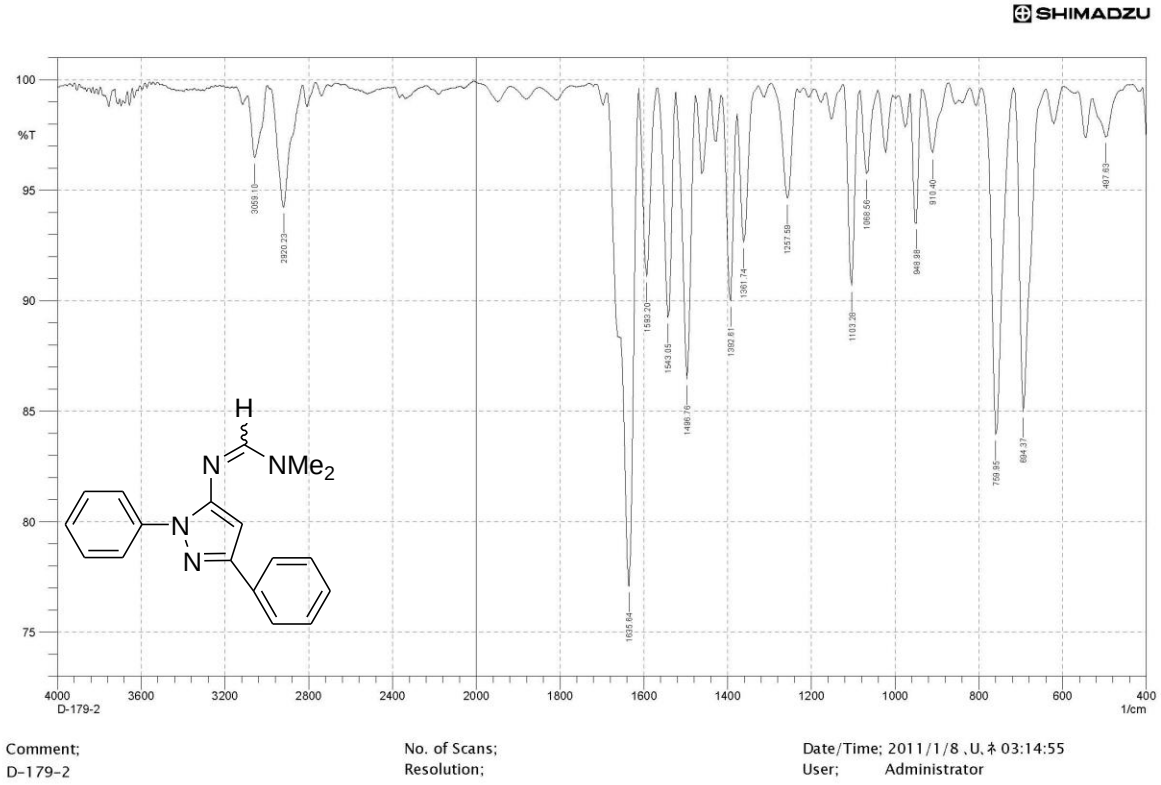

Figure 30 IR spectrum of compound **3a**

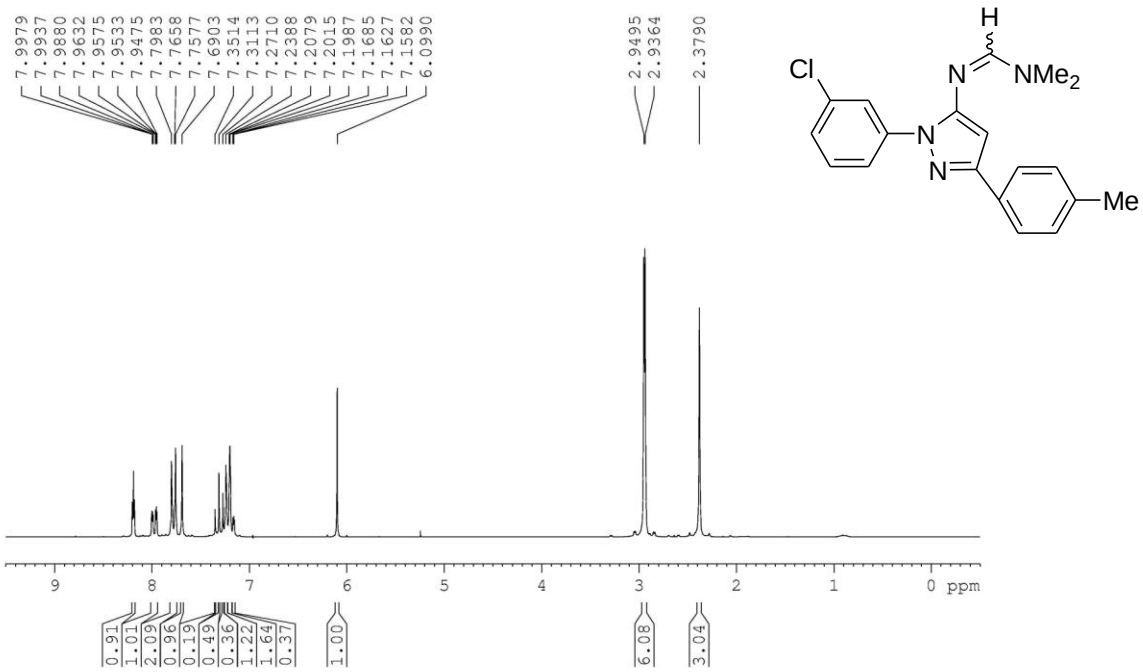

Figure 31 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **3b**

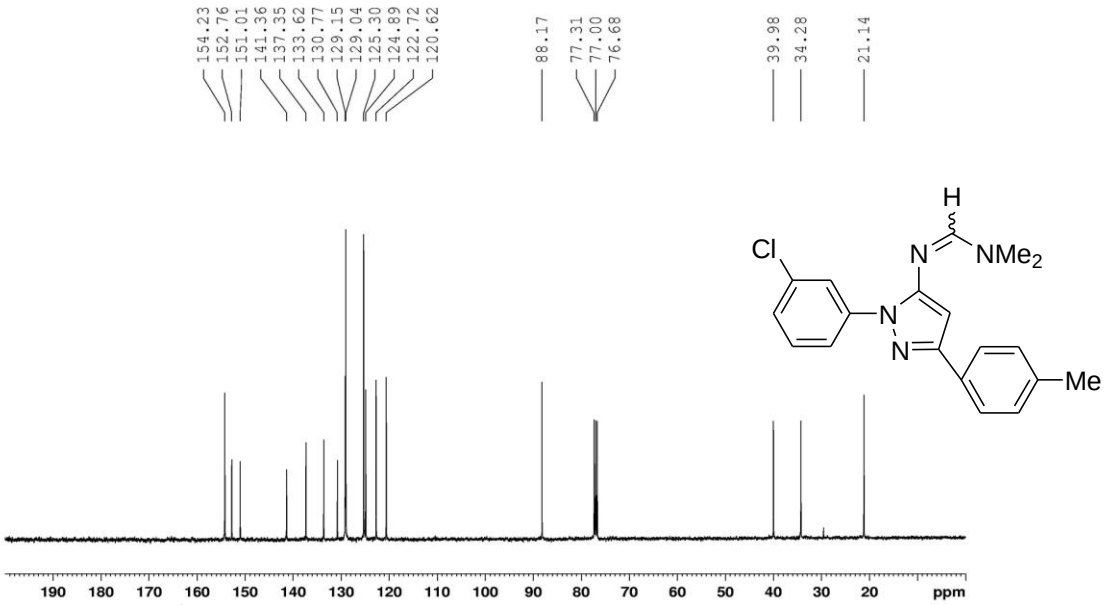

Figure 32 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **3b**

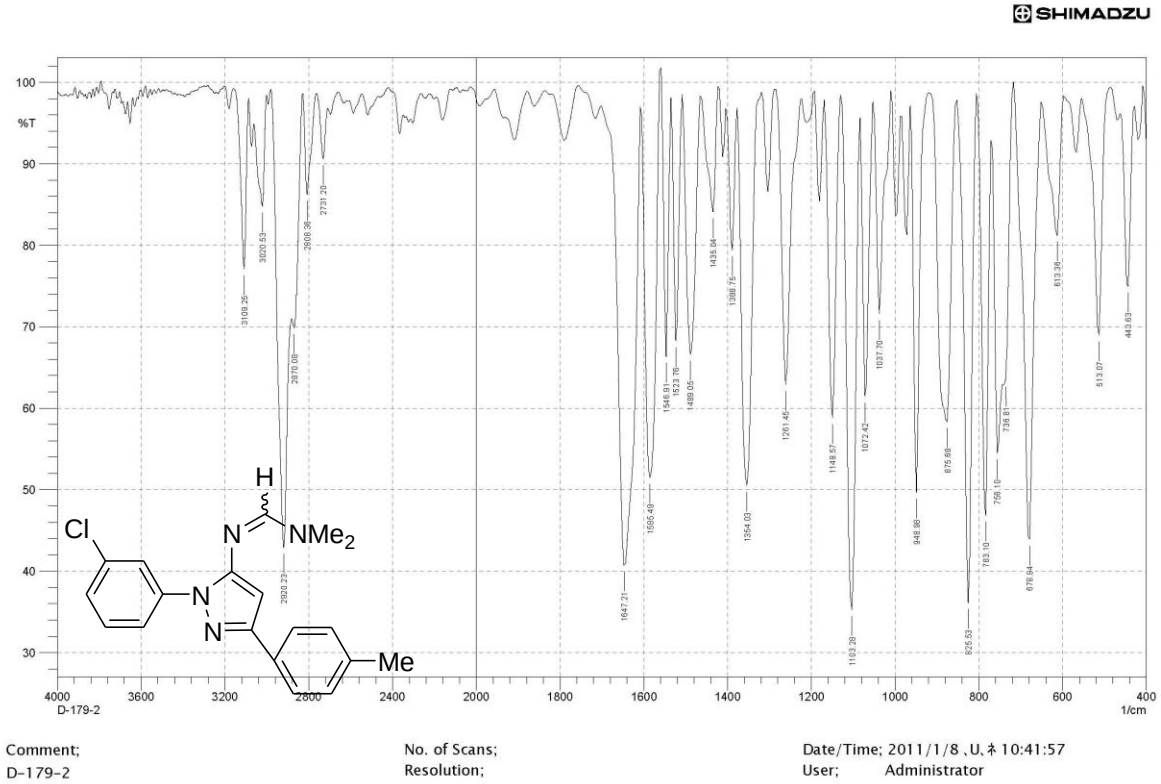

Figure 33 IR spectrum of compound **3b**

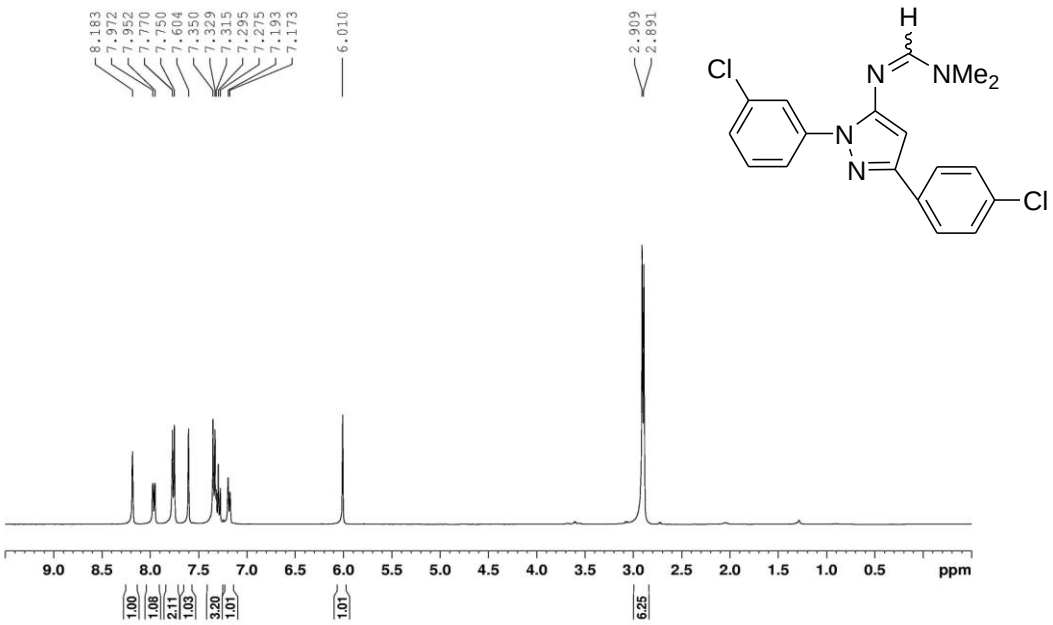

Figure 34 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **3c**

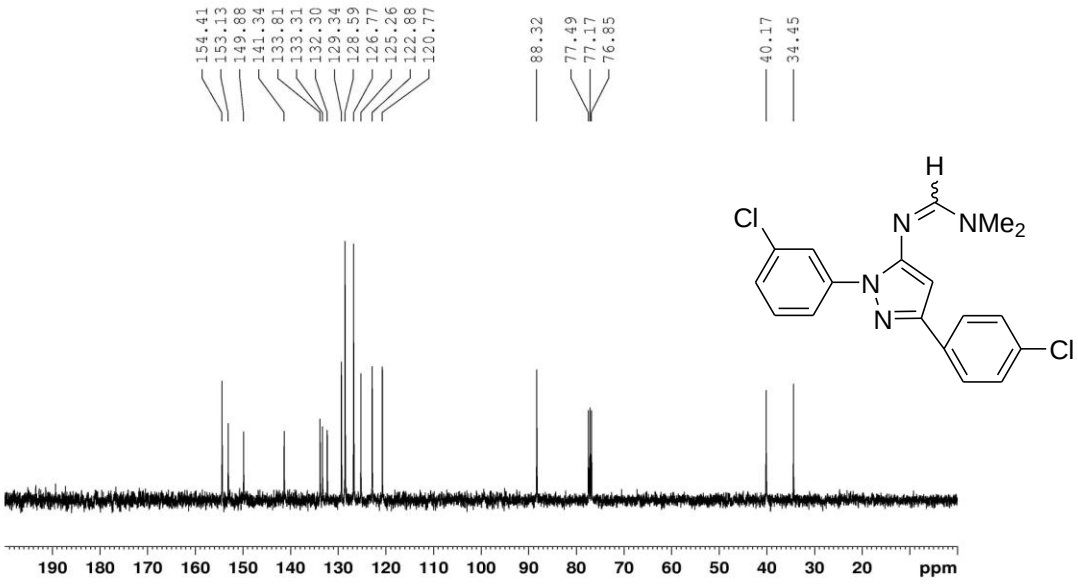

Figure 35 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **3c**

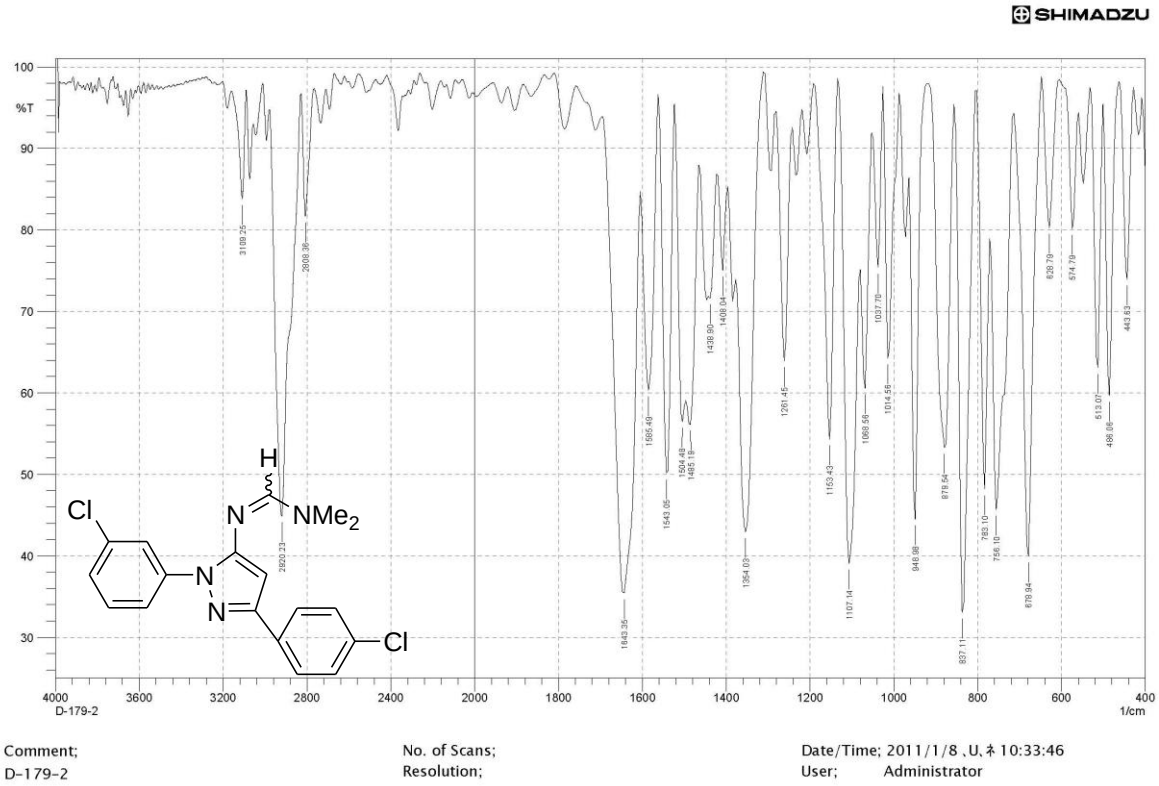

Figure 36 IR spectrum of compound **3c**

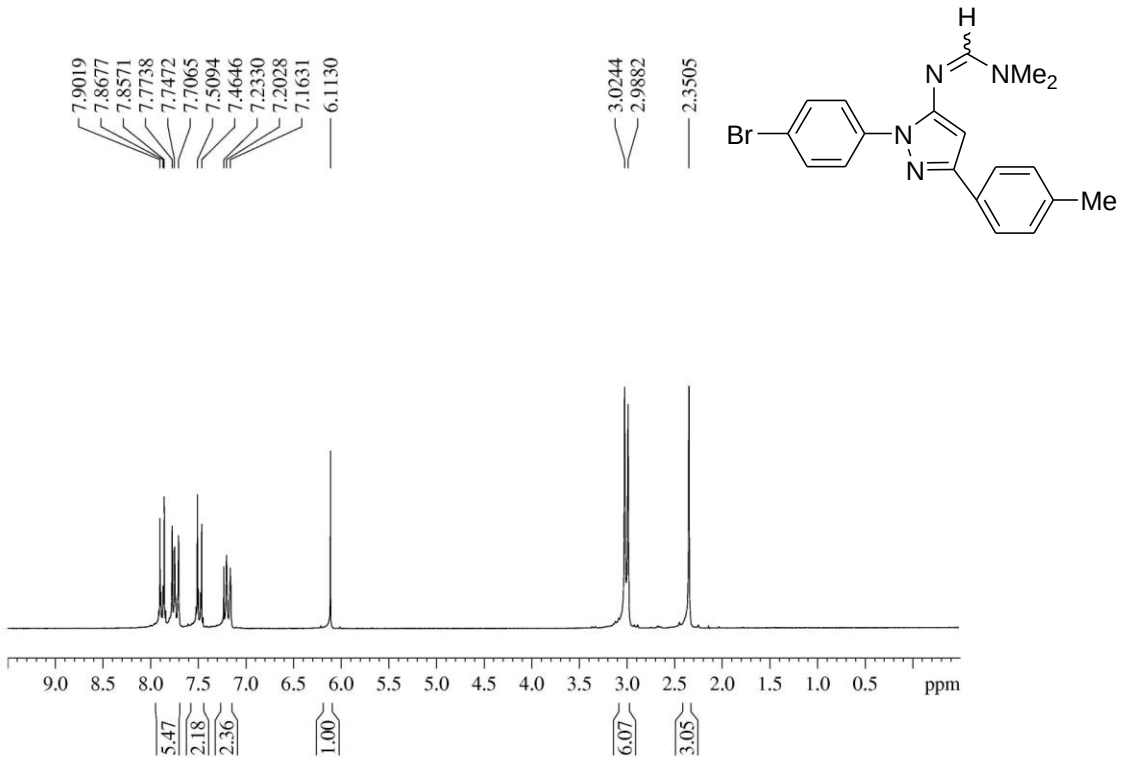

Figure 37 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **3d**

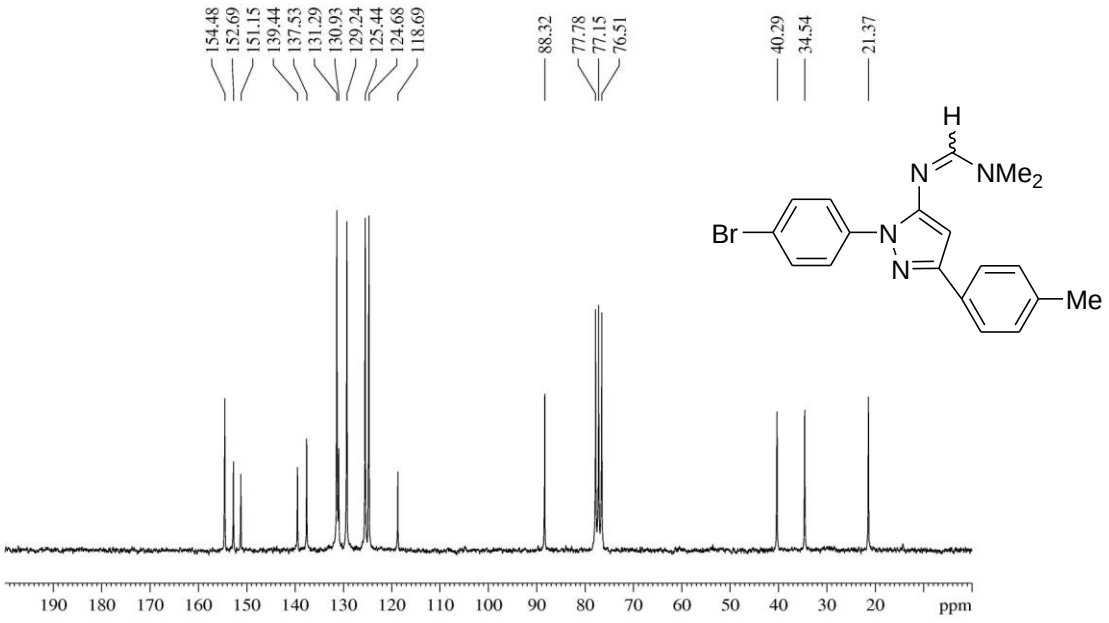

Figure 38 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **3d**

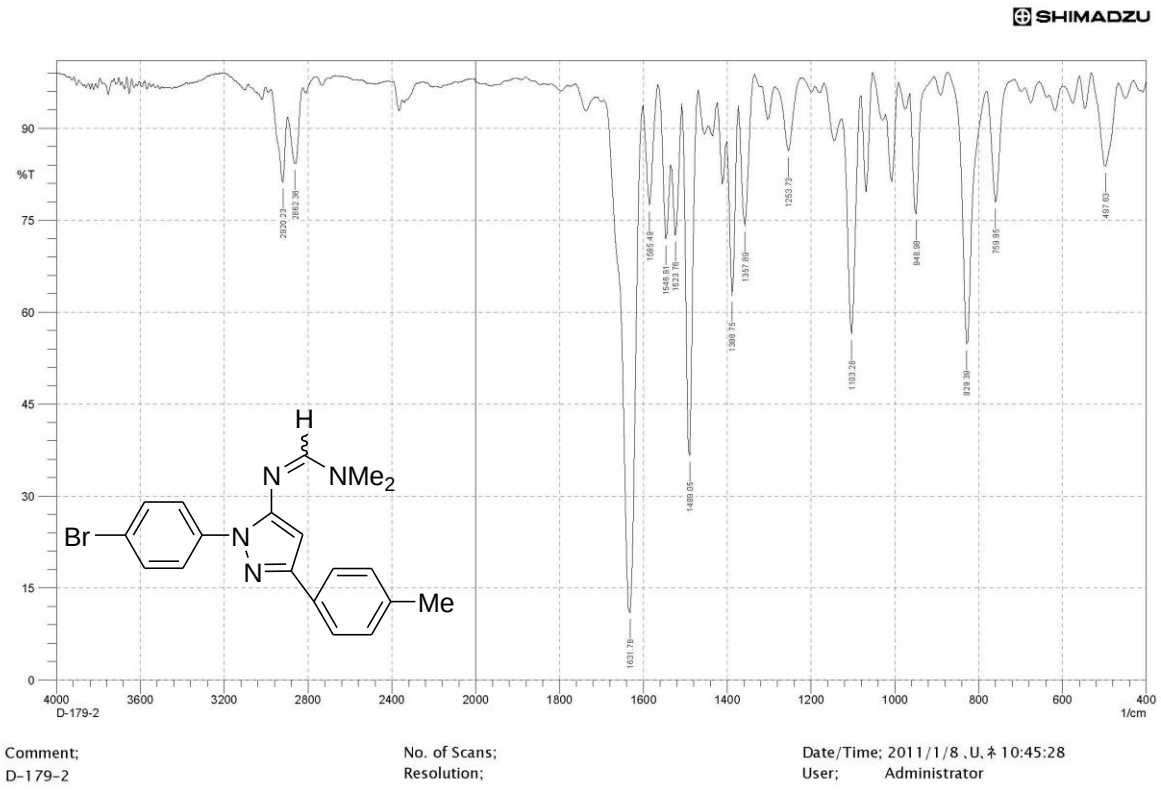

Figure 39 IR spectrum of compound **3d**

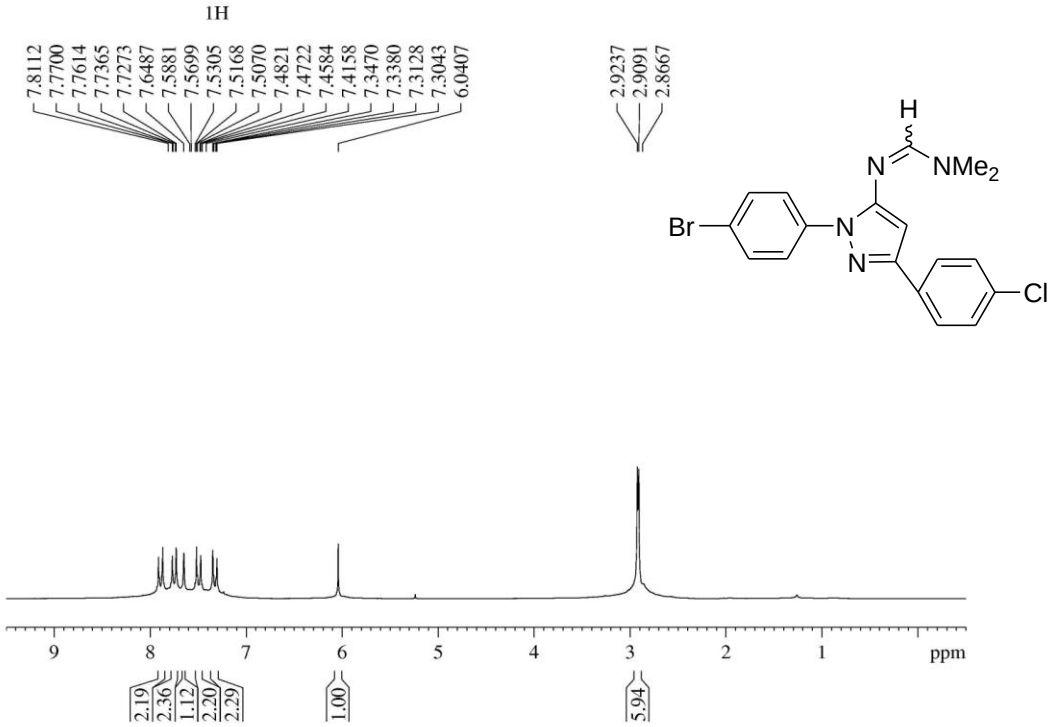

Figure 40 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **3e**

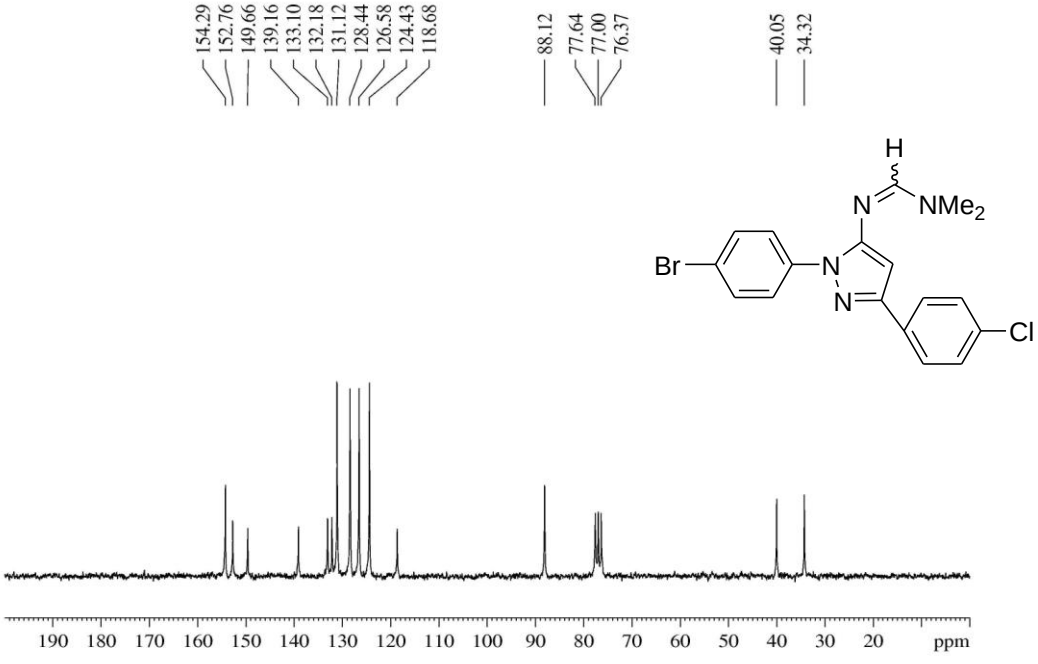

Figure 41 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **3e**

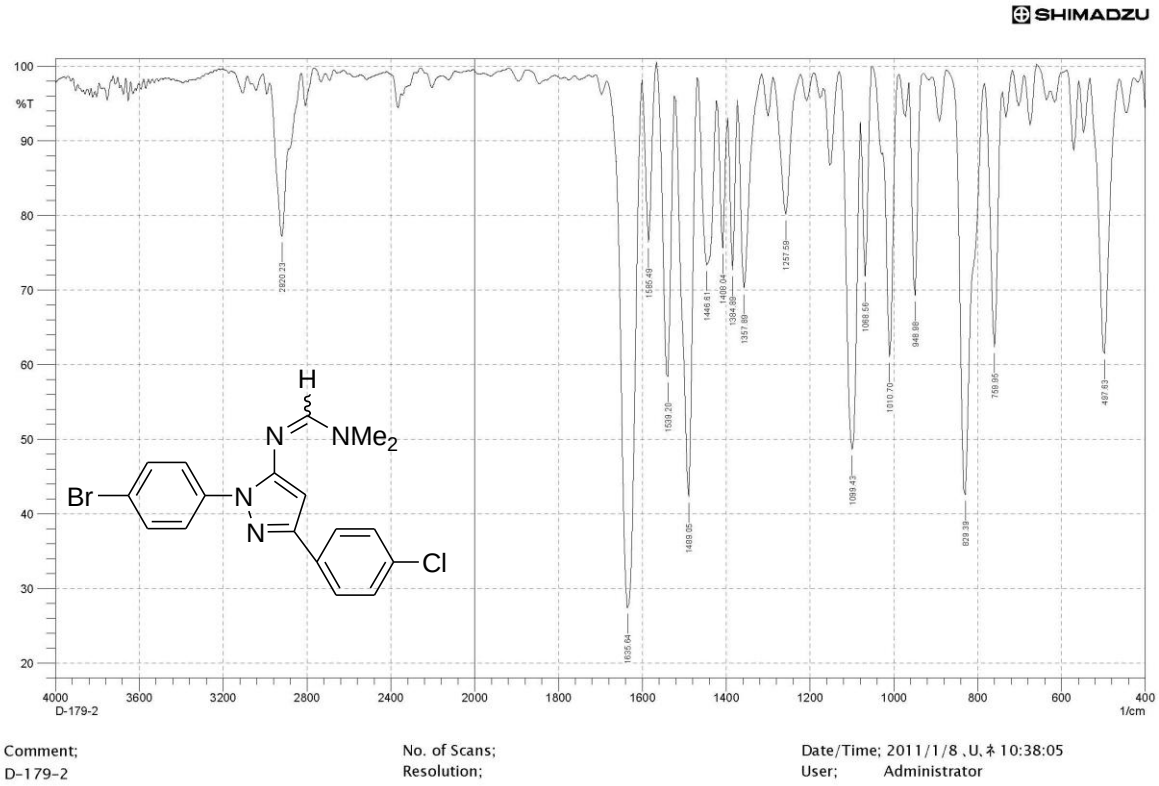

Figure 42 IR spectrum of compound **3e**

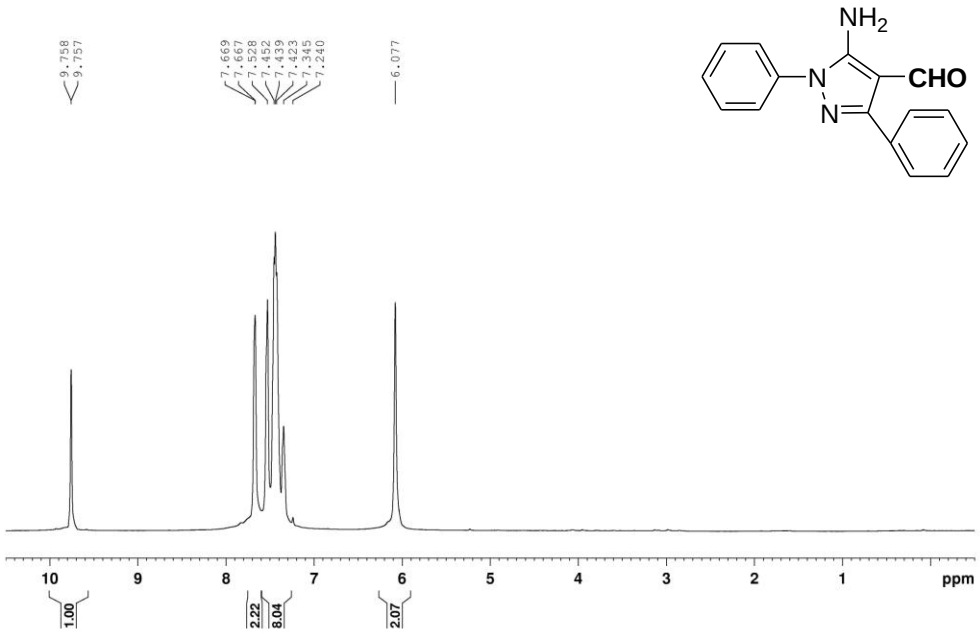

Figure 43 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **4a**

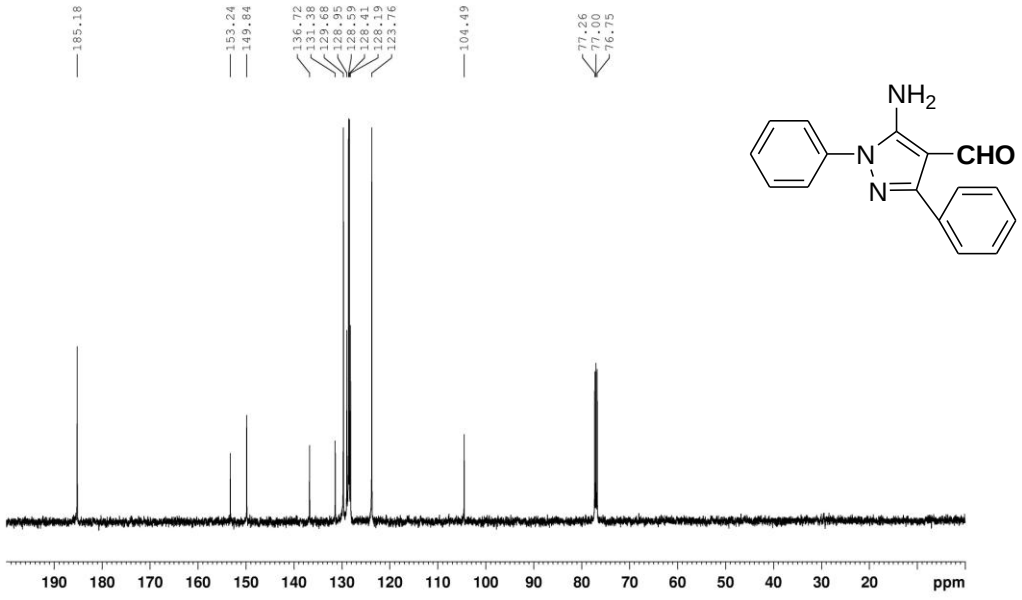

Figure 44 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **4a**

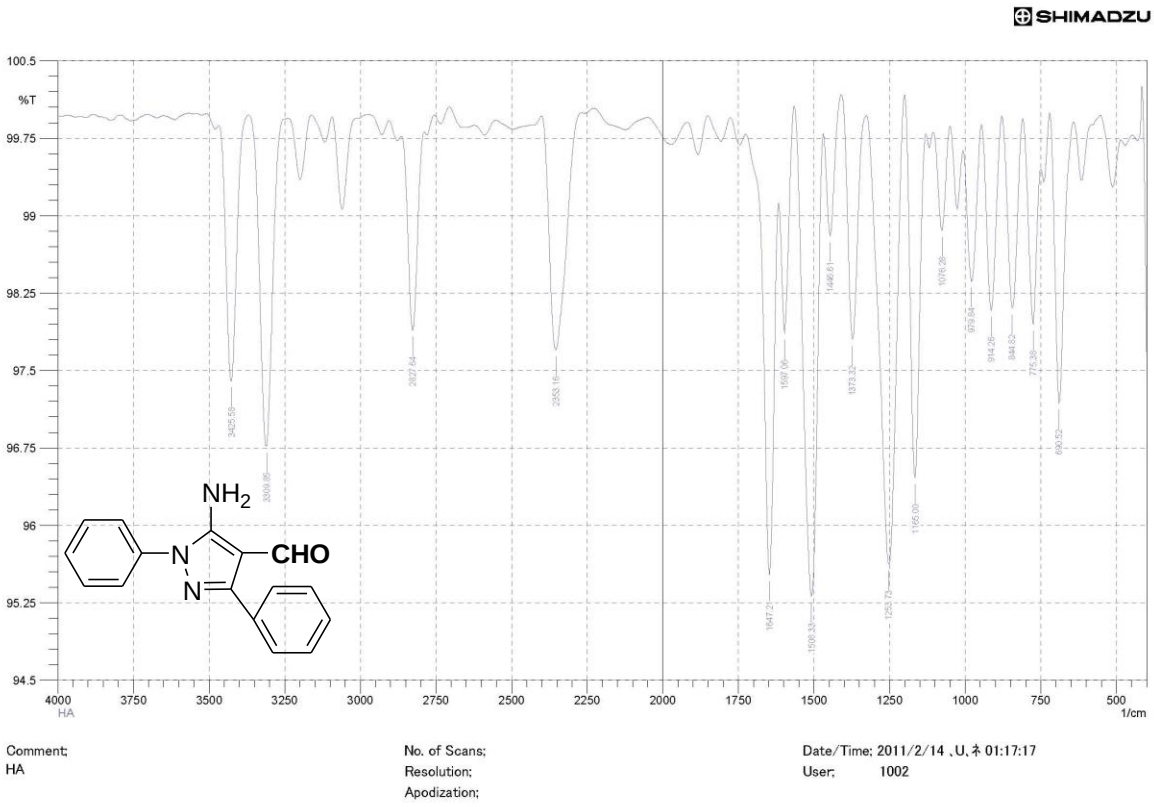

Figure 45 IR spectrum of compound **4a**

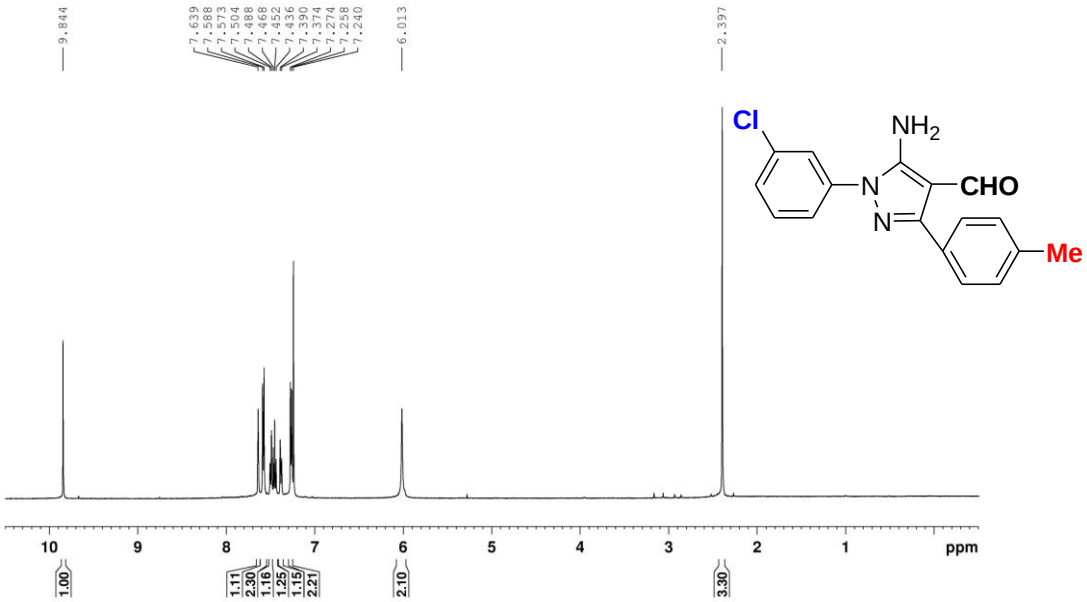

Figure 46 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **4b**

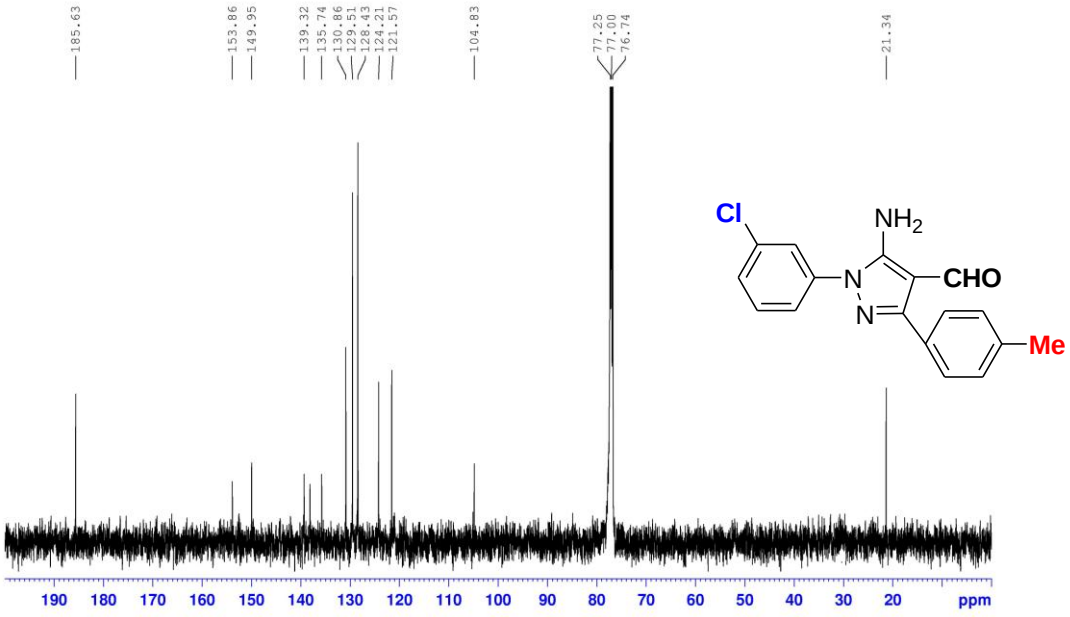

Figure 47 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **4b**

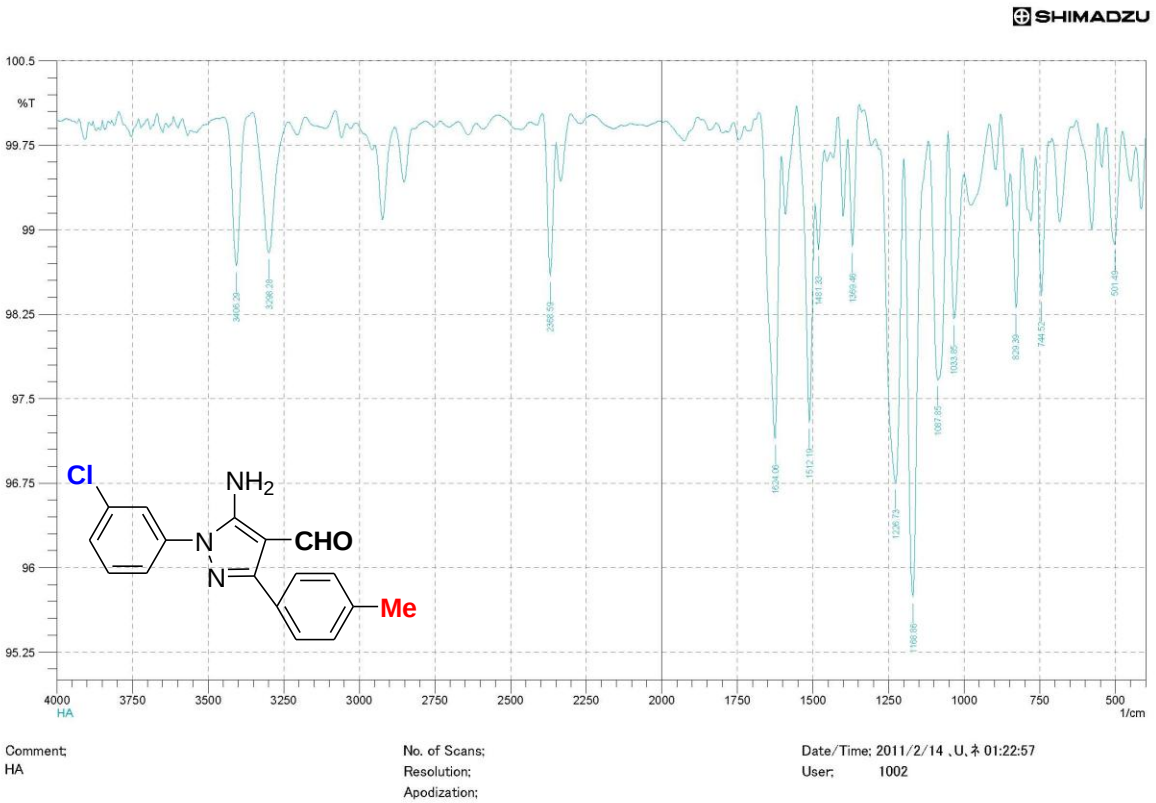

Figure 48 IR spectrum of compound **4b**

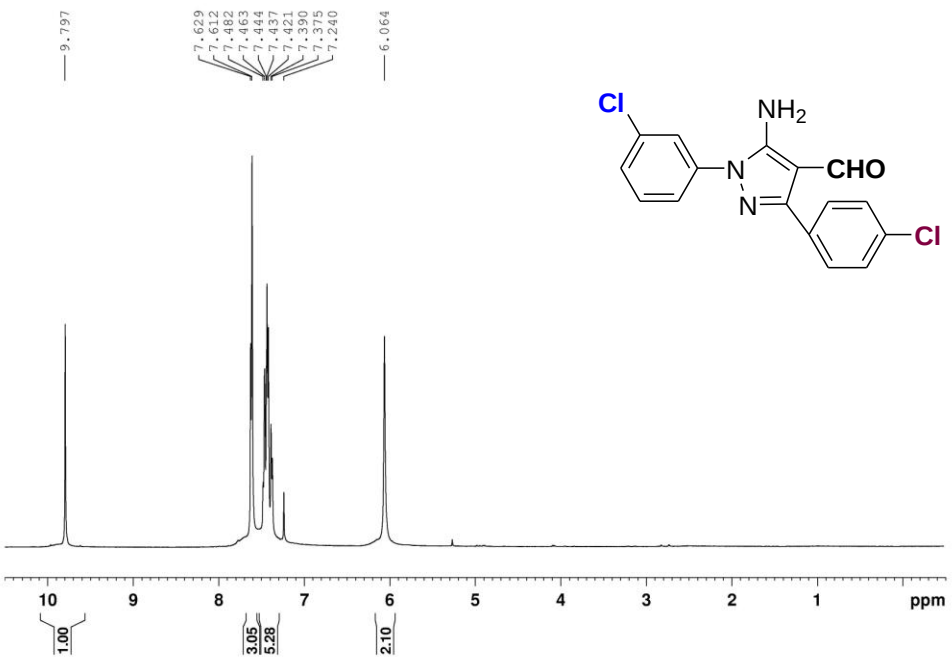

Figure 49 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **4c**

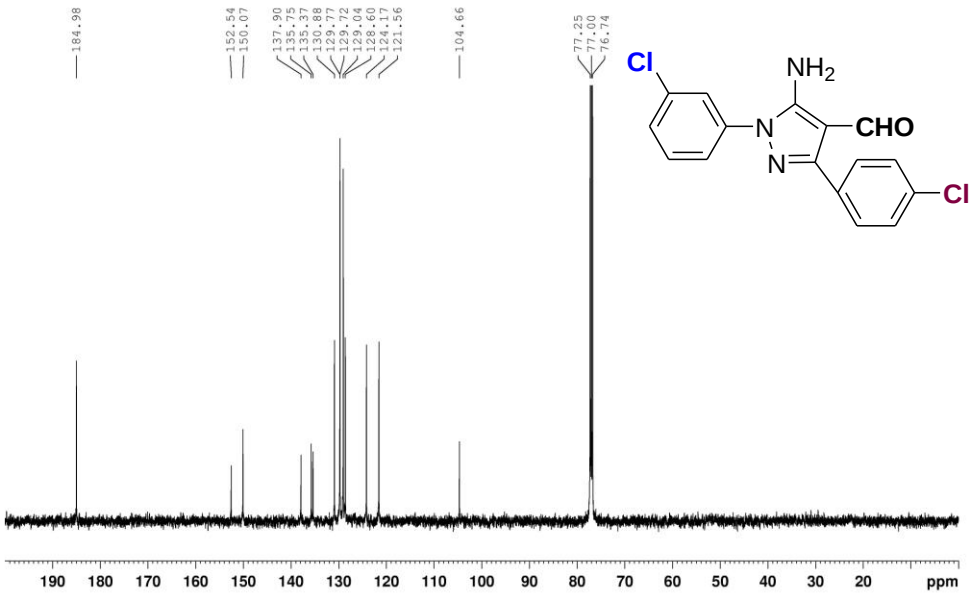

Figure 50 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **4c**

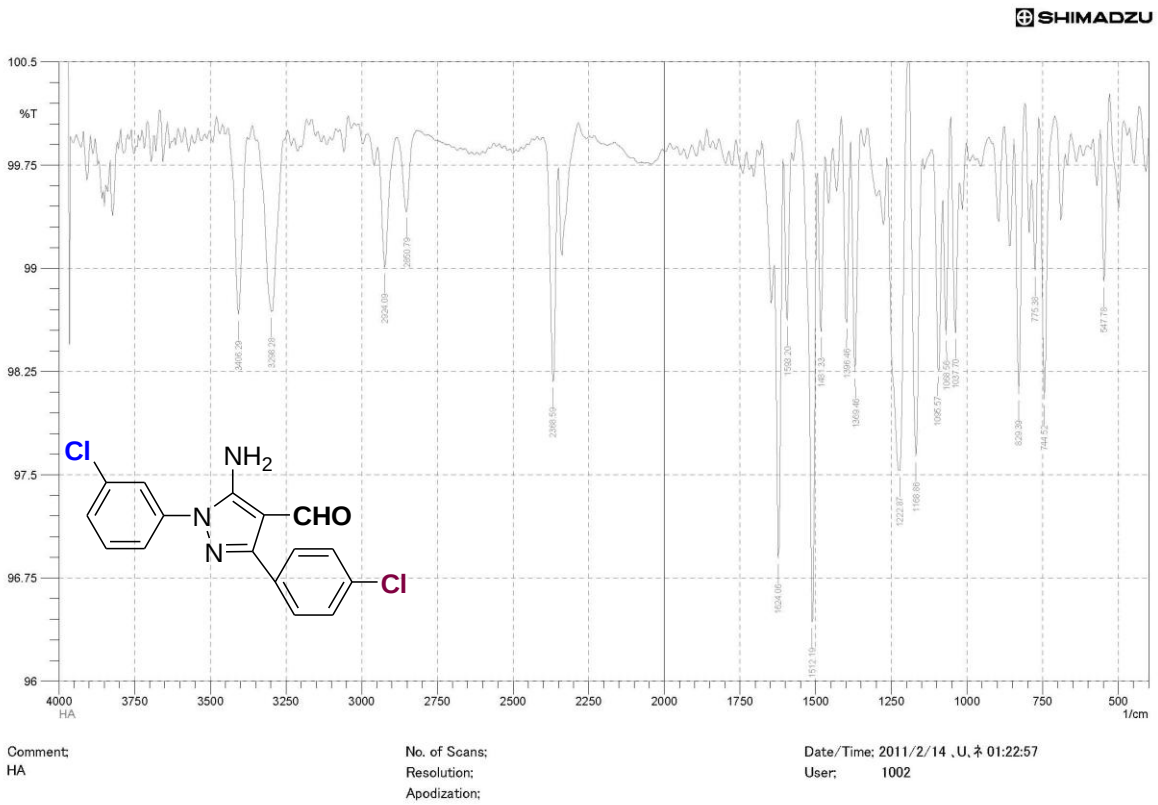

Figure 51 IR spectrum of compound **4c**

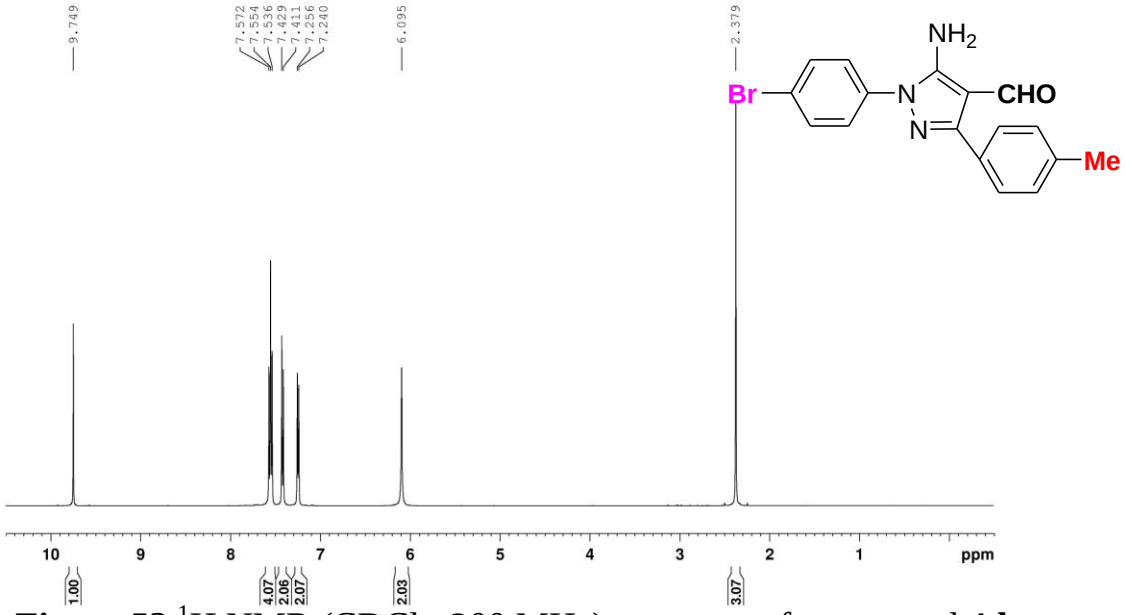

Figure 52 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **4d**

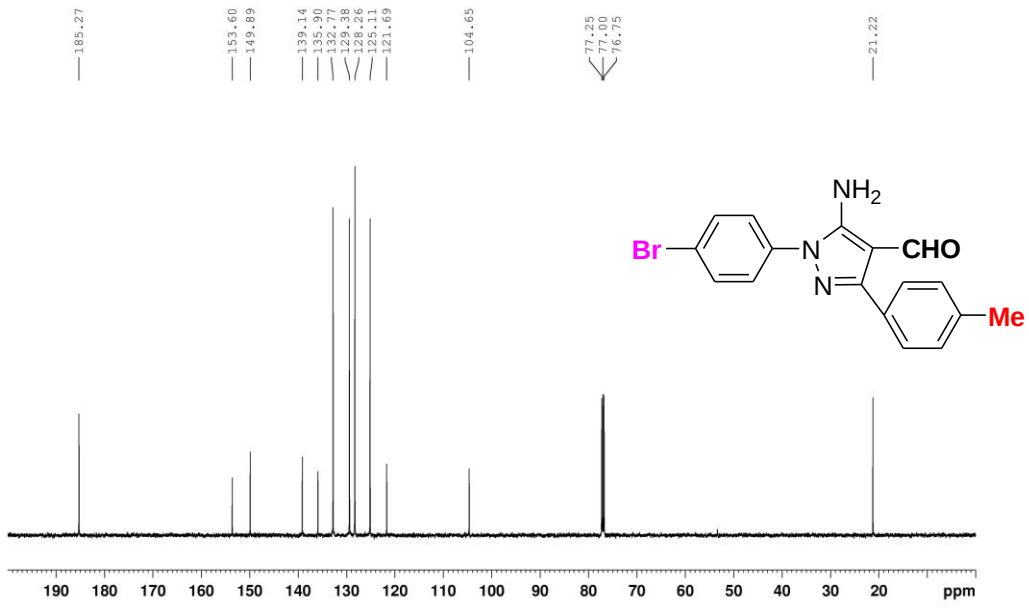

Figure 53 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **4d**

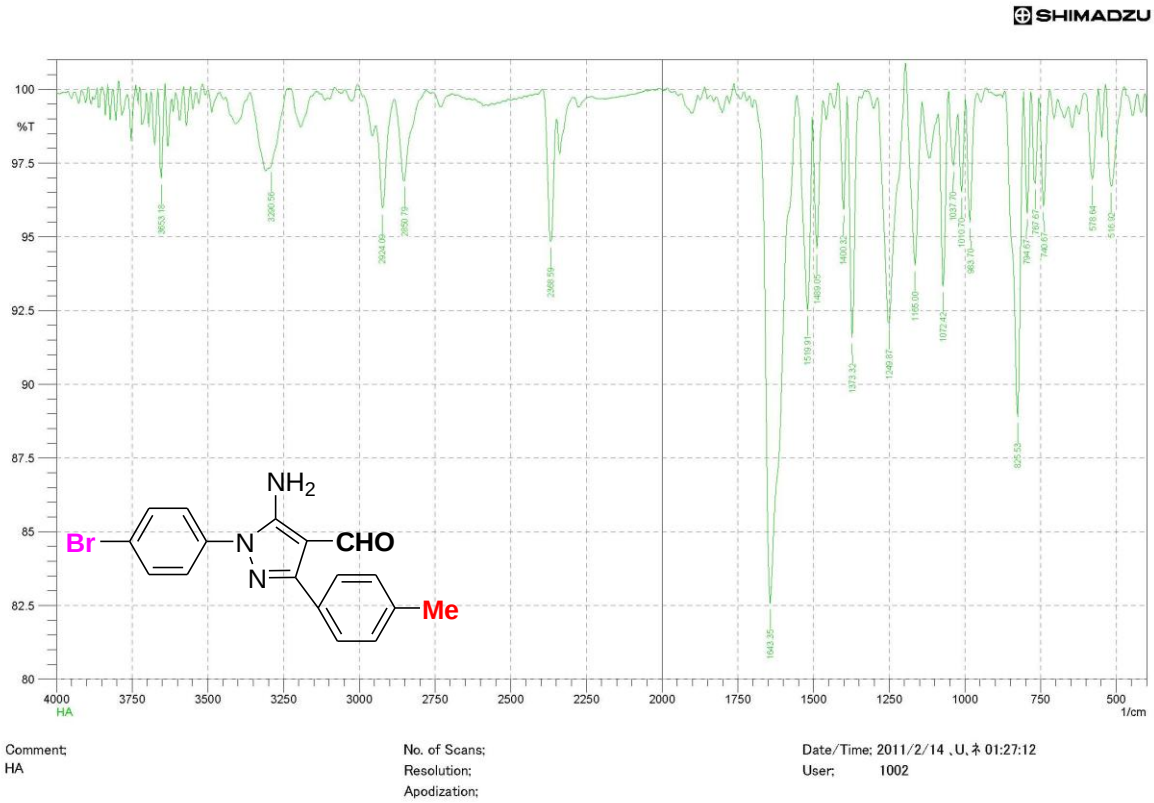

Figure 54 IR spectrum of compound **4d**

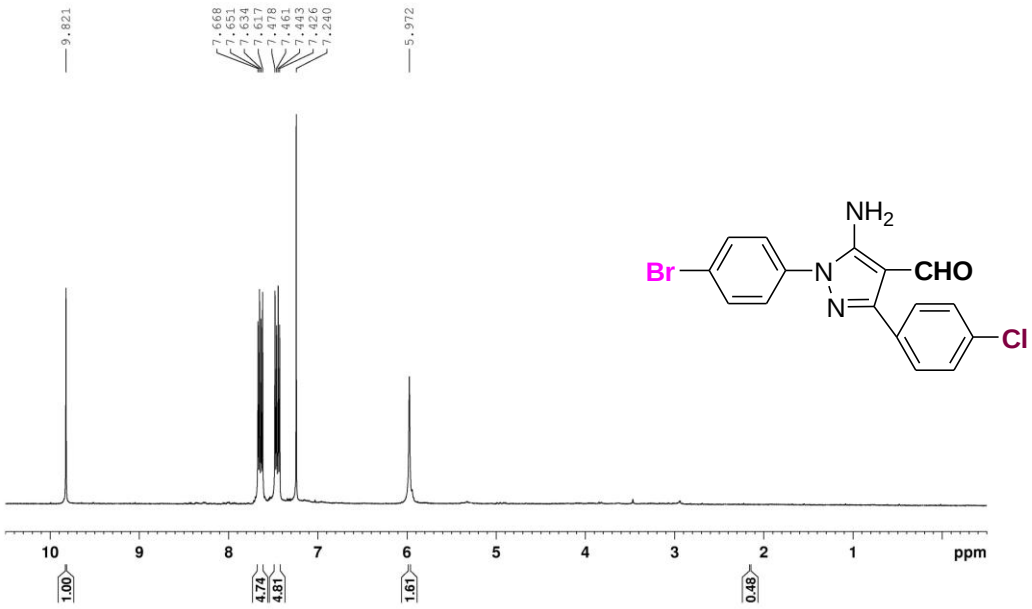

Figure 55 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of compound **4e**

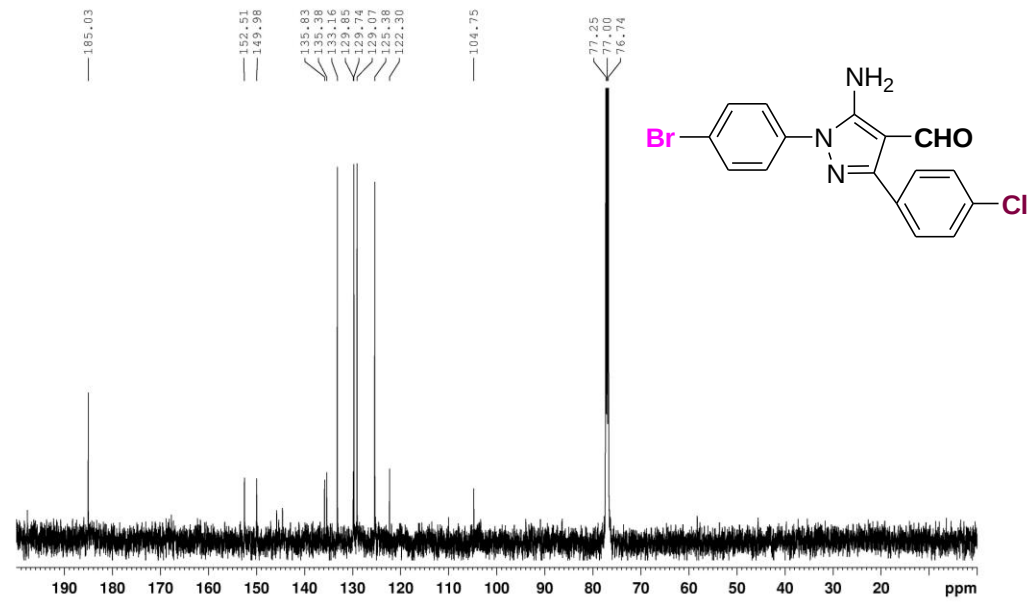

Figure 56 ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **4e**

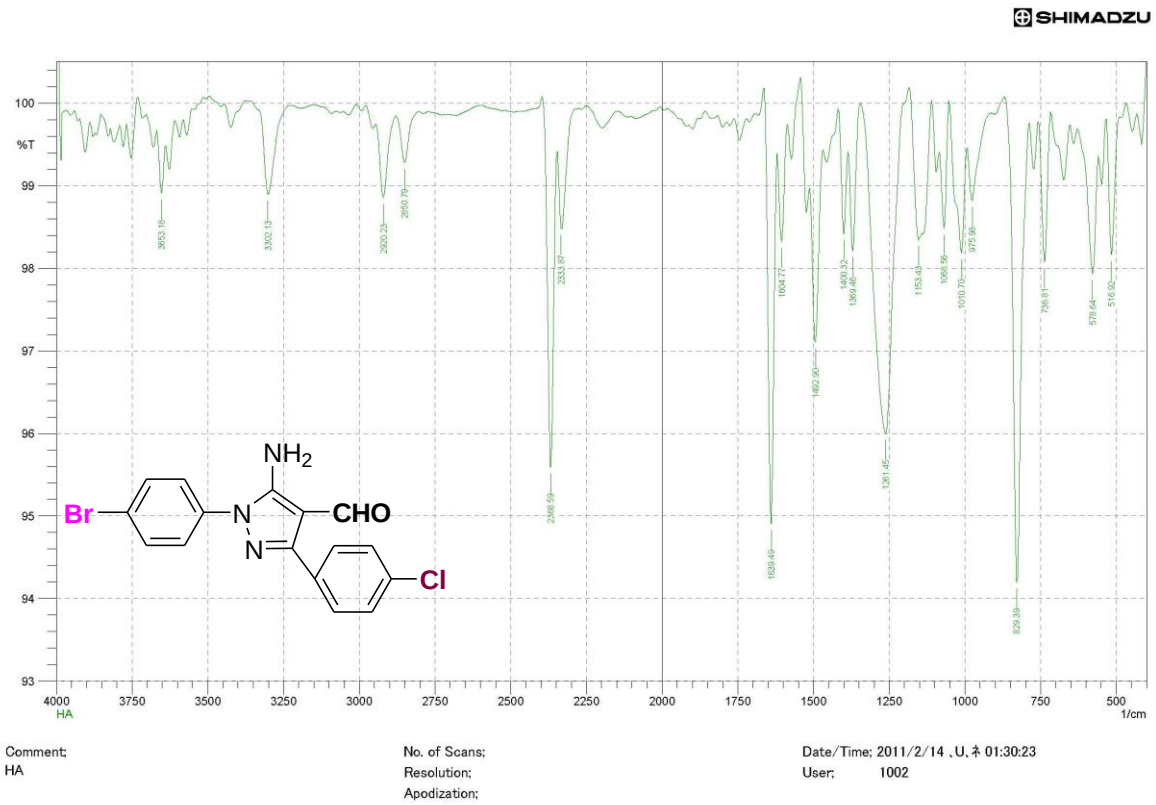

Figure 57 IR spectrum of compound **4e**