Chemoselective synthesis, antiproliferative activities and SAR study of 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines and pyrazolyl-2-azadienes

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Abstract

Chemoselective microwave-assisted amidination was successfully developed to synthesize 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines and pyrazolyl-2-azadienes. All of the starting materials and resulting products were tested against NCI-H226, NPC-TW01, and Jurkat cancer cells to evaluate their antiproliferative activities. 1*H*-Pyrazol-5-yl-*N*,*N*-dimethylformamidines **2b, 2c** and **2d** were most potent with IC_{50} values in low micromolar range. 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.

Introduction

Pyrazoles attract attentions due to their wide range of pharmacological properties (Haddad *et al*., 2004; Ramesh Kakarla *et al*. 2007). The bioactivities of functionalized *N*-arylpyrazoles are extensively studied (Elguero *et al*., 1984, 1996; Huang *et al*., 2000; Kost *et al*., 1966; Lee *et al*., 2003) and the C-5 substituted pyrazoles are also explored in the design of pharmaceuticals and agrochemical agents (Sakya *et al*., 2003). As a result, we focus to develop the new methodology for the synthesis a series of *N*-arylpyrzoles containing aminidyl group derivatives by microwave irradiation. These compounds have represented an attractive target in view of versatile biological activity (Cheng *et al*., 2010).

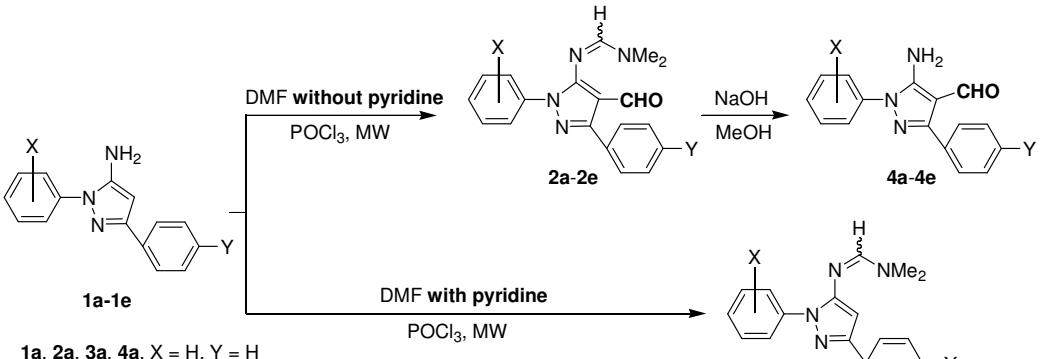
Amidinyl groups are intensively studied since they contribute to the activities of many biologically important compounds (Bielawska *et al*., 2004; Collins *et al*., 1998; Fastier *et al*., 1962; Grout R. J., 1975; Kreutzberger A, 1968; Panico *et al*., 2002; Sielecki *et al*., 2001; Sienkiewich *et al*., 2005). Except for acting as valuable pharmacophore, amidines are also important building blocks for the preparation of various heterocyclic compounds (Boyd G. V. 1991; Croce *et al*., 1997; Mason *et al*., 2001; Palacios *et al*., 2006, 2009), protecting groups for primary amines (Pocha *et al*., 1986; Rudyk *et al*., 2003), support linkers in solid phase synthesis (Furth *et al*.,1997), and auxiliaries in asymmetric synthesis (Matulenko *et al*., 1996; Meyers *et al*.,1991, 1992, 1993, 1996). The introduction of an amidinyl group into a known biological molecule is consequently of interest in the field of medicinal chemistry and demonstrates good results in several models (Lund *et al*., 1972; Oszczapowicz *et al*., 1997). Herein, we reported a new chemoselective microwave-assisted amidination method to synthesize 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines and pyrazolyl-2-azadienes by using the suitable amount of basic pyridine as the basic catalyst. The reactivity and bioactivity for the different skeletal of methnimidamides

and starting material 5-amino-1,3-disubstituted pyrazole were also explored.

Result and Discussion

Chemistry

Scheme 1 illustrates a newly developed chemoselective microwave-assisted amidination (Oszczapowicz *et al*., 1997) of 5-amino-1,3-disubstituted pyrazoles **1** to give the corresponding 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines **2** and pyrazolyl-2-azadienes **3**. A series of 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines **2b**–**2e** were designed based on our previous study (Oszczapowicz *et al*., 1997) with enhanced antiproliferative activity. 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 15–20 min. 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 1 and Scheme 1). 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 (Besan *et al*., 1980). Compounds **2a**–**2e** were fully characterized by spectroscopic methods.



Scheme 1

Table 1. The results of synthesis of methnimidamides **2a**–**2e** and 5-amino-4-formylpyrazoles **4a**–**4e**

5-Amino-1,3-*N*,*N*-disubstituted pyrazoles			Methnimidamide (**2a**–**2e**)		5-Amino-4-formylpyrazoles (**4a**–**4e**)	
S.M. (**1a**–**1e**)	X	Y	Products	Yields (%)	Products	Yields (%)
1a	H	H	**2a**	94	**4a**	92
1b	*m*-Cl	Me	**2b**	82	**4b**	85
1c	*m*-Cl	Cl	**2c**	81	**4c**	87
1d	*p*-Br	Me	**2d**	90	**4d**	83
1e	*p*-Br	Cl	**2e**	92	**4e**	96

For the investigation of the effecy 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** without 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 2). For

cesium carbonate, the methnimidamide product **3a** was obtained in 71% 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.

Table 2. 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.

When the same condition was applied to the commercially available organic bases including triethylamine (NEt_3), dimethylaminopyrium (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 2). 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 the use 3.0 equivalent of pyridine can give pyrazolyl-2-azadiene product **3a** in the best yield (97% yield, see entry 9 of Table 2). 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 3). The reliable chemoseletive 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 3). Following the aforementioned studies, the chemoseletive amidination reaction seemed determinate to the suitable amount of pyridine basic agent.

Table 3. 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

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-amidinaion, only one method was found by using HCl aqueous solution (Mason *et al*., 2001). 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 1 and Scheme 1).

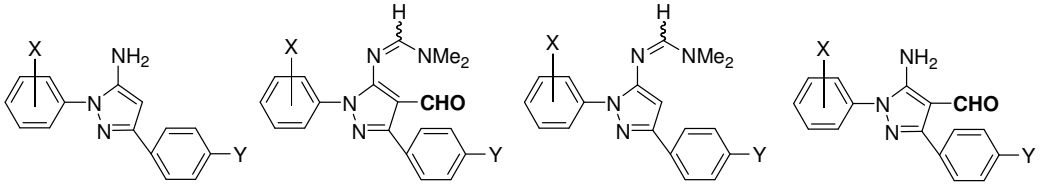
Biological evaluations

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 4].

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 4). 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 4. Antiproliferative activity of the pyrazole derivatives



Compounds	Prozoles (**1a**–**1e**, **2a**–**2e**, **3a**–**3e**, and **4a**–**4e**)		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

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
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.

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 4 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**.

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. 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. 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.

Experimental

Chemistry

All chemicals were reagent grade and use as purchased. All reactions were carried out under argon or nitrogen atmosphere and monitored by 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.

Analytical thin-layer chromatography (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.

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

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.

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, 128.4 (2 × CH), 128.5 (2 × CH), 128.8, 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), 7.51–7.55 (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, 129.2 (6 × 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) 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-dimethyl-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, 154.5, 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) 386 (100), 385 (M^+, 19), 371 (16), 357 (9), 342 (14), 330 (9), 316 (8), 220 (16), 111 (18), 83 (9); Anal. Calcd for $C_{19}H_{16}ClN_4O$; 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-dimethyl-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), 7.21–7.23 (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), 128.3, 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) 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-dimethyl-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 (2 × CH), 120.3, 125.6 (2 × CH), 128.7 (2 × CH), 130.5 (2 × CH), 131.5 (2 × CH), 135.0, 138.1, 154.5 (2 × CH), 158.9, 184.4; IR (KBr) 2364 (m), 2333 (m), 1666 (m), 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) 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, 127.6, 128.3 (2 × CH), 128.5 (3 × 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 $C_{19}H_{19}ClN_4$; C: 67.35; H: 5.65; N: 16.54, 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-dimethyl-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, 5 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, 122.3, 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) 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-dimethyl-methanimidamide (**3c**)*

mp (purified by column chromatography on silica gel) 108–110 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.93 (s, 3 H, CH_3), 3.02 (s, 3 H, CH_3), 6.03 (s, 1 H), 7.18–7.35 (m, 3 H, ArH), 7.65 (s, 1 H), 7.73–7.78 (m, 2 H, ArH), 7.77–7.82 (m, 1 H, ArH), 7.90–7.95 (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) 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-dimethyl-methanimidamide (**3d**)*

mp (purified by column chromatography on silica gel) 115–117 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.35 (s, 3 H, CH_3), 2.97 (s, 3 H, CH_3), 3.00 (s, 3 H, CH_3), 6.11 (s, 1 H), 7.16–7.20 (m, 2 H, ArH), 7.71–7.75 (m, 3 H, ArH), 7.85–7.92 (m, 2 H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.3 (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) 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-dimethyl-methanimidamide (**3e**)*

mp (purified by column chromatography on silica gel) 152–154 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.86 (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), 7.64 (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 34.5 (CH_3), 40.3 (CH_3), 88.3, 118.9, 124.6 (2 × CH), 126.8 (2 × CH), 128.6 (2 × CH), 131.3 (2 × CH), 132.4, 133.3, 139.4, 149.9, 153.0, 154.5; 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) 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), 7.39–7.72 (m, 10 H, ArH), 9.81 (s, 1 H, CHO); ^{13}C NMR (50MHz, $CDCl_3$) δ 104, 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), 6.01 (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, 64), 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; ^1H NMR ($CDCl_3$, 200 MHz) δ6.10 (s, 2 H, NH_2), 7.24–7.25 (m, 2 H, ArH), 7.41–7.42 (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, 98), 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; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.97 (s, 2 H, NH_2), 7.42–7.47 (m, 4 H, ArH), 7.61–7.66 (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) 356 (M + 1, 32), 375 (M^+, 100); 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.

Antiproliferative activity

Cell lines

Human non-small cell lung carcinoma (NCI-H226) was purchased from American Type Culture Collection (ATCC; Rockville, MD). T-cell leukemia (MT-2) 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.

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 0.4 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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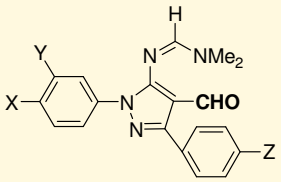
Chemoselective synthesis, antiproliferative activities and SAR study of 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines and pyrazolyl-2-azadienes



			GI_{50} (μM) for Antiproliferative activity		
X	Y	Z	NCI-H266	Jurkat	NPC-TW01
H	**Cl**	**Me**	8.9	7.8	7.2
H	**Cl**	**Cl**	9.2	7.7	7.4
Br	H	**Me**	8.2	6.7	6.0