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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 IC₅₀ 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.

Keywords Amidination · Pyrazoles · Antiproliferative activity · Microwave-assisted reaction · Methnimidamide

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Introduction

Pyrazoles attract attentions due to their wide range of pharmacological properties (Haddad *et al.*, 2004; Ramesh Kakarla and Gerritz, 2007). The bioactivities of functionalized *N*-arylpyrazoles are extensively studied (Elguero, 1984, 1996; Huang and Katzenellenbogen, 2000; Kost and Grandberg, 1966; Lee *et al.*, 2003) and the C-5 substituted pyrazoles are also explored in the design of pharmaceuticals and agrochemical agents (Sakya and Rast, 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, 1975; Kreutzberger, 1968; Panico et al., 2002; Sielecki et al., 2001; Sienkiewich et al., 2005; Stephens et al., 2001). Except for acting as valuable pharmacophore, amidines are also important building blocks for the preparation of various heterocyclic compounds (Boyd, 1991; Croce et al., 1997; Mason et al., 2001a, b; Palacios et al., 2006, 2009), protecting groups for primary amines (Pocha, 1986; Rudyk et al., 2003), support linkers in solid phase synthesis (Furth et al., 1997a, b), and auxiliaries in asymmetric synthesis (Matulenko and Meyers, 1996; Meyers et al., 1991; Meyers, 1992; Meyers and Elworthy, 1992; Meyers and Hutchings, 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 and Tybring, 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-2azadienes 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 1H-pyrazol-5-yl-N,N-dimethylformamidines 2 and pyrazolyl-2-azadienes 3. A series of 1H-pyrazol-5-yl-N,Ndimethylformamidines 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 1H-pyrazol-5-yl-N,N-dimethylformamidines 2a-2e were readily obtained in 81–94% yields (see Table 1; 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.

For the investigation of the effect of formyl group on activity, we evaluated the chemoselective microwaveassisted 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 2a. The bases included sodium hydroxide (NaOH), potassium carbonate (K₂CO₃), cesium carbonate $(CsCO_3)$, triethylamine (NEt_3) , dimethylaminopyridine (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 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.

When the same condition was applied to the commercially available organic bases including triethylamine (NEt₃), dimethylaminopyridine (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



Scheme 1 Synthesis of methnimidamides 2a-2e, pyrazolyl-2-azadienes 3a-3e, and 5-amino-4-formylpyrazoles 4a-4e

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

5-Amino-1,3- <i>N</i> , <i>N</i> - disubstituted pyrazoles			Methnimidamides (2a–2e)		5-Amino-4- formylpyrazoles (4a–4e)	
S.M. (1a–1e)	Х	Y	Products	Yields (%)	Products	Yields (%)
1a	Н	Н	2a	94	4 a	92
1b	<i>m</i> -Cl	Me	2b	82	4 b	85
1c	<i>m</i> -Cl	Cl	2c	81	4c	87
1d	<i>p</i> -Br	Me	2d	90	4d	83
1e	<i>p</i> -Br	Cl	2e	92	4e	96

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 ₂ CO 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

compounds **1a–1e** to provide the corresponding pyrazolyl-2azadiene products **3a–3e** without formyl group in 78–98% yields (see Table 3). The reliable chemoselective procedure involved the treatment of 5-amino-1,3-disubstituted pyrazoles **1a–1e** with ~1.2 equivalent of POCl₃ 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 chemoselective amidination reaction seemed determinate to the suitable amount of pyridine basic agent.

To identify the essentiality of amidinyl group for the inhibitory activity study, a series of de-amidination

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

5-Amino-1,3- <i>N</i> , <i>N</i> pyrazoles	/-disubstitute	Pyrazolyl-2-azadienes (3a–3e)		
S.M. (1a–1e)	Х	Y	Products	Yields (%)
1a	Н	Н	3a	97
1b	<i>m</i> -Cl	Me	3b	91
1c	<i>m</i> -Cl	Cl	3c	98
1d	<i>p</i> -Br	Me	3d	93
1e	<i>p</i> -Br	Cl	3e	78

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 (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 methnimidamides **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; 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 GI₅₀ 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 GI₅₀ values of **1d** are 54.3 μ M (NCI-H226), 80.2 μ M (NPC-TW01), and 45.0 μ M (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 GI₅₀ values between 7.2 and 9.2 μ M (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

Table 4 Antiproliferative activity of the pyrazole derivatives



Compounds	Pyrazoles (1a-1e, 2a	a–2e , 3a–3e , and 4a–4e)	$GI_{50} \; \left(\mu M\right)^{a,b}$	$GI_{50} (\mu M)^{a,b}$		
	X (N-1)	Y (C-3)	NCI-H226	NPC-TW01	Jurkat	
1a	Н	Н	72.2	>100	83.0	
1b	<i>m</i> -Cl	Me	63.5	>100	56.6	
1c	m-Cl	Cl	75.1	>100	>100	
1d	<i>p</i> -Br	Me	54.3	80.2	45.0	
1e	<i>p</i> -Br	Cl	58.7	64.4	61.3	
2a	Н	Н	31.4	9.3	23.5	
2b	<i>m</i> -Cl	Me	8.9	7.2	7.8	
2c	<i>m</i> -Cl	Cl	9.2	7.4	7.7	
2d	<i>p</i> -Br	Me	8.2	6.0	6.7	
2e	<i>p</i> -Br	Cl	62.9	>100	38.9	
3a	Н	Н	>100	>100	>100	
3b	<i>m</i> -Cl	Me	80.9	>100	>100	
3c	<i>m</i> -Cl	Cl	75.6	92.7	74.5	
3d	<i>p</i> -Br	Me	73.9	>100	>100	
3e	<i>p</i> -Br	Cl	59.8	60.7	84.9	
4a	Н	Н	>100	>100	87.3	
4b	<i>m</i> -Cl	Me	71.8	>100	86.3	
4c	<i>m</i> -Cl	Cl	79.7	>100	78.9	
4d	<i>p</i> -Br	Me	8.5	28.2	34.4	
4e	<i>p</i> -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 \pm SD of three independent experiments

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 GI₅₀ values between 6.0 and 8.2 μ M. 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.

For the further the structure–activity relationship investigation, pyrazolyl-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** [GI₅₀: >59.8 μ M (NCI-H226), >60.7 μ M (NPC-TW01), and >74.5 μ M (Jurkat)] and **4a–4e** [GI₅₀: >8.5 μ M (NCI-H226), >28.2 μ M (NPC-TW01), and >34.4 μ M (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 1H-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 structureactivity 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 CaH₂. Toluene (reagent grade, from Merck Chemical Co.) was dried by distillation from CaH₂ under nitrogen. 4-Methylbenzoylacetonitrile, phenylhydrazine was purchased from Acros Chemical Co. 4-Bromophenylhydrazine hydrochloride, 4-chlorobenzoylacetonitrile, and 3-chlorophenylhydrazine hydrochloride were purchased from Alfa Aesar Chemical Company. Benzoylacetonitrile was purchased from TCI. N,N-Dimethylformamide and pyridine were purchased from Scharlau Chemical Co. Phosphorylchloride was 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 or 400 MHz) spectrometer by use of CDCl₃ as solvent. Carbon-13 NMR spectra were obtained on a Bruker (75 or 100 MHz) spectrometer by used of CDCl₃ as solvent. Carbon-13 chemical shifts are referenced to the center of the CDCl₃ 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 POCl₃ (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 CH₂Cl₂ (4 × 20 ml). The organic extracts were washed with saturated NaHCO₃, dried over MgSO₄, 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-1H-pyrazol-5-yl-N,N-dimethylmethanimidamide (2a) Mp (purified by column chromatography on silica gel) 120-122°C; ¹H NMR (CDCl₃, 200 MHz) & 3.01 (s, 3 H, CH₃), 3.12 (s, 3 H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 34.3 (CH₃), 40.7 (CH₃), 108.4, 124.3 (2 \times CH), 126.8, 128.4 $(2 \times CH)$, 128.5 $(2 \times CH)$, 128.8, 129.4 $(2 \times CH)$, 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 C₁₉H₁₈N₄O; 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)-1Hpyrazol-5-yl]-N,N-dimethyl-methanimidamide (2b) Mp (purified by column chromatography on silica gel) 166–168°C; ¹H NMR (CDCl₃, 200 MHz) δ 2.40 (s, 3 H, CH₃), 3.01 (s, 3 H, CH₃), 3.11 (s, 3 H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 21.4 (CH₃), 34.4 (CH₃), 40.8 (CH₃), 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 C₂₀H₁₉ClN₄O; 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; ¹H NMR (CDCl₃, 200 MHz) δ 3.03 (s, 3 H, CH₃), 3.13 (s, 3 H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 34.5 (CH₃), 40.8 (CH₃), 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 C₁₉H₁₆ClN₄O; 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)-1Hpyrazol-5-yl]-N,N-dimethyl-methanimidamide (2d) Mp (purified by column chromatography on silica gel) 198–200°C; ¹H NMR (CDCl₃, 200 MHz) δ 2.36 (s, 3 H, CH₃), 2.98 (s, 3 H, CH₃), 3.09 (s, 3 H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 21.4 (CH₃), 34.4 (CH₃), 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 C₂₀H₁₉BrN₄O; 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; ¹H NMR (CDCl₃, 200 MHz) δ 3.01 (s, 3 H, CH₃), 3.13 (s, 3H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 34.5 (CH₃), 40.8 (CH₃), 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 C₁₉H₁₆ClN₄O; 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-2azadiene compounds (**3a–3e**)

A solution of pyrazol-5-amine derivatives (**1a–1b**, 1.0 equiv), POCl₃ (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 CH₂Cl₂ (4 × 20 ml). The organic extracts were washed with saturated NaHCO₃, dried over MgSO₄, 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*-1*H*-*pyrazol*-5-*yl*)-*N*,*N*-*dimethylmethanimidamide* (*3a*) Mp (purified by column chromatography on silica gel) 113–115°C; ¹H NMR (CDCl₃, 200 MHz) δ 2.29 (s, 3 H, CH₃), 3.01 (s, 3 H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 34.5 (CH₃), 40.2 (CH₃), 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 C₁₉H₁₉ClN₄; 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; ¹H NMR (CDCl₃, 200 MHz) δ 2.38 (s, 3 H, CH₃), 2.94 (s, 3 H, CH₃), 2.95 (s, 3 H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 21.1 (CH₃), 34.3 (CH₃), 40.0 (CH₃), 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 $C_{19}H_{19}CIN_4$; 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 (CDCl₃, 200 MHz) δ 2.93 (s, 3 H, CH₃), 3.02 (s, 3 H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 34.5 (CH₃), 40.2 (CH₃), 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 C₁₈H₁₆Cl₂N₄; 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*-*pyr*azol-5-yl]-*N*,*N*-dimethyl-methanimidamide (**3d**) Mp (purified by column chromatography on silica gel) 115–117°C; ¹H NMR (CDCl₃, 200 MHz) δ 2.35 (s, 3 H, CH₃), 2.97 (s, 3 H, CH₃), 3.00 (s, 3 H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 21.3 (CH₃), 34.5 (CH₃), 40.3 (CH₃), 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 C₁₈H₁₉BrN₄; 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 (CDCl₃, 200 MHz) δ 2.86 (s, 3 H, CH₃), 2.92 (s, 3H, CH₃), 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); ¹³C NMR (50 MHz, CDCl₃) δ 34.5 (CH₃), 40.3 (CH₃), 88.3, 118.9, 124.6 (2 × CH), 126.8 (2 × CH), 128.6 (2 × CH), 131.3 $(2 \times CH)$, 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 C₁₈H₁₆BrClN₄; 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-4formylpyrazoles (**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 NaHCO₃, dried over MgSO₄, 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; ¹H NMR (CDCl₃, 200 MHz) δ 6.13 (s, 2 H, NH₂), 7.39–7.72 (m, 10 H, ArH), 9.81 (s, 1 H, CHO); ¹³C NMR (50 MHz, CDCl₃) 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 C₁₆H₁₃N₃O; 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-carbaldehyde (**4b**) Mp (purified by column chromatography on silica gel) 147–148°C; ¹H NMR (CDCl₃, 200 MHz) δ 2.40 (s, 3 H, CH₃), 6.01 (s, 2 H, NH₂), 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); ¹³C NMR (50 MHz, CDCl₃) δ 21.4 (CH₃), 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 C₁₇H₁₄ClN₃O; 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; ¹H NMR (CDCl₃, 200 MHz) δ 6.06 (s, 2 H, NH₂), 7.37–7.48 (m, 5 H, ArH), 7.61–7.62 (m, 3 H, ArH), 9.80 (s, 1 H, CHO); ¹³C NMR (50 MHz, CDCl₃) 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 C₁₆H₁₁Cl₂N₃O; 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 (CDCl₃, 200 MHz) δ 6.10 (s, 2 H, NH₂), 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); ¹³C NMR (50 MHz, CDCl₃) δ 21.2 (CH₃), 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 C₁₇H₁₄BrN₃O; 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*)-1*H*-*pyrazole*-4-*carbaldehyde* (4e) Mp (purified by column chromatography on silica gel) 191–192°C; ¹H NMR (CDCl₃, 200 MHz) δ 5.97 (s, 2 H, NH₂), 7.42–7.47 (m, 4 H, ArH), 7.61–7.66 (m, 4 H, ArH), 9.82 (s, 1 H, CHO); ¹³C NMR (50 MHz, CDCl₃) 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 C₁₆H₁₁BrClN₃O; 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% CO₂/95% 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 (GI₅₀).

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