Synthesis and antiproliferative evaluation of *N*,*N*-disubstituted-*N*'-[1-aryl-1*H*-pyrazol-5-yl]-methnimidamides

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ABSTRACT

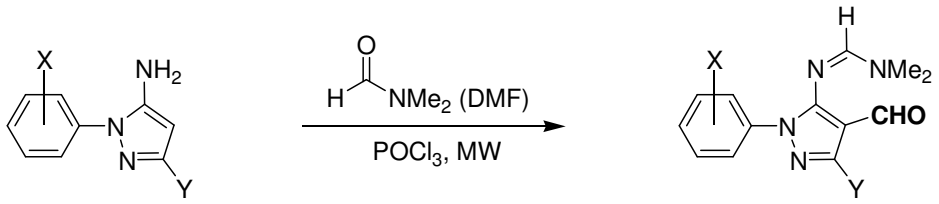
A series of *N*,*N*-disubstituted-*N*'-[1-aryl-1*H*-pyrazol-5-yl]-methnimidamides was synthesized by a newly developed microwave reaction and their antiproliferative activities were evaluated. Microwave irradiation of 5-amino-1,3-disubstituted pyrazoles with various amide solvents in the presence of $POCl_3$ provided the corresponding **2a**–**2k**, **3a**–**3c**, and **4a**–**4f** in good to excellent yields. The obatnied methnimidamides were tested against NCI-H661, NPC-TW01, and Jurkat cancer cell lines and the results indicated that compounds **2d** and **2e** were the most potent with IC_{50} values in low micromolar range.

Amidinyl groups are intensively studied since they contribute to the activities of many biologically important compounds including anticancer,[1-2] anti-degenerative,[3] anti-platelet,[4] antimicrobial,[5] antibacterial, antiprotozoal, anti-tumour drugs,[6-8] serine protease inhibitors,[5] and nitric oxide synthase inhibitors.[9] 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. The grafting of the aminidyl group is found to enhance the antibacterial activity of penicillin,[10-12] and improve the therapeutic window of anthracyclines.[13] Except for acting as valuable pharmacophore, amidines are also important building blocks for the preparation of various heterocyclic compounds,[14] protecting groups for primary amines,[15] support linkers in solid phase synthesis,[16] and auxiliaries in asymmetric synthesis.[17]

Pyrazoles are among the important scaffolds possessing various biological activities. The bioactivity of functionalized *N*-arylpyrazole were extensively studied[19-21] and the C-5 substituted pyrazoles are also exploited in the design of pharmaceuticals and agrochemical agents.[20] *N*,*N*-Dimethyl-*N*'-[1-aryl-3-phenyl-1*H*-pyrazol-5-yl]-methnimidamide derivatives are C-5 substituted *N*-arypyrazoles extensively utilized precursors to construct the heterocyclic rings, such as fused pyrazolo[3,4-*b*]pyridines, pyrazolo[3,4-*b*]pyridones, and pyrazolo[3,4-*d*]pyrimidines.[18] In this paper, we reported an efficient synthesis for the introduction of a amindinyl group into the C-5 position of *N*-arylpyrzoles by use of commercially available amide solvents and $POCl_3$[22] to provide the compound. The antiproliferative activities of the methnimidamide derivatives were explored on cancer cells and the structure-activity relationship was established.

Scheme 1 illustrates the amidination of 5-amino-1,3-disubstituted pyrazoles **1a**–**1k** to the corresponding **1a**–**1k** and the optimization of the reaction. A model

procedure involved the treatment of 5-amino-1,3-disubstituted pyrazoles **1a** with a catalytic amount of $POCl_3$ (~1.2 equivalent) in DMF at 30–40 °C with 100 W of microwave energy within 10–15 min. After work-up and purified by column chromatography on silica gel, the corresponding amidination product **2a** was obtained in 94% yields (see Table 1).In addition to generating the amidinyl group on the primary amine, the formylation also took place on the C-4 position of pyrazolic ring.[22]



Scheme 1

Table 1. The results of the amidination of 5-amino-1,3-*N*,*N*-disubstituted pyrazoles with DMF

5-Amino-1,3-*N*,*N*-disubstituted pyrazoles			Methnimidamides (**2a**–**2k**)	
S.M. (**1a**–**1k**)	X	Y	Products	Yields (%)
1a	H	Ph	**2a**	94
1b	*o*-Cl	Ph	**2b**	82
1c	*m*-Me	Ph	**2c**	81
1d	*m*-Cl	Ph	**2d**	90
1e	*m*-NO_2	Ph	**2e**	86
1f	*p*- Br	Ph	**2f**	83
1g	*p*- OMe	Ph	**2g**	92
1h	H	*p*-Me-Ph	**2h**	97
1i	H	*p*-Cl-Ph	**2i**	91
1j	H	*p*-OMe-Ph	**2j**	95

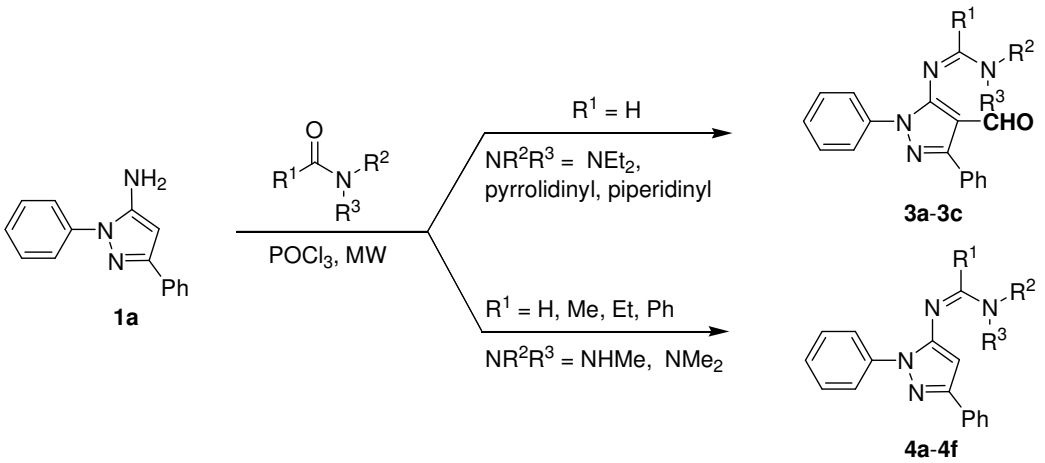
1k	H	*t*-Butyl	**2k**	77

The synthetic strategy was applicable to 5-amino-1-aryl-3-phenylpyrazoles **1b**–**1g** bearing an electron withdrawing or electron donating group on phenyl ring at the N-1 position, such as *o*-Cl, *m*-Me, *m*-Cl, *p*-Br, *p*-OMe, and *m*-NO_2. The reaction provided the corresponding **2b**–**2g** in good to excellent yields (81–94% yields, see Table 1). Compounds **2a**–**2g** were fully characterized by spectroscopic method. For example, compound **2a** presented a peak at δ 8.69 ppm for N=C–C^1H(NMe_2) and a peak at δ 9.69 ppm for O=C–^{1}H in the proton NMR spectrum. In ^{13}C NMR spectrum, compound **2a** possessed characterization absorptions at δ 185.1 ppm for aldehyde carbon O=^{13}C and at δ 159.0 ppm for imine carbon N=^{13}C–NMe_2. Its IR spectrum showed absorption at 1671 cm^{-1} for –C=O stretching and 1658 cm^{-1} for –C=N stretching.

To realize the effect of the substitutent on the pyrazole ring for the reaction, 5-amino-1-phenyl-3-substituted pyrazoles **1h**–**1k** containing the *t*-butyl, *p*-Me-Ph, *p*-Cl-Ph, or *p*-OMe-Ph groups at the C-3 position of the pyrazolic ring were served as the starting material for studying. The corresponding desired products **2h**–**2k** were produced in 77–97% yields. As a result, this efficient microwave-assisted amidination method can be successfully applied to synthesize a series of 1,3-disubstituted-methnimidamides **2a**–**2k**.

To investigate the reactivity of the amide solvents, 5-amino-1-3-diphenylpyrazole (**1a**) was used as model to react with various amide solvents, including *N*,*N*-diethylformamide (DEF), 1-pyrrolidinecarboxaldehyde and 1-piperidinecarboxaldehyde under the same microwave-assisted condition (see Scheme 2). The corresponding products **3a**–**3c** were produced in 91–96% yields (see the entry 1–3 of Table 2). Extension of this strategy to 5-amino-1-3-diphenylpyrazole (**1a**) with a series of amide solvents including *N*-methylacetamide,

N-methylformamide, *N*-methylpropanamide, *N*,*N*-dimethylacetamide, *N*,*N*-dimethylformamide, and *N*,*N*-dimethylbenzamide, however, provided the unexpected amidination products **4a**–**4f** in good to excellent yields without forming a formyl group (86–97%, see the entry 4–12 of Table 2). The yielding formyl amidinylation pyrazole products seemed determinate to the dissociation of the amide solvents.[23-25]



Scheme 2

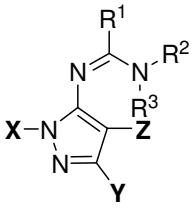
Table 2. The results of the amidination of 5-amino-1,3-*N*,*N*-diphenylpyrazoles (**1a**) with amide solvents

Entry	Substrates	Amide solvents $R^1C(=O)NR^2R^3$			Methnimidamides (**3a**–**3c** and **4a**–**4f**)	
		R^1	R^2	R^3	Products	Yields (%)
1	**1a**	H	Et	Et	**3a**	91
2	**1a**	H	Pyrrolidinyl		**3b**	96
3	**1a**	H	Piperidinyl		**3c**	92
4	**1a**	H	H	Me	**4a**	86
8	**1a**	Me	H	Me	**4b**	94
9	**1a**	Et	H	Me	**4c**	93

10	**1a**	Me	Me	Me	**4d**	90
11	**1a**	Ph	Me	Me	**4e**	97
12	**1a**	Et	Me	Me	**4f**	95

The growth inhibitory activity of all methnimidamide compounds is evaluated against a panel of human cancer cell lines, including lung carcinoma (NCI-H661), 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. Compound **2a** was selected as the compared model for the inhibitory activity study. The GI_{50} values of **2a** are 31.4 μM (NCI-H661), 9.3 μM (NPC-TW01), and 23.5 μM (Jurkat), respectively. For compounds **2b**–**2g** containing various *N*-1 substituted groups, **2d** and **2f** with *m*-Cl-Ph and *p*-Br-Ph groups possessed the best inhibitory activity against the three cancer cell lines with GI_{50} values between 6.4 μM and 8.3 μM. The results also demonstrated they were more active against NPC-TW01 and NCI-H661 than Jurkat. Among of methnimidamide derivatives **2h**–**2k** with *p*-Me-Ph, *p*-Cl-Ph, *p*-OMe-Ph or *t*-butyl groups at C-3 position on the pyrazolic ring, same tendency was found. The antiproliferative activities of **2h** and **2i** were more better than **2a,** but similar to that of **2d** and **2f.**

Table 3. Antiproliferative activity of the methnimidamide derivatives



Comp-ounds	Methnimidamides (**2a**–**2k**, **3a**–**3c**, and **4a**–**4e**)						GI$_{50}$ (μM)[a,b]		
	X	Y	Z	R^{1}C(=N)NR^{2}R^3			NCI-H661	NPC-TW01	Jurkat
				R^1	R^2	R^3			

2a	Ph	Ph	CHO	H	Me	Me	31.4	9.3	23.5
2b	*o*-Cl-Ph	Ph	CHO	H	Me	Me	31.4	23.3	26.3
2c	*m*-Me-Ph	Ph	CHO	H	Me	Me	31.0	8.9	9.7
2d	*m*-Cl-Ph	Ph	CHO	H	Me	Me	6.9	6.4	8.3
2e	*m*-NO_2-Ph	Ph	CHO	H	Me	Me	>50	36.7	>50
2f	*p*-Br-Ph	Ph	CHO	H	Me	Me	6.7	7.4	7.3
2g	*p*-OMe-Ph	Ph	CHO	H	Me	Me	33.4	20.2	19.7
2h	Ph	*p*-Me-Ph	CHO	H	Me	Me	11.9	9.7	9.5
2i	Ph	*p*-Cl-Ph	CHO	H	Me	Me	8.6	8.1	7.9
2j	Ph	*p*-OMe-Ph	CHO	H	Me	Me	9.9	27.2	12.5
2k	Ph	*t*-Bu	CHO	H	Me	Me	42.8	>50	43.5
3a	Ph	Ph	CHO	H	Et	Et	28.8	30.5	30.0
3b	Ph	Ph	CHO	H	Pyrrolidinyl		17.6	>50	31.3
3c	Ph	Ph	CHO	H	Piperidinyl		17.7	24.4	29.0
4a	Ph	Ph	H	H	H	Me	>50	>50	>50
4b	Ph	Ph	H	Me	H	Me	>50	>50	>50
4c	Ph	Ph	H	Et	H	Me	>50	>50	>50
4d	Ph	Ph	H	Me	Me	Me	35.1	> 50	> 50
4e	Ph	Ph	H	Ph	Me	Me	46.7	20.8	36.1

[a]NCI-H661: 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 further investigation of the structure activity relationship, the antiproliferative activities of the modified amidinyl compounds **3a**–**3c** were tested. Compound **3c** was more potent [GI_{50}: 17.7 μM (NCI-H661), 24.4 μM (NPC-TW01), and 29.0 μM (Jurkat)] than compounds **3a** and **3b**, due to the flexible six-membered piperidinyl ring which may promote the activity. In comparison with model compound **2a** (R^2, R^3 = methyl) and **3a**–**3c** (R^2, R^3 = ethyl, piperidinyl, or pyrrolidinyl), the bulky groups on the amidinyl moiety may not favor to reach the blocking side. As a result, the antiproliferative activity of compounds **3a**–**3c** was less potent than the model compound **2a**. On the other hand, compounds **4a**–**4e** without the formyl group at C-4 position on the pyrazolic ring showed poor activity on NCI-H661, NPC-TW01, and Jurkatcells with GI_{50} values greater than 50 μM. They were not regarded as target for the future investigation. From the results in Table 3, we believed the formyl group at C-4 position in pyrazolic ring is necessary for the inhibitory activity. Furthermore, the data indicated that tendency for sensitivity is nasopharyngeal (NPC-TW01) > T-cell leukemia (Jurkat) cell > lung carcinoma (NCI-H661) for methnimidamide compounds **2a**–**2k**, **3a**–**3c**, and **4a**–**4e**.

In conclusion, we have developed a newly microwave-assisted amidination method to prepare a series of methnimidamide compounds by using 5-amino-1,3-disubstituted pyrazoles, amide solvents and $POCl_3$. Based on the growth inhibitory activity data, compounds **2d** and **2e** with *m*-Cl-Ph and *p*-Br-Ph groups at *N*-1 position and **2h** and **2i** with *p*-Me-Ph and *p*-Cl-Ph groups at C-3 position in pyrazolic ring possessed the most potent activity. For the structure activity relationship study, the formyl group at C-4 position in the core pyrazolic ring is necessary for the inhibitory activity.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:

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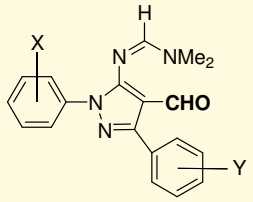

X	Y	GI_{50} (μM) for Antiproliferative activity		
		NCI-H661	NPC-TW01	Jurkat
m-Cl	H	6.9	6.4	8.3
p-Br	H	6.7	7.4	7.3
H	*p*-Me	11.9	9.7	9.5
H	*p*-Cl	8.6	8.1	7.9