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Synthesis and preliminary biological evaluation of novel pyrazolo[1,5-*a*]pyrazin-4(5*H*)-one derivatives as potential agents against A549 lung cancer cells

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ABSTRACT

A series of novel pyrazolo[1,5-*a*]pyrazin-4(5*H*)-one derivatives were synthesized by the reaction of ethyl 3-aryl-1-(2-bromoethyl)-1*H*-pyrazole-5-carboxylate and amine in the general heating condition and microwave-assisted condition. The structures of the compounds were determined by IR, ^{1}H NMR and mass spectroscopy, in addition, representative single-crystal structures were characterized by using X-ray diffraction analysis. Preliminary biological evaluation showed that the compounds could inhibit the growth of A549 cells in dosage- and time-dependent manners. The study on structure-activity relationships showed that compounds with 4-chlorophenyl group at pyrazole moiety, such as 5-benzyl-2-(4-chlorophenyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (**3o**) had much more inhibitory effects. Compound **3o** was the most effective small molecule in inhibiting A549 cell growth and might perform its action through modulating autophagy.

1. Introduction

Lung cancer is one of the leading causes of death worldwide.[1] Our understanding of the biology of cancer has undoubtedly improved in the last decade. One characteristic of cancer cells is their highly proliferative nature. Consequently, inhibition of proliferative pathways is considered an effective strategy to fight cancer and much attention has recently been paid to the discovery and development of new, more selective anticancer agents.[2–4]

Many pyrazole derivatives are known to exhibit a wide range of biological properties such as cannabinoid hCB1 and hCB2 receptor, anti-inflammatory, inhibitors of p38 Kinase, CB1 receptor antagonists, antimicrobial activity.[5–9] Extensive studies have been devoted to arylpyrazole derivatives such as Celecoxib, a well-known cyclooxygenase-2 inhibitor.[10–12] The incorporation of heterocyclic rings into prospective pharmaceutical candidates is a major strategy to obtain activity and safety advantages. As a consequence, much attention has been paid to the design and synthesis of fused-pyrazole derivatives.[13–17] However, a search of the literature revealed very few reports concerning pyrazolo-pyrazinones.[18–20] In our previous papers, we synthesized a series of novel fused-pyrazole derivatives including 6-(aroxymethyl)-2-aryl-6,7-dihydropyrazolo[5,1-*c*][1,4]oxazin-4-one derivatives and 5-alkyl-2-ferrocenyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one.[21,22] The evaluation of biological activity showed that these compounds can inhibit A549 lung cancer cell growth. To extend the diversity of fused-pyrazole skeleton and screen anticancer agents, the modification of structure is needed.

Microwave-assisted chemistry has blossomed into a useful technique for a variety of applications in organic synthesis. There are some excellent reviews and reports on the broad use of microwave irradiation in organic synthesis. It has been demonstrated that the use of microwave heating can dramatically cut down reaction time, increase product purity and yields, and allow precise control of reaction conditions, all of which make it suited to meet the increased demands of high throughput chemistry.[23–30] However, reports concerning microwave-assisted rapid synthesis of pyrazole-fused pyrazinone derivatives has not been reported.

Herein, we would like to report the microwave-assisted synthesis, structural characterization and preliminary biological evaluation of novel pyrazole-fused pyrazinone derivatives.

2. Results and discussion

2.1. Chemistry

The synthesis of pyrazolo[1,5-*a*]pyrazin-4(5*H*)-one derivatives **3** has been accomplished as outlined in Scheme 1 starting from

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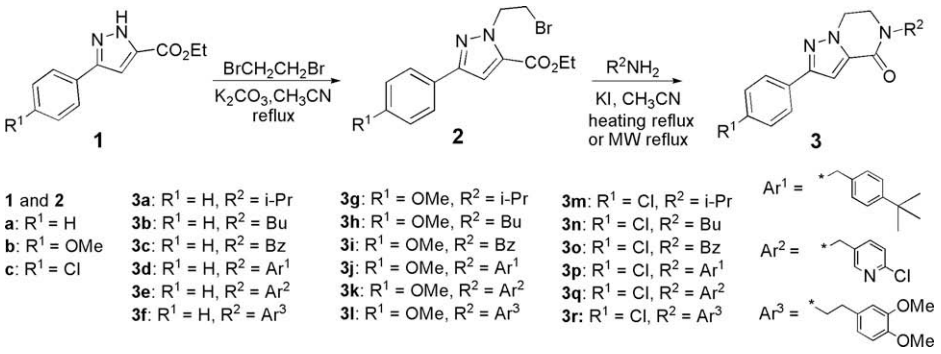

Scheme 1. Synthesis of pyrazolo[1,5-*a*]pyrazin-4(5*H*)-one derivatives.

ethyl 3-aryl-1-(2-bromoethyl)-1*H*-pyrazole-5-carboxylate **2** and amine. Firstly, the N-alkylation reaction of ethyl 3-aryl-1*H*-pyrazole-5-carboxylate **1** with excess 1,2-dibromoethane was achieved in the presence of potassium carbonate as the base in acetonitrile. After flash chromatography on silica gel, the ethyl 3-aryl-1-(2-bromoethyl)-1*H*-pyrazole-5-carboxylate **2** and the isomer, ethyl 5-aryl-1-(2-bromoethyl)-1*H*-pyrazole-3-carboxylate were obtained in 85 and 13% yields, respectively. The isomers can be easily distinguished by comparing the chemical shift in ^{1}H NMR spectra as described in our previous paper.[22] Thus, for example, 5-butyl-2-phenyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (**3b**) was synthesized in 78.1% yield by the reaction of ethyl 1-(2-bromoethyl)-3-phenyl-1*H*-pyrazole-5-carboxylate (**2a)** with butylamine in acetonitrile over a 5 h reflux period. The structures of pyrazolo[1,5-*a*]pyrazin-4(5*H*)-one **3** were determined by IR, ^{1}H NMR and mass spectroscopy. For example, 5-((6-chloropyridin-3-yl)-methyl)-2-(4-methoxyphenyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (**3k**), obtained in 67.3% yield as white crystal, gave a [M]-ion peak at m/z 368.8 in the ESI-MS, in accord with the molecular formula $C_{19}H_{17}ClN_4O_2$. In the IR spectra, the carbonyl group absorption was observed in the 1659 cm^{-1}. The ^{1}H NMR spectra indicated the chemical shift of the protons in methylene at δ = 4.75 (s, CH_2) and methyl at δ = 3.84 (s, CH_3). Two methylene protons in the pyrazine moiety appeared at 3.71 and 4.39 as triplet peaks (J = 6.2 Hz). A proton signal in pyrazole moiety appeared at 7.11 as singlet peak. Two *ortho*- aromatic protons signals in 4-methoxybenzene moiety appeared at the range of δ = 6.95 and 7.72 ppm as doublet peaks (J = 8.7 Hz), respectively. The signals of three protons in pyridine appeared at δ = 8.38 (d, J = 2.1 Hz), 7.71 (dd, J = 2.1, 8.2 Hz) and 7.34 (d, J = 8.2 Hz), respectively.

We focused our attention on the microwave-assisted synthesis technique after obtaining compounds **3** by classical heating method. These reactions are performed in a modified domestic microwave oven due to its low cost and ready availability. In a typical experiment, ethyl 3-aryl-1-(2-bromoethyl)-1*H*-pyrazole-5-carboxylate **2** and amine were mixed in acetonitrile and added in a flask with a condenser, and irradiated under refluxing condition for 0.8–5.7 h. After work-up, desired compounds were obtained. Comparing two methods, microwave-assisted synthesis technique dramatically cut down reaction time, increase product yields as shown in Table 1. The yields of compounds **3** were depended on the structure of reagent amine regardless of classical heating or microwave-assisted. From the Table 1 we can find that the more the steric hindrance of amine was, the lower the yield of compounds **3**. For example, in the case of **3a**, **3g** and **3m**, because the steric hindrance of isopropylamine is larger than *n*-butylamine, the yields of **3a**, **3g** and **3m** are 24.7, 22.8 and 36.6%, respectively, which are less than the yields of **3b**, **3h** and **3n** (78.1, 84.3 and 64.0**%**) in the classical heating condition. In the microwave-assisted condition, we did not obtained **3a**, **3g** and **3m** in good yields as other compounds.

Table 1
The yields of compounds **3** in the condition of classical heating and microwave-assisted.

Entry	Compounds	Classical heating		Microwave-assisted	
		Time (h)	Yields (%)	Time (h)	Yields (%)
1	**3a**	7	24.7	—	—
2	**3b**	5	78.1	2.5	75.8
3	**3c**	8	84.2	1.3	88.5
4	**3d**	9	64.0	3.2	54.3
5	**3e**	15	43.1	5.7	59.1
6	**3f**	10	74.3	0.8	94.4
7	**3g**	7	22.8	—	—
8	**3h**	7	84.3	2.7	80.9
9	**3i**	7	87.1	1.2	84.8
10	**3j**	8	68.7	3.2	67.6
11	**3k**	17	67.0	4	70.6
12	**3l**	4	68.2	1.2	71.3
13	**3m**	6	36.6	—	—
14	**3n**	5	64.0	2.4	66.7
15	**3o**	8	70.2	1.7	88.5
16	**3p**	9	54.8	3	83.1
17	**3q**	17	55.2	5	59.1
18	**3r**	3	56.6	1.2	86.4

In the case of the amine with electron-donating group, such as benzylamine (entry 3, 9 and 15) as well as 3,4-dimethoxyphenylethylamine (entry 6, 12 and 18), products **3** could be obtained in shorter time and satisfactory yields. On the other hand, in the case of amine with electron-withdrawing group, such as (6-chloropyridin-3-yl)methanamine (entry 5, 11 and 17), the reaction time were longer and the yields were lower in the both classical heating condition and microwave-assisted condition.

2.2. Single-crystal structural characterization by X-ray

The spatial structures of compounds **3k** and **3l** were determined by using X-ray diffraction analysis. The single crystals were grown from ethyl acetate at room temperature. The molecular views of **3k** and **3l** are shown in Figures 1 and 2. Crystal data and structure refinement for **3k** and **3l** are shown in Table 2.

The molecule of **3k** (Fig. 1) consists of four fragments, a planar pyrazole ring, aryl ring bonded to pyrazole, pyrazinone ring and arylalkyl group. An optimal electronic overlap of the π-system demands a coplanar arrangement. Indeed, the pyrazole ring and pyrazinone ring are approximately coplanar besides C7, which distance to the plane is 0.575 Å. The coplanar makes dihedral angles of 3.27(8)° and 73.65(8)° with the phenyl and pyridine rings, respectively, while that between the phenyl and pyridine rings is 76.91(9)°. In compound **3l** (Fig. 2), which is a close analogue of **3k**, the pyrazole ring and pyrazinone ring are approximately coplanar besides C12, which distance to the plane is 0.538 Å. The coplanar makes dihedral angles of 12.85(11)° and 88.23(10)° with