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Microwave-assisted rapid synthesis of sugar-based pyrazole derivatives with anticancer activity in water

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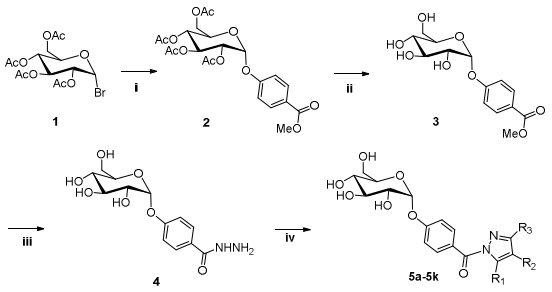
A rapid, efficient and green method has been developed for the synthesis of some novel sugar-based pyrazole derivatives in eco-friendly water under microwave irradiation in good yields. Most of these new compounds displayed good antitumor activity.

As an important class of five-membered nitrogen-containing heterocycles, pyrazole derivatives have exhibited wide range of biological activities, including antiviral,[1] anticonvulsant,[2, 3] and anti-inflammatory [4-6]. Furthermore, many new compounds containing pyrazole moieties with good anticancer activities were synthesized and reported in recent years[7, 8]. However, the bad water solubility and huge toxicity to normal cells compromised its application in the drug discovery. On the other hand, D-glucose has been used as the important sugar moiety in modifying of compounds with potential biological activity[9, 10]. It is not only because it is non-toxic to the human body and can transform into nutrition and energy, but it also can improve the absorbing capacity and bioavailability of some drug molecules.[11-14] Based on the advantages above, the synthesis of sugar-modified pyrazole derivatives have draw more attention from researchers and many new strategies have been developed.[15, 16] And some sugar–pyrazole hybrids with good anticancer activity were reported recently. [17, 18] It provided inspiration for us to develop more convenient and quickly method for novel sugar-based pyrazole derivatives with potential biological activity.

The widely accepted synthetic way for acyl pyrazoles was the coupling of an appropriate hydrazide with corresponding 2, 4-pentanedione analogues. However, the reaction should proceed in the presence of hydrochloric acid, with poor yield and long time.[19] In the past decades, microwave irritation has been chosen as an more effectively method to obtained pyrazole derivatives. Compared with conventional heating, microwave irritation method displayed significant advantages in shorting reaction time, increasing product yields and purities.[20-22] Furthermore, selecting water as the clean and green solvent has drawn widespread concern from researchers.[23-26] In continuation of our interest in the synthesis of novel sugar-based molecules with potential anticancer activity, herein we have reported a new strategy to achieve the sugar-based pyrazole derivatives with good yields under microwave irradiation in water.

The nitrogen-containing precursor of glucose: sugar-based phenyl hydrazide (**4**) was synthesized in our previous work.[27] Compound **4** was treated with various 2, 4-pentanedione analogues under microwave irradiation in water for about fifteen minutes. Then a series of sugar-based pyrazole derivatives **5a-5k** were obtained in good yields (**Scheme 1**).



Scheme 1. General procedure for the preparation of sugar-based pyrazole derivatives **5a-5k**. Reagents and conditions: (i) K_2CO_3/KI, methyl p-hydroxybenzoate, anhydrous acetonitrile, 55℃, 5h; (ii) sodium methoxide, reflux, 2h; (iii) 98% $NH_2NH_2{\cdot}H_2O$, methanol, reflux, 12 h; (iv) 2,4-pentanedione analogues, microwave irradiation, water, 100℃, 10min.

The reaction solvent was optimized as shown in **table 1**. Based on the reaction of compound **4** with 2, 4-pentandione, the commonly used solvent such as ethanol, methanol, THF, DMF, water and mixed solvent were chosen. Interestingly, the reaction using water as the solvent resulted in the corresponding compound in yield of 88% (**Table 1, Entry 2**).

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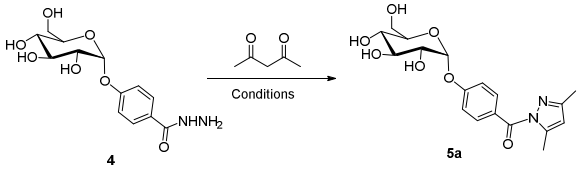
Furthermore, compared with the traditional heating mode, microwave irritation can effectively improve the yield and shorten the reaction time (**Table 1，Entry 1 and 2**). Then the factors which affecting the yield were investigated. The solubility of compound **4** in CH_3OH, CH_3CH_2OH, THF, DMF and H_2O was conducted. The results displayed that compound 4 is very soluble in water with 400mg dissolving in 10mL of water at 20 ℃. However, the solubility of compound **4** in CH_3OH, CH_3CH_2OH or THF is relative bad, except in DMF. Based on the experiments and reported articles [28, 29], the water played an important role in enhancing the yield as the reaction medium at absence of acidic reagent.

Table 1. Optimization of solvent [a]



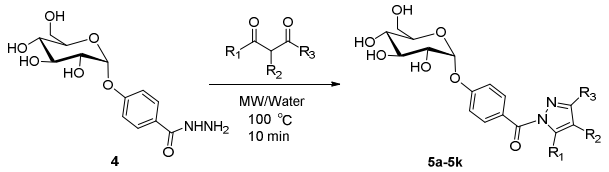
Entry	Solvent	Condition	Time	Yield（%） [b]
1	H_2O	reflux	5h	0
2	H_2O	MW	15min	88
3	CH_3OH	MW	30min	trace
4	CH_3CH_2OH	MW	30min	trace
5	THF	MW	30min	trace
6	H_2O+ CH_3OH(1:1)	MW	30min	22
7	H_2O+ CH_3OH(1:1)	MW	30min	31
8	DMF	MW	30min	42

[a] Reaction conditions: **4** (0.5mmol), 2, 4-pentandione (0.75mmol), solvent (10 mL), UWave-1000, open reaction vessel, temperature-time control (500 W), 100 ℃ [b] isolated yield

With the optimized reaction conditions in hand, some target compounds **5a-b**, **5e-f** and **5i-5k** were obtained in yields of 71-88%. However, the yields of compounds **5c-d** and **5g-h** were relatively lower because of the poor chemoselectivity as shown in the ^{1}H NMR spectrum. Furthermore, the great differences of yields also were observed in the reactants with different substituent groups. When the substituent groups were changed by aromatic hydrocarbon, the products can hardly be detected(**Table 2，5l and 5n**). We supposed that steric has a great impact on the yield of the reaction. In addition, when selecting an electron-withdrawing substituent group, such as trifluoromethyl, the desired product can't be detected. (**Table 2，5m**).

All of the new compounds were characterized through means of ^{1}H NMR, ^{13}C NMR, HRMS, IR spectra. The IR spectra of pyrazoles showed characteristic absorption peaks around 1690 (C=O) and 1580 cm^{-1} (C=N). The ^{1}H NMR of compound **1** was shown in the supporting information, conformed the alpha acetobromoglucose. Furthermore, coupling constants (J values) for H-1 of glycosyl part are around 4.0 Hz, indicated that alpha linkage due to twice configurations with the KI as the catalyst. In the ^{1}H NMR spectrum of compounds **5a-d**, **5g-h** and **5k**, the presence of sugar-based pyrazole derivatives were confirmed from the appearance of a sharp singlet at δ 6.27-6.35 ppm which corresponds to the methine proton of the target pyrazole ring. Moreover, the ^{13}C spectra of compounds **5a-k** also confirmed the presence of pyrazole ring with the carbon signal of the pyrazole ring at δ 145-160 ppm and δ 111 ppm.

Table 2. Synthesis of sugar-based pyrazole derivatives[a]



Entry	R_1	R_2	R_3	Yield (%)[b]
5a	CH_3	H	CH_3	88
5b	CH_2CH_3	H	CH_2CH_3	86
5c/5d	CH_3 /CH_2CH_3	H	CH_2CH_3 / CH_3	57/19[b]
5e	CH_3	CH_3	CH_3	85
5f	CH_3	Cl	CH_3	80
5g/5h	CH_3/ $CH_2CH(CH_3)_2$	H	$CH_2CH(CH_3)_2$ / CH_3	56/14[c]
5i	CH_3	$(CH_2)_3CH_3$	CH_3	74
5j	CH_3	CH_2CH_3	CH_3	82
5k	$CH(CH_3)_2$	H	$CH(CH_3)_2$	71
5l	CH_3	H	C_6H_5	0
5m	CH_3	H	CHF_3	0
5n	C_6H_5	H	C_6H_5	0

[a] Reaction condition: **4** (0.5mmol), 2, 4-pentandione (0.75mmol), H_2O (10 mL), UWave-1000, open reaction vessel, temperature-time control(500 W), 100 ℃10min. [b] isolated yield. [c] NMR yield

All the synthesized sugar-based pyrazole derivatives were evaluated for their cytotoxicity against HepG2 cells, A549 cells and normal cells (RTE) using MTS proliferation assay method (**Table 3**).5-Fluorouracil was chosen as the positive control according to some reported articles about the anticancer activity of pyrazole derivatives.[30-32] The antiproliferative activities of these compounds at 100 μM against A549 cells, HepG2 cells and normal cells (RTE) were displayed in **Table 3**. As indicated in **Table 3,** most of the new compounds **5a-5k** exhibited some appreciable anti-proliferative activity against the two cancer cell lines. Interestingly, all the compounds nearly have no effect on RTE cell growth. These results suggested that selectivity of novel sugar-based pyrazole derivatives **5a-k** towards tumor cells compared with normal RTE cells. Furthermore, compared with the positive control 5-Fluorouracil, these compounds shown broader spectrum anti-proliferative

activity. Although, **5a-5k** displayed relative lower anti-proliferative activity against A549 cells than 5-Fluorouracil, the anti-proliferative activity against HepG2 cells is better.

Table 3. In vitro cytotoxicity of **5a-5k** on different cell lines at 100 μM and at 24 h

Compounds	Inhibition (%) 100 (μM)		
	HepG2	A549	Normal cells
5a	56.31%±1.21	53.22%±1.01	17.16%±0.64
5b	55.47%±0.98	30.03%±0.78	15.23%±0.49
5c/5d	55.74%±1.01	39.25%±0.92	13.20%±0.62
5e	56.81%±1.34	38.70%±0.84	16.11%±0.73
5f	55.34%±1.12	42.14%±1.28	12.83%±0.35
5g/5h	70.97%±2.58	52.25%±1.54	25.10%±0.93
5i	62.48%±1.14	43.22%±1.48	24.64%±0.84
5j	72.02%±1.95	23.16%±0.87	17.73%±0.57
5k	65.32%±1.06	45.18%±1.04	14.25%±0.46
5-Fluorouracil	47.41%±1.01	87.41%±1.29	13.07%±0.36

Furthermore, IC_{50} values of these compounds along with the positive control 5-Fluorouracil were determined (**Table 4)**. The results indicated that these new sugar-based pyrazole derivatives displayed higher inhibition for the HepG2 cell lines with the IC_{50} values ranging from 0.24 to 13.9μM than they did for the A549 cell lines. Compounds **5g/5h** and **5j** (IC_{50} values 0.24μM and 0.25μM) were superior to 5-Fluorouracil (IC_{50} value 159μM) against HepG2 cells. Although, the anticancer activity against A549 cell lines of compound **5a** (IC_{50} value 5.52μM) is relative worse than 5-Fluorouracil (IC_{50} value 1.96μM), **5a** still shown good anti-lung cancer activity. These results suggested that introducing of longer alky chain seem to have positive effect on enhancing the biological activity. The further structure-activity-relationship studies will be continued in our laboratory to determine how the substituent affects the anti-tumor activity and design the best chemical structure with more active biological activity in the future.

Table 4. The IC_{50} values of **5a-5k** against HepG2 cells and A549 cells.

Test compounds	IC_{50} μM	
	HepG2	A549
5a	2.12±0.23	5.52±1.08
5b	2.76±0.36	>100
5c/5d	7.68±1.12	25.17±2.12
5e	11.5±1.98	>100
5f	2.06±0.38	54.32±4.52
5g/5h	0.24±0.13	13.78±2.18
5i	1.94±0.19	23.16±3.47
5j	0.25±0.08	>100
5k	2.83±0.26	26.42±2.83
5-Fluorouracil	159.43±6.15	1.96±0.14

In summary, a series of novel sugar-based pyrazole derivatives with good anticancer activity have been synthesized under microwave irradiation in eco-friendly water. Most of the new compounds displayed good inhibitory activity against HepG2 cells or A549 cells, especially compound 5a. The in vitro anti-proliferation assay and IC_{50} values support our design and ensure these sugar-based pyrazole derivatives are promising lead compound for the discovery of new anticancer drugs.

Acknowledgements

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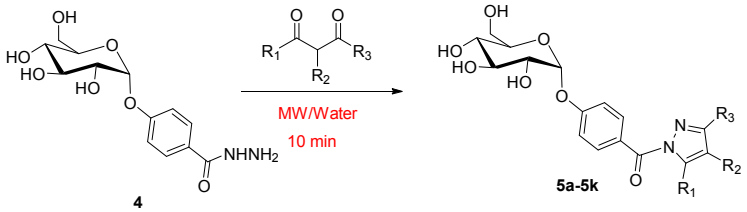

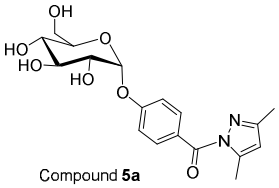

Compound **5a**

Compound **5a** IC_{50} =2.12±0.23μM (against HepG2)

IC_{50} = 5.52±1.08μM (against A549)