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Substrate-controlled chemoselective synthesis and potent cytotoxic activity of novel 5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4-one derivatives

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ABSTRACT

The substrate-controlled chemoselective synthesis of novel 5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4-one derivatives has been successfully achieved via microwave-assisted three-component reactions of 2,6-diaminopyrimidin-4(3*H*)-one, aromatic aldehydes and 1,2-diphenylethanone. This approach has the prominent features of chemoselectivity, diasteroselectivity, atom economy, short reaction time, high yield as well as operational simplicity. Moreover, these novel compounds were subject to the test of in vitro cytotoxicity to carcinoma SW1116 and SGC7901 cells. Most of the tested compounds showed significant cytotoxicity to SW1116 cells and compound **4b** exhibited more potent and efficacious cytotoxicity to SGC7901 cells than doxorubicin hydrochloride as positive control.

It is known to all that cancer is a leading cause of death. Apart form the use of surgical treatment and irradiation, chemotherapy still remains an important option for the treatment of cancer. In this context, the search for novel chemotherapeutic agents and approaches to cancer treatment is an active research field stimulated by the discovery of new biological targets and by the possibility of obtaining new drugs with less undesirable side effects.

Among potential chemotherapeutic agents, heterocyclic compounds represent an outstanding type of anti-cancer drug candidate. Pyrido[2,3-*d*]pyrimidin-4-one derivatives, containing two fused heterocyclic scaffolds in one molecule, exhibit significant biological properties such as antitumor[1] and antifungal activities.[2] In spite of the much attention paid to the synthesis of this class of poly-functionalized compounds from organic and medicinal chemists,[2,3] a survey of the literature reveals no report on the synthesis and bioactivity evaluation of 5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4-one derivatives (Fig. 1), which have high steric hindrance resulted from the three adjacent aromatic rings. Therefore, developing an efficient approach to the synthesis of this novel class of heterocycles as well as evaluation on their cytotoxic activity is very important for the sake of discovering new anticancer drug candidates.

On the other hand, in the skeleton of 5,6,7-triarylpyrido-[2,3-*d*]pyrimidin-4-one derivatives, a unit of 1,4-dihydropyridine is embedded. It is well known that 1,4-dihydropyridine and 3,4-dihydropyridine are easy to change into each other by tautomerizm, in which the equilibrium tends toward the 1,4-dihydro form in most cases.[4] This fact resulted in our observations of either exclusive formation of the 1,4-dihydro derivative or a mixture where it predominated over the 3,4-dihydro form. Can 3,4-dihydropyridines be exclusively synthesized? Compared with the vast attentions and numerous synthetic approaches to 1,4-dihydropyridines, the investigations on the synthesis of 3,4-dihydropyridines are rather rare.[5]

In view of the prominent merits of microwave-assisted multi-component reactions[6] and as a continuation of our efforts on chemoselective synthesis of heterocyclic compounds with potential bioactivities,[7] we investigated the three-component reactions of 2,6-diaminopyrimidin-4(3*H*)-one **1**, aromatic aldehydes **2** and 1,2-diphenylethanone **3** under microwave irradiation (MW) (Scheme 1). To our delight, the chemoselective synthesis of 5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4(3*H*,5*H*,8*H*)-ones **4** and *trans*-5,6-dihydro-5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4(3*H*)-ones **5** as single diastereoisomers, which include the units of 1,4-dihydropyridine and 3,4-dihydropyridine respectively, were achieved by the control of different substrates **2**.

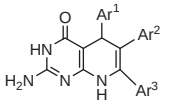

Fig. 1. Structure of 5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4-one derivatives.

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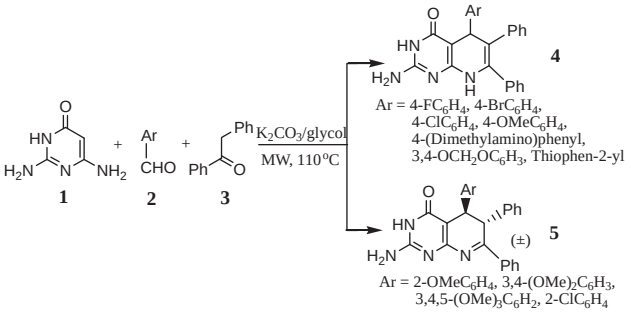


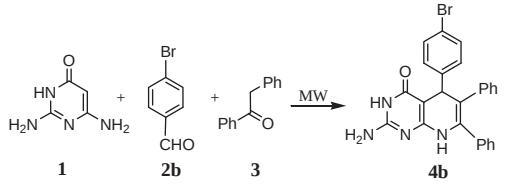
Scheme 1. Chemoselective synthesis of heterocycles **4** and **5**.

It goes without saying that chemoselectivity is a key issue to be controlled in the fields of organic and medicinal syntheses. In recent years, many studies have focused on the chemoselectivity of reactions controlled by metal catalysts and solvents.[8] In spite of these achievements, the investigations on substrate-controlled chemoselective reactions are not well documented. Therefore, these chemoselective syntheses of 5,6,7-triarylpyrido[2,3-*d*]pyrimidine-4-one derivatives **4** and **5** provide good examples of substrate-controlled chemoselective reactions.

In this paper, we report this efficient substrate-controlled chemoselective synthesis of novel 5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4-one derivatives via microwave-assisted three-component reactions and the evaluation of their cytotoxic activity, leading to the discovery of some new heterocycles with potent cytotoxicity higher than or similar to doxorubicin hydrochloride, a powerful anticancer drug.

Initially, the reaction of 2,6-diaminopyrimidin-4(3*H*)-one **1** (1 mmol), 4-bromobenzaldehyde **2b** (1 mmol) and 1,2-diphenylethanone **3** (1 mmol) was employed to optimize the reaction conditions. As illustrated in Table 1, K_2CO_3/glycol was preferred as the optimal catalyst/solvent system and 110 °C was chosen as the most suitable reaction temperature (Table 1, entry 6) in view of the highest yield of **4b**.

Table 1
Reaction condition optimization for the synthesis of **4b**[a]



Entry	Catalyst/solvent	*T* (°C)	Time (min)	Yield[b] (%)
1	Glycol	110	12	77
2	95%EtOH	110	18	58
3	Water	110	24	Trace
4	DMF	110	15	64
5	AcOH	110	14	72
6	K_2CO_3/glycol	110	9	89
7	Et_3N/Glycol	110	15	81
8	$NaHCO_3$/glycol	110	12	83
9	K_2CO_3/glycol	90	16	79
10	K_2CO_3/glycol	100	12	84
11	K_2CO_3/glycol	120	9	87

[a] All the reactions were carried out with 1 mmol of **1**, 1 mmol of **2b** and 1 mmol of **3** in 2 mL of solvent without or with the presence of catalyst (0.1 mmol) under MW at the initial/maximum power of 100 W/250 W.
[b] Isolated yields.

Table 2
Chemoselective synthesis of **4** and **5** under MW[a]

Entry	2	4 or 5	Ar	Time (min)	Yield[b] (%)
1	**2a**	**4a**	4-FC_6H_4	10	82
2	**2b**	**4b**	4-BrC_6H_4	9	89
3	**2c**	**4c**	4-ClC_6H_4	12	85
4	**2d**	**4d**	4-$CH_3OC_6H_4$	10	86
5	**2e**	**4e**	4-(Dimethylamino)phenyl	12	83
6	**2f**	**4f**	3,4-$OCH_2OC_6H_3$	12	81
7	**2g**	**4g**	Thiophen-2-yl	14	80
8	**2h**	**5a**	2-$CH_3OC_6H_4$	10	83
9	**2i**	**5b**	3,4-$(CH_3O)_2C_6H_3$	12	84
10	**2j**	**5c**	3,4,5-$(CH_3O)_3C_6H_2$	14	81
11	**2k**	**5d**	2-ClC_6H_4	11	82

[a] All the reactions were carried out with 1 mmol of **1**, 1 mmol of **2** and 1 mmol of **3** in 2 mL of glycol with the presence of K_2CO_3 (0.1 mmol) at 110 °C under MW at the initial/maximum power of 100 W/250 W.
[b] Isolated yields.

Under the optimized reaction conditions, various aromatic aldehydes **2** bearing electron-withdrawing or electron-donating groups on the aromatic ring were reacted with 2,6-diaminopyrimidin-4(3*H*)-one **1** and 1,2-diphenylethanone **3** (Table 2). Surprisingly, aside from the expected products **4** (Table 2, entries 1–7) in their 1,4-dihydropyridine forms, several undesired products **5** (Table 2, entries 8–11) in the 3,4-dihydropyridine forms were generated when using different substrates **2**.

The results indicate that the Ar group in dihydropyridine skeletons of **4** and **5** serves as a convenient tautomeric 'switch'. It seems that the selectivity of this reaction has no necessary relationship with the electronic nature of the substituents since compounds **4** and **5** can be prepared not only by aromatic aldehydes with electron-donating groups (Table 2, entries 4–6 and 8–10), but also by those with electron-withdrawing groups (Table 2, entries 1–3 and 11). Obviously, the aromatic group in the undesired products **5** is ortho-substituted or poly-substituted with either electron-donating (Table 2, entries 8–10) or electron-withdrawing groups (Table 2, entry 11). Thus, **4** exists exclusively in the 1,4-dihydro form, while **5** is entirely in the 3,4-dihydro form, presumably due to a steric effect.[4c,4d]

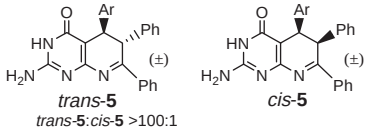
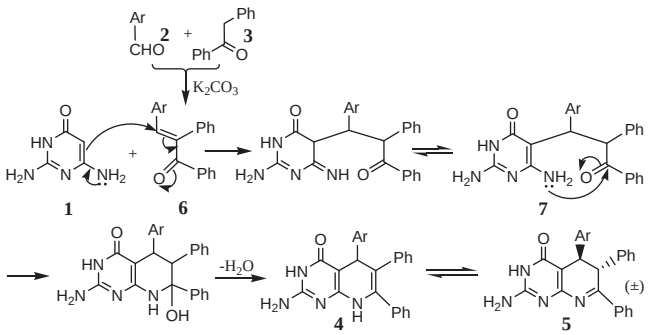


Fig. 2. Configurations of compound **5**.



Scheme 2. Supposed mechanism of this reaction.

Table 3
Cytotoxicity of new compounds **4** and **5**

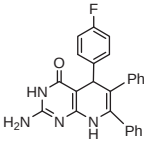
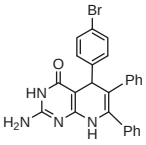
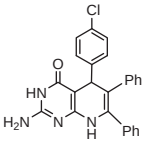
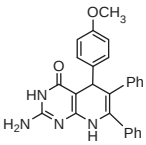
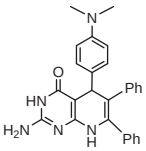
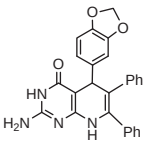
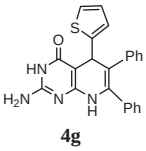
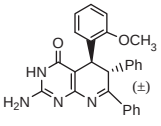
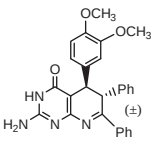
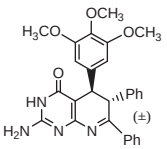
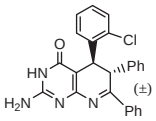
Entry	Compound	Inhibition rate on SW1116[a] (%)			IC_{50} to SGC7901[b] (μg/mL)
		0.1 μg/mL	1 μg/mL	10 μg/mL	
1	 **4a**	31.55 ± 7.79	17.60 ± 3.91	50.29 ± 7.33	13.035
2	 **4b**	19.78 ± 2.61	19.27 ± 7.35	11.96 ± 2.68	0.074
3	 **4c**	14.88 ± 1.72	25.14 ± 5.63	25.54 ± 1.16	0.104
4	 **4d**	43.01 ± 1.28	29.12 ± 8.44	42.84 ± 5.39	0.341
5	 **4e**	20.54 ± 2.03	27.89 ± 7.55	13.99 ± 2.58	0.790
6	 **4f**	15.12 ± 4.63	34.63 ± 0.74	20.55 ± 8.17	0.090
7	 **4g**	22.39 ± 1.20	37.24 ± 0.64	48.32 ± 0.67	0.108
8	 **5a**	22.35 ± 4.32	6.47 ± 2.57	31.55 ± 4.23	0.359
9	 **5b**	2.24 ± 0.19	13.81 ± 1.23	23.90 ± 1.75	0.606
10	 **5c**	21.82 ± 0.88	16.69 ± 1.83	36.89 ± 3.13	2.979

Table 3 (*continued*)

Entry	Compound	Inhibition rate on SW1116[a] (%)			IC_{50} to SGC7901[b] (μg/mL)
		0.1 μg/mL	1 μg/mL	10 μg/mL	
11	 **5d**	31.77 ± 7.54	27.31 ± 2.46	38.73 ± 2.27	1.565
12	Doxorubicin hydrochloride[c]			28.52 ± 1.47	0.078

[a] The inhibition rate on SW1116 was represented as mean ± S.D.
[b] The IC_{50} value to SGC7901 corresponded to the compound concentration causing 50% mortality in SGC7901 cells.
[c] Doxorubicin hydrochloride was used as a positive control.

The structures of compounds **4** and **5** were unambiguously characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS (ESI).[9] It is worth-noting that the configurations of the two adjacent tertiary hydrogen atoms in all the products **5** were exclusively *trans* (Fig. 2) and the ratios of *trans*-**5** to *cis*-**5** were more than 100:1, which was determined by ^{1}H NMR spectroscopy. The *trans* stereochemistry of compounds **5** was established by the coupling constants (J = 0 Hz) between the two adjacent methine protons, which was reported in the literature to be 0 Hz for the *trans* diastereoisomers of 3,4-dihydropyridine derivatives[10] because of the nearly orthogonal positions of the two protons confirmed by an X-ray structure determination.[10b]

A plausible mechanism for the formation of compounds **4** and **5** is shown in Scheme 2. Firstly, 2,6-diaminopyrimidin-4(3*H*)-one **1** underwent Michael addition with the intermediate **6**, which was formed from Knoevenagel condensation of aromatic aldehydes **2** and 1,2-diphenylethanone **3**, to give an open-chain intermediate **7**. Subsequently intramolecular cyclization and dehydration afforded products **4** or tautomers **5**.

In order to survey the possible bioactivity of this class of novel compounds, 5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4-one derivatives **4** and **5** were subject to the test of in vitro cytotoxicity to colon carcinoma cell line SW1116 and stomach carcinoma cell line SGC7901.[9]

The cytotoxic activity of compounds **4** and **5** to colon carcinoma cell line SW1116 was assayed in three concentrations of 0.1, 1 and 10 μg/mL (Table 3). The results suggested that all the tested compounds in the three concentrations inhibited proliferation of SW1116 cells with inhibition rate (IR) from 11.96% to 50.29%. In the three tested concentrations, most of the tested compounds with the concentration of 10 μg/mL have the highest cytotoxic activity to SW1116 cells. Compared with doxorubicin hydrochloride (IR = 28.52%), a powerful anticancer drug as positive control, compounds **4a** (IR = 50.29%), **4d** (IR = 42.84%), **4g** (IR = 48.32%), **5a** (IR = 31.55%), **5c** (IR = 36.89%) and **5d** (IR = 38.73%) inhibited the growth of SW1116 cells in higher rates at the same concentration of 10 μg/mL.

With the preliminary results, the IC_{50} values of compounds **4** and **5** to stomach carcinoma cell line SGC7901 were tested to further evaluate their cytotoxicity (Table 3). In general, the tested compounds exhibited significant cytotoxicity to SGC7901 cells with fairly low IC_{50} values from 13.035 to 0.074 μg/mL. Notably, compound **4b** (IC_{50} value = 0.074 μg/mL) is more potent and efficacious than doxorubicin hydrochloride (IC_{50} value = 0.078 μg/mL). Besides, compound **4f** (IC_{50} value = 0.090 μg/mL) is nearly as active as doxorubicin hydrochloride.

In summary, this study has achieved the chemoselective synthesis of highly sterically hindered 5,6,7-triarylpyrido[2,3-*d*]-pyrimidin-4-one derivatives **4** and **5** as single diastereoisomers by controlling the different substrates in microwave-assisted three-component reactions. This approach has the prominent features of chemoselectivity, diasteroselectivity, atom economy, short reaction time, high yield as well as operational simplicity. Moreover, the in vitro cytotoxic activity of these novel 5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4-one derivatives **4** and **5** were assayed, which resulted in the finding of compound **4b** with more potent and efficacious cytotoxicity than doxorubicin hydrochloride. Although there is no obvious relationship between the structure and the cytotoxic activity of the tested compounds, the results indicated that this novel class of 5,6,7-triarylpyrido[2,3-*d*]pyrimidin-4-one derivatives may become promising anti-cancer drug candidates after further investigations.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.114.

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