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Green chemoselective synthesis of thiazolo[3,2-*a*]pyridine derivatives and evaluation of their antioxidant and cytotoxic activities

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ABSTRACT

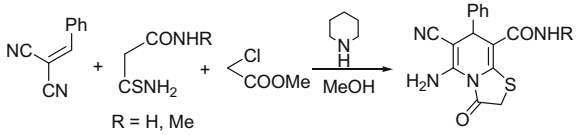
The green chemoselective synthesis of thiazolo[3,2-*a*]pyridine derivatives was achieved in water via microwave-assisted three-component reactions of malononitrile, aromatic aldehydes and 2-mercaptoacetic acid with molar ratios of 2:1:1.5 and 2:2.2:1, respectively. These compounds were subject to the experiments of antioxidant activity and cytotoxicity to carcinoma HCT-116 cells and mice lymphocytes. Nearly all of the tested compounds possessed potent capacities for scavenging free radicals. In addition, most of these compounds showed cytotoxicity to HCT-116 cells and mice lymphocytes with no selectivity. Of these, only thiazolo[3,2-*a*]pyridine derivative **5d** suggested selective cytotoxicity to tumor cell line HCT-116 cells.

Thiazolo[3,2-*a*]pyridines, containing two fused heterocyclic motifs in one molecule, have many important bioactivities such as inhibiting beta-amyloid production,[1] potent CDK2-Cyclin A inhibitor,[2] α-glucosidase inhibitor,[3] potential uterus stimulant,[4] antibacterial and antifungal activities.[5] 5-Amino-7-aryl-6,8-dicyano-3-oxo-2,3-dihydro-7*H*-thiazolo[3,2-*a*]pyridines **1** (Scheme 2), representing a novel class of thiazolo[3,2-*a*]pyridine derivatives, possess electron acceptors and donors and are assumed to scavenge free radicals. However, to the best of our knowledge, the synthetic methods for this class of thiazolo[3,2-*a*]pyridines and their bioactivities including antioxidant and cytotoxic activities have not been well investigated.

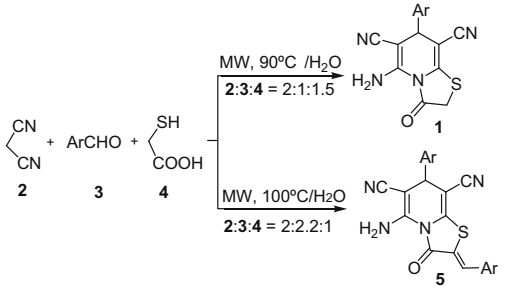
Previous studies showed that the synthesis of the compounds with similar structures can be conducted by multi-step reactions or from complicated starting materials.[6] A relatively simple synthesis was achieved by the three-component reactions of benzylidenemalononitrile, N-substituted thiocarbamoylacetamides and methyl 2-chloroacetate catalyzed by piperidine in methanol (Scheme 1).[7]

Unfortunately, synthesis of these compounds involves the use of volatile and toxic organic catalyst and solvent. In addition, starting materials of benzylidenemalononitrile and N-substituted thiocarbamoylacetamides must be synthesized beforehand. This leads to the elongation of reaction time and reduction in the yield of target products. Thus attempts to develop green and facile approaches to synthesize this class of novel thiazolo[3,2-*a*]pyridines **1** are of theoretical and practical significance.

In recent years, microwave-assisted synthesis using water as solvent has become an active topic because of the use of greener and inexpensive solvents and energy efficiency, which is in well accordance with the two prominent green chemistry principles.[8]



Scheme 1.



Scheme 2.

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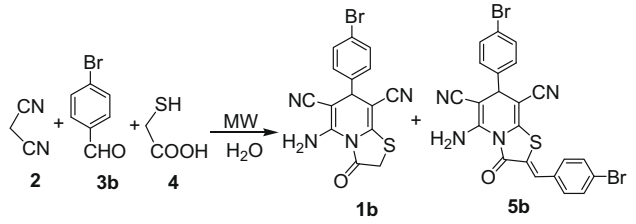
On the other hand, multi-component reactions (MCR) occupy an outstanding position in organic and medicinal chemistry by virtue of the intrinsic atom economy and selectivity, simpler procedures and equipments, time and energy savings, as well as environmental friendliness.[9] Hence, microwave-assisted multi-component reactions in water has become a green and facile approach to the synthesis of biologically important heterocyclic compounds.

As a continuation of our efforts on the green synthesis and potential bioactivities of heterocyclic compounds,[10] we report the green chemoselective synthesis of thiazolo[3,2-*a*]pyridines **1** and **5** via microwave-assisted three-component reactions of malononitrile **2**, aromatic aldehydes **3** and 2-mercaptoacetic acid **4** with different molar ratios in water (Scheme 2) and their antioxidant and cytotoxic activities.

The synthesis of thiazolo[3,2-*a*]pyridine **1b** was initiated by the three-component reaction of malononitrile **2** (2 mmol), 4-bromophenyl aldehyde **3b** (1 mmol) and 2-mercaptoacetic acid **4** (1 mmol) with molar ratio of 2:1:1 at 90 °C under microwave irradiation (MW) in water. Yet the end products of this reaction turned out to be the mixture of compound **1b** and **5b** (Table 1, entry 1). After conducting numerous experiments (Table 1, entries 2–8), the molar ratio of the three reactants for exclusively synthesizing compound **1b** was optimized to be 2:1:1.5.

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



Entry	**2**:**3b**:**4**[b]	*T* (°C)	**1b**:**5b**[b]	Yield of **1b**[c] (%)
1	2:1:1	90	1.8:1	57
2	2:1:1.1	90	2.2:1	62
3	2:1:1.2	90	3.1:1	70
4	2:1:1.3	90	5.2:1	77
5	2:1:1.4	90	7.8:1	82
6	2:1:1.5	90	>99:1	89
7	2:1:1.6	90	11.3:1	73
8	2:1:1.7	90	11.1:1	70
9	2:1:1.5	70	10.6:1	74
10	2:1:1.5	80	>99:1	81
11	2:1:1.5	100	>99:1	84
12	2:1:1.5	110	11.6:1	78

[a] All the reactions were carried out in the presence of **2** (2 mmol), **3b** and **4** with various molar ratios in 2 mL water under MW at the initial power of 100 W and the maximum power of 250 W.
[b] Molar ratios.
[c] Isolated yields.

In order to optimize the reaction temperature, the reaction of **2** (2 mmol), **3b** and **4** in a molar ratio of 2:1:1.5 was conducted in water under MW at temperatures ranging from 70 °C to 110 °C, with an increment of 10 °C. The results (Table 1, entries 6 and 9–12) showed that the reaction at 90 °C coincided with the highest yield of **1b**.

Similar attempts were also made to exclusively synthesize thiazolo[3,2-*a*]pyridine **5b** using different molar ratio of the same three reactants for synthesizing **1b**, which resulted in the optimization of molar ratio being 2:2.2:1 and the reaction temperature at 100 °C.

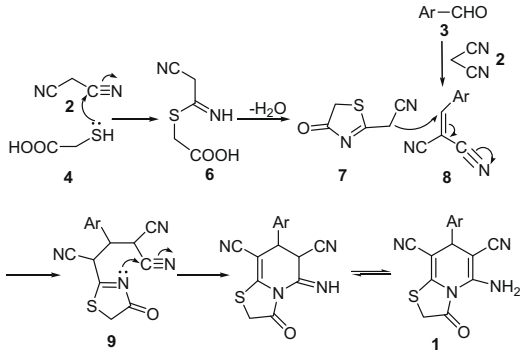
A series of thiazolo[3,2-*a*]pyridine derivatives **1** and **5** were chemoselectively synthesized (Scheme 2) under their respective optimal reaction conditions and the results are summarized in Table 2. Obviously, this protocol can be applied not only to aromatic aldehydes with electron-withdrawing groups, but also to those with electron-donating groups in high yields. Therefore, this green chemoselective approach has wide scope of applicability in synthesizing thiazolo[3,2-*a*]pyridine derivatives.

The structures of thiazolo[3,2-*a*]pyridines **1** and **5** were unambiguously characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS (ESI).[11] For example, in the ^{1}H NMR spectrum of **1f**, the two doublets at 6.95 and 7.24 ppm with a coupling constant of 8.4 Hz were assigned to the four protons of the *p*-methoxyphenyl group, which were verified by a singlet of 3H at 3.78 ppm designated as the methoxy group. The singlet at 7.32 ppm was identified as the two protons of the amino group and that at 4.40 ppm was assigned to the tertiary proton in the pyridine ring. Another singlet at 4.21 ppm was designated as the two methylene protons in the thiazole ring. In the ^{13}C NMR spectrum of **1f**, the pyridine carbons showed chemical shifts at 158.8, 148.2, 85.4, 63.9 and 40.3 ppm, while the carbonyl carbon and methylene carbon contained in the thiazole ring were located at 172.5 and 33.2 ppm, respectively. Besides, the carbons in the symmetric aromatic ring and the attached methoxyl exhibited chemical shifts at 147.7, 134.3, 128.9 (2C), 114.1 (2C) and 55.1 ppm, while the two cyano-group carbons were positioned at 118.8 and 116.4 ppm. The IR spectrum of compound **1f** showed strong absorbance at 3394 and 3284 cm^{-1} due to the presence of NH_2 group, two sharp absorptions at 2208 and 2197 cm^{-1} suggesting the existence of two CN groups and strong absorption at 1734 cm^{-1} contributed by the C=O group.

Table 2
Synthesis of **1** and **5** under microwave irradiation[a]

Entry	Product	Ar	Time (min)	Yield[b] (%)	Mp (lit.) (°C)
1	**1a**	4-FC_6H_4	6	87	267–269
2	**1b**	4-BrC_6H_4	6	89	292–294
3	**1c**	2-ClC_6H_4	6	83	234–236
4	**1d**	2,4-$Cl_2C_6H_3$	7	80	269–271
5	**1e**	C_6H_5	8	81	238–240
6	**1f**	4-$CH_3OC_6H_4$	8	82	236–238
7	**1g**	3,4-$OCH_2OC_6H_3$	9	85	271–273
8	**1h**	4-$CH_3C_6H_4$	8	83	265–267
9	**5a**	4-$NO_2C_6H_4$	7	87	258–260 (243–245)[5b]
10	**5b**	4-BrC_6H_4	6	89	272–273 (278–280)[5b]
11	**5c**	3-$NO_2C_6H_4$	6	84	278–279
12	**5d**	4-OH-3-$NO_2C_6H_3$	6	87	269–271
13	**5e**	2-Thienyl	7	82	247–248

[a] All the synthesis of **1** were carried out in the presence of 2 mmol of **2**, 1 mmol of **3** and 1.5 mmol of **4** in 2 mL water under MW at 90 °C with the initial power of 100 W and the maximum power of 250 W. All the synthesis of **5** were carried out in the presence of 2 mmol of **2**, 2.2 mmol of **3** and 1 mmol of **4** in 2 mL water under MW at 100 °C with the initial power of 100 W and the maximum power of 250 W.
[b] Isolated yields.



Scheme 3.

A plausible mechanism for the formation of compounds **1** is suggested in Scheme 3. Firstly, the nucleophilic addition of 2-mercaptoacetic acid **4** to malononitrile **2** yielded the intermediate **6**, which further gave thiazolinone derivatives **7** via intramolecular dehydration. Then, the intermediate **7** underwent Michael addition with arylidenemalononitrile **8** formed from Knoevenagel condensation of malononitrile **2** and aldehyde **3** to give an open-chain intermediate **9**, which was subsequently intramolecular cyclized and isomerized to afford the product **1**. The formation mechanism of compound **5** is similar to that of compound **1**, which has been demonstrated in literature.[5b,12]

In order to survey the possible biological activities of this class of compounds, derivatives of thiazolo[3,2-*a*]pyridines **1** and **5** were subject to the test of antioxidant activity and cytotoxicity to carcinoma cell line HCT 116 (ATTC CCL 247) and mice lymphocytes. The antioxidant activity is represented by their capacities for scavenging 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) and hydroxyl free radical (OH) using protocols described in our previous works,[10d,e] and the results are summarized in Table 3. Nearly all the tested compounds showed strong capacities for scavenging DPPH and OH compared with those of the positive control L-Ascorbic acid. The top three for scavenging DPPH radical are compounds **1e**, **1a** and **1h** with phenyl, 4-fluorobenzyl and 4-methylbenzyl as Ar group, respectively. While the top three for scavenging OH are compounds **5e**, **1a** and **1h** with 2-thienyl, 4-fluorobenzyl and 4-methylbenzyl as Ar group, separately. It is noted that the substituents of Ar group in these potent antioxidant compounds include both electron-withdrawing and electron-donating group. These aromatic groups possibly play as electron receptors and donors that underlie their antioxidant activities.

The cytotoxic assay[11] (Table 4) showed that the tested compounds inhibited proliferation of HCT 116 cells up to 63.8%.

Table 3
Free radicals scavenging capacities of compounds **1** and **5**[a]

Entry	Compound	Ar	DPPH (%/mg)	OH (%/mg)
1	**1a**	4-FC_6H_4	880.99 ± 7.00	654.55 ± 12.03
2	**1b**	4-BrC_6H_4	67.65 ± 3.08	250.00 ± 19.42
3	**1c**	2-ClC_6H_4	811.85 ± 3.92	570.45 ± 29.81
4	**1d**	2,4-$Cl_2C_6H_3$	708.64 ± 23.77	486.36 ± 6.01
5	**1e**	C_6H_5	884.94 ± 32.87	431.82 ± 15.91
6	**1f**	4-$CH_3OC_6H_4$	140.25 ± 3.08	207.58 ± 18.23
7	**1g**	3,4-$OCH_2OC_6H_3$	444.44 ± 13.17	431.06 ± 15.96
8	**1h**	4-$CH_3C_6H_4$	858.77 ± 16.85	609.85 ± 28.60
9	**5a**	4-$NO_2C_6H_4$	387.65 ± 23.63	297.73 ± 12.03
10	**5b**	4-BrC_6H_4	107.65 ± 6.68	125.00 ± 7.87
11	**5c**	3-$NO_2C_6H_4$	45.93 ± 2.96	56.82 ± 6.82
12	**5d**	4-OH-3-$NO_2C_6H_3$	108.64 ± 12.60	201.52 ± 15.13
13	**5e**	2-Thienyl	717.53 ± 22.29	665.91 ± 15.75
14	L-Ascorbic acid[b]		188.12 ± 5.13	108.65 ± 4.48

[a] The scavenging capacities were represented as percentage inhibition (mean ± SD, $n = 3$) of the free radicals by 1 mg tested compound.
[b] L-Ascorbic acid was used as a positive control and purchased locally with purities higher than 98.5%. Other chemicals for bioassay of antioxidant activity are obtained from Sigma.

Meanwhile these compounds also inhibited the proliferation of mice lymphocytes up to 71.28%. In general, these compounds inhibited the growth of mice lymphocytes in a rate higher than they did to HCT-116 cells. Only compound **5d** suggested higher selective cytotoxicity to HCT 116 cells. Thus the simultaneously existence of 4-OH and 3-NO_2 in Ar group of **5d** might be the necessity for selectively inhibiting the growth of tumor cells.

It has been reported that most natural products inhibiting the growth of tumor cells also possess antioxidant activity[13], and antioxidant natural products are believed to be the pharmaceuticals able to treat or prevent oxidative stress-induced cancers.[14] In our study, however, high antioxidant activity of thiazolo[3,2-*a*]pyridine derivatives **1** and **5** does not coincide with their selective cytotoxicity to tumor cell line HCT-116, which seems to indicate that highly antioxidant activity of these compounds does not guarantee their selective cytotoxicity to tumor cells.

This study has achieved the green chemoselective synthesis of thiazolo[3,2-*a*]pyridine derivatives and examined their in vitro antioxidant and cytotoxic activities. Further experiments are needed to determine the selective cytotoxicity to other tumor cell lines. Regardless, this study does provide a green shortcut for the synthesis of thiazolo[3,2-*a*]pyridine derivatives with high chemoselectivity, environmental friendliness, short reaction time and high yields of target products. More importantly, bioassay of these compounds resulted in the finding of thiazolo[3,2-*a*]pyridine derivative **5d** with

Table 4
Cytotoxicity of compounds **1** and **5**[a]

Entry	compound[b]	Ar	Inhibition rate on HCT-116 (%)	Inhibition rate on lymphocytes (%)
1	**1a**	4-FC_6H_4	47.77 ± 2.85	66.88 ± 0.88
2	**1b**	4-BrC_6H_4	43.55 ± 0.88	69.59 ± 3.57
3	**1c**	2-ClC_6H_4	54.06 ± 2.54	60.36 ± 3.81
4	**1d**	2,4-$Cl_2C_6H_3$	29.93 ± 4.66	55.35 ± 6.79
5	**1e**	C_6H_5	50.84 ± 1.92	64.84 ± 2.97
6	**1f**	4-$CH_3OC_6H_4$	50.17 ± 2.61	70.60 ± 2.08
7	**1g**	3,4-$OCH_2OC_6H_3$	51.78 ± 2.57	62.02 ± 3.35
8	**1h**	4-$CH_3C_6H_4$	47.42 ± 2.35	63.77 ± 4.76
9	**5a**	4-$NO_2C_6H_4$	35.45 ± 1.87	51.69 ± 1.83
10	**5b**	4-BrC_6H_4	56.65 ± 2.07	71.28 ± 0.84
11	**5c**	3-$NO_2C_6H_4$	52.99 ± 1.61	60.35 ± 4.40
12	**5d**	4-OH-3-$NO_2C_6H_3$	41.22 ± 1.84	5.31 ± 0.26
13	**5e**	2-Thienyl	63.81 ± 4.22	55.42 ± 3.31

[a] The cytotoxicity was represented as percentage inhibition (mean ± SD, $n = 3$) of HCT-116 cells and lymphocytes.
[b] The concentration of the tested compounds is 1 mg/mL.

potent antioxidant activity and selective tumor cytotoxicity, which gains some insights into the synthesis of the compounds able to inhibit tumor growth without evident side-toxicities.

Acknowledgments

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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.2009.08.046.

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