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Design and diversity-oriented synthesis of novel 1,4-thiazepan-3-ones fused with bioactive heterocyclic skeletons and evaluation of their antioxidant and cytotoxic activities

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ABSTRACT

This study has achieved the design and diversity-oriented synthesis of novel 1,4-thiazepine derivatives embedded with carbazole, pyrazole or isoxazole motif via microwave-assisted multicomponent reactions under solvent-free condition, thus providing a green and facile access to 1,4-thiazepine derivatives with prominent features of high structural diversity, short reaction time, high yields and environmental friendliness. More importantly, these novel compounds have been subjected to the test of in vitro antioxidant and cytotoxic activities, resulting in the finding that these 1,4-thiazepine derivatives not only have significant antioxidant activity, but also exhibit remarkably selective cytotoxicity to carcinoma cell line HCT 116.

The 1,4-thiazepine ring is one of important moieties in nitrogen- and sulfur-containing heterocycles and has been widely used as key building block for pharmaceutical agents as well as biologically active compounds.[1] Besides, carbazole, pyrazole and isoxazole skeletons constitute the core structural element of many natural and synthesized products, which exhibit diverse pharmacological properties as exemplified by antitumor,[2] antimicrobial,[3] insecticidal,[3] and anti-inflammatory activitites.[4]

Based on the versatile bioactivities of the above mentioned structures, it is promising that the integration of carbazole, pyrazole or isoxazole scaffold with 1,4-thiazepine segment might result in the discovery of new drug candidates with unknown or enhanced bioactivities. However, the design of 1,4-thiazepine compounds implanted with carbazole, pyrazole and isoxazole frameworks for medicinal purpose has been less recognized and only very few reports describe the synthesis of related compounds.[5] Therefore, the development of facile approaches to access these novel targets with structural diversity is highly desirable and valuable for medicinal chemistry and drug discovery.

Diversity-oriented synthesis (DOS)[6,7] via multicomponent reactions (MCRs)[8] has become a powerful protocol to access pharmaceutically relevant heterocycles because of their combined prominent features such as high reaction rate and efficiency, atom economy and selectivity, time and energy savings, target product specificity and minimal environmental impact.[7,8]

In view of the significance of 1,4-thiazepines fused with bioactive heterocyclic skeletons and as our continuous efforts on synthesizing heterocycles with potential bioactivities,[9] herein, we report the design and diversity-oriented synthesis of novel 1,4-thiazepine derivatives **4** embedded with carbazole, pyrazole or isoxazole motif via microwave-assisted multicomponent reactions of aldehydes **1**, mercaptoacetic acid **3** and various heteroaromatic amines **2** (Scheme 1). Furthermore, we also report the evaluation of the antioxidant and cytotoxic activities of the synthesized 1,4-thiazepine compounds **4**.

Initially, the reaction of *p*-methoxybenzaldehyde **1b**, 3-amino-9-ethylcarbazole **2a** and mercaptoacetic acid **3** under microwave irradiation (MW) was employed to optimize the reaction conditions (Table 1). The screen of solvent (entries 1–7) revealed that solvent-free condition was the best suitable condition for this reaction (entry 7). Then, under solvent-free condition, the optimal reaction temperature was further investigated (entries 7–12), and 120 °C with the highest yield of 92% (entry 11) was chosen as the best suitable condition for all further microwave-assisted reactions.

With optimal conditions in hand, we then carried out the design and diversity-oriented synthesis of novel 1,4-thiazepine derivatives **4** embedded with carbazole, pyrazole and isoxazole motifs (Table 2). Different heteroaromatic amines **2a**–**2d** containing carbazole, pyrazole and isoxazole skeletons were utilized to the

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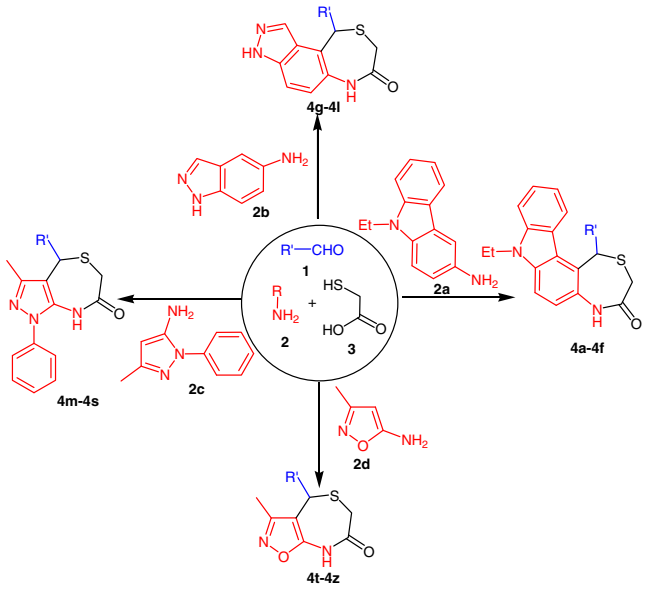
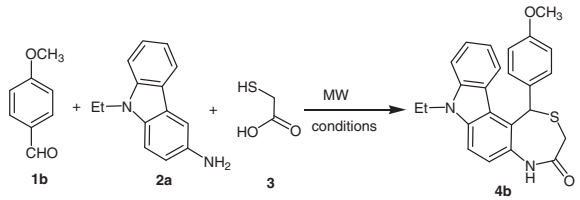


Scheme 1. Synthesis of 4-aza-podophyllotoxin analogs.

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



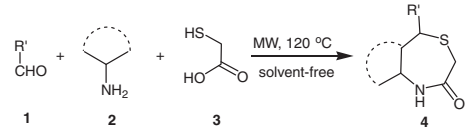
Entry	Solvent	*T* (°C)	Time (min)	Yield[b] (%)
1	THF	100	14	Trace
2	Ethanol	100	14	10
3	Glycol	100	14	15
4	DMF	100	14	27
5	HOAc	100	14	39
6	H_2O	100	14	48
7	—[c]	100	14	57
8	—	105	12	66
9	—	110	11	75
10	—	115	10	80
11	—	120	9	92
12	—	125	9	89

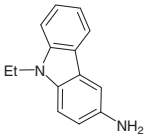
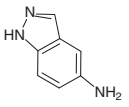
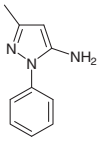
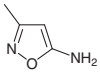
[a] Unless otherwise indicated, all the reactions were carried out in 1 mmol scale in 2 mL of solvent under MW with the initial power of 200 W and the maximum power of 350 W, and the ratio of **1b**/**2a**/**3** was 1:1:1.
[b] Isolated yield.
[c] Solvent-free condition.

three-component reactions in order to obtain target molecules incorporated with bioactive units of 1,4-thiazepine and carbazole, pyrazole or isoxazole. On the other hand, various aldehydes **1** including electronically poor or rich aromatic aldehydes and heteroaromatic aldehyde were applied to this reaction for the aim to access target molecules with structural diversity. As shown in Table 2, the protocol is amenable to a wide scope of aldehydes **1** and heteroaromatic amines **2a**–**2d**, affording a library of novel 1,4-thiazepine derivatives **4** with high yields in short reaction time.

The structures of 1,4-thiazepine derivatives **4** were unambiguously characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS (ESI).[10] A

Table 2
Design and synthesis of 1,4-thiazepine derivatives **4**[a]



Entry	4	2	R′	Time (min)	Yield[b] (%)
1	**4a**		*p*-Tolyl (**1a**)	9	92
2	**4b**		4-Methoxyphenyl (**1b**)	9	93
3	**4c**		Benzo[*d*][1,3]dioxol-6-yl (**1c**)	8	91
4	**4d**		3,4-Dimethoxyphenyl (**1d**)	9	90
5	**4e**		Thiophen-2-yl (1e)	9	91
6	**4f**		4-Bromophenyl (1f)	10	76
7	**4g**		4-Methoxyphenyl (**1b**)	9	93
8	**4h**		*p*-Tolyl (**1a**)	9	92
9	**4i**		Benzo[*d*][1,3]dioxol-6-yl (**1c**)	8	91
10	**4j**		3,4,5-Trimethoxyphenyl (**1g**)	9	92
11	**4k**		4-Bromophenyl (**1f**)	10	88
12	**4l**		4-Chlorophenyl (**1h**)	10	89
13	**4m**		4-Methoxyphenyl (**1b**)	9	92
14	**4n**		*p*-Tolyl (**1a**)	9	91
15	**4o**		Benzo[*d*][1,3]dioxol-6-yl (**1c**)	8	90
16	**4p**		4-Chlorophenyl (**1h**)	9	93
17	**4q**		2-Chlorophenyl (**1i**)	9	92
18	**4r**		2,4-dichlorophenyl (**1j**)	10	90
19	**4s**		3-Nitrophenyl (**1k**)	10	90
20	**4t**		4-Methoxyphenyl (**1b**)	9	93
21	**4u**		*p*-Tolyl (**1a**)	9	92
22	**4v**		Benzo[*d*][1,3]dioxol-6-yl (**1c**)	9	91
23	**4w**		3,4-Dimethoxyphenyl (**1d**)	9	94
24	**4x**		Thiophen-2-yl (**1e**)	9	94
25	**4y**		2-Chlorophenyl (**1i**)	10	90
26	**4z**		4-Bromophenyl (**1f**)	10	91

[a] Unless otherwise indicated, all the reactions were carried out in 1 mmol scale in the absence of solvent at 120 °C under MW with the initial power of 200 W and the maximum power of 350 W, and the ratio of **1**/**2**/**3** was 1:1:1.
[b] Isolated yield.

plausible mechanism for the reaction is similar to what has been reported in the literaute.[5]

In order to survey the possible biological activities of this class of novel compounds, 1,4-thiazepine derivatives **4** 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), superoxide anion (O_2^-) and hydroxyl free radical (OH),[10] and the results are summarized in Table 3. Nearly all the tested compounds showed strong capacities for scavenging DPPH, O_2^- and OH compared with those of the positive control L-ascorbic acid. The top

Table 3
Free radicals scavenging capacities of compounds **4**[a]

Entry	4	DPPH (%/mg)	O_2^- (%/mg)	OH (%/mg)
1	**4a**	1038.46 ± 8.81	2395.11 ± 72.79	994.21 ± 31.88
2	**4b**	528.84 ± 18.71	1210.84 ± 34.48	476.77 ± 10.99
3	**4c**	235.57 ± 11.24	578.39 ± 29.62	348.06 ± 5.64
4	**4d**	261.21 ± 7.28	552.40 ± 27.35	370.89 ± 2.36
5	**4e**	519.23 ± 12.57	1018.47 ± 22.27	770.48 ± 7.48
6	**4f**	291.66 ± 5.36	559.40 ± 40.55	271.38 ± 5.22
7	**4g**	394.23 ± 8.54	1204.01 ± 19.83	393.92 ± 3.73
8	**4h**	381.41 ± 11.39	1211.93 ± 25.82	368.45 ± 6.65
9	**4i**	201.92 ± 5.84	610.65 ± 9.00	187.37 ± 4.25
10	**4j**	195.51 ± 6.12	590.22 ± 8.87	247.07 ± 3.90
11	**4k**	238.78 ± 6.12	609.00 ± 10.73	200.05 ± 4.59
12	**4l**	193.91 ± 5.57	600.80 ± 4.90	195.16 ± 1.89
13	**4m**	161.85 ± 5.36	644.75 ± 16.47	477.55 ± 28.92
14	**4n**	176.28 ± 3.27	600.33 ± 33.52	676.76 ± 10.27
15	**4o**	209.93 ± 6.56	604.97 ± 18.15	643.89 ± 9.10
16	**4p**	346.15 ± 12.27	1248.83 ± 10.96	1103.30 ± 59.24
17	**4q**	225.96 ± 11.56	589.07 ± 12.96	640.16 ± 25.2
18	**4r**	179.48 ± 5.57	592.46 ± 10.08	767.33 ± 6.87
19	**4s**	384.61 ± 7.63	1247.46 ± 12.48	994.46 ± 61.51
20	**4t**	448.71 ± 14.78	1231.44 ± 20.69	407.17 ± 21.73
21	**4u**	375.00 ± 14.35	1295.39 ± 28.77	331.01 ± 11.55
22	**4v**	674.67 ± 8.06	626.11 ± 8.93	248.93 ± 21.99
23	**4w**	195.51 ± 7.45	613.31 ± 13.67	191.49 ± 3.04
24	**4x**	352.56 ± 10.93	1264.57 ± 27.94	929.75 ± 9.46
25	**4y**	173.07 ± 5.84	621.91 ± 10.04	221.15 ± 7.47
26	**4z**	1794.87 ± 68.38	6181.25 ± 25.47	4189.50 ± 66.65
27	PC[b]	182.82 ± 0.98	104.17 ± 1.10	97.25 ± 0.83

[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 (PC).

Table 4
Cytotoxicity of compounds **4**[a]

Entry	4[b]	Inhibition rate on HCT-116 (%)	Viability rate of lymphocytes (%)
1	**4a**	53.42 ± 1.59	93.21 ± 2.51
2	**4b**	58.88 ± 2.91	51.42 ± 5.23
3	**4c**	51.57 ± 1.84	157.59 ± 3.33
4	**4d**	56.16 ± 1.93	101.15 ± 4.41
5	**4e**	60.33 ± 2.04	44.99 ± 5.43
6	**4f**	62.00 ± 0.98	136.07 ± 4.44
7	**4g**	61.84 ± 1.40	44.81 ± 0.74
8	**4h**	66.82 ± 0.42	20.46 ± 0.82
9	**4i**	55.90 ± 4.33	154.81 ± 5.18
10	**4j**	58.23 ± 0.09	166.22 ± 2.04
11	**4k**	63.35 ± 1.21	42.60 ± 2.36
12	**4l**	60.93 ± 1.62	67.90 ± 3.82
13	**4m**	57.75 ± 2.34	78.81 ± 2.97
14	**4n**	58.44 ± 3.71	25.67 ± 3.808
15	**4o**	51.34 ± 3.80	66.94 ± 4.96
16	**4p**	50.18 ± 2.22	82.30 ± 4.51
17	**4q**	55.29 ± 0.44	68.66 ± 5.665
18	**4r**	53.27 ± 4.42	156.06 ± 6.03
19	**4s**	54.26 ± 4.09	154.00 ± 3.25
20	**4t**	60.34 ± 2.85	164.84 ± 3.26
21	**4u**	64.68 ± 1.10	102.59 ± 4.57
22	**4v**	50.43 ± 6.72	87.25 ± 2.81
23	**4w**	54.58 ± 3.04	165.37 ± 5.08
24	**4x**	62.59 ± 4.14	159.91 ± 3.25
25	**4y**	54.58 ± 1.72	170.13 ± 0.79
26	**4z**	63.05 ± 3.48	156.87 ± 3.09

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

three for scavenging DPPH radical are 1,4-thiazepines **4z**, **4a** and **4v** containing carbazole and isoxazole skeletons with 4-bromophenyl, *p*-tolyl and benzo[*d*][1,3]dioxol-6-yl as R′ group, respectively. And the top three for scavenging O_2^- radical are 1,4-thiazepines **4z**, **4u** and **4a** containing carbazole and isoxazole skeletons with 4-bromophenyl and *p*-tolyl as R′ group. While the top three for scavenging OH are compounds **4z**, **4p**, **4s** and **4a** (**4s** and **4a** almost have the same capacity of scavenging OH) imbedded with carbazole, pyrazole and isoxazole units with 4-bromophenyl, 4-chlorophenyl, 3-nitrophenyl and *p*-tolyl as R′ group, separately. It is worth-noting that compound **4z** has the most extraordinary capacities for scavenging all the three radicals, which are dozens of times higher than those of the positive control. Although there is no regular relationship between the structure and the antioxidant activity of the tested compounds, it is obvious that these 1,4-thiazepine derivatives **4** fused with carbazole, pyrazole and isoxazole skeletons have remarkable antioxidant activity.

The cytotoxic assay[10] (Table 4) showed that all the tested compounds inhibited the proliferation of HCT 116 cells with inhibition rate ranging from 50.18% to 64.68%. However, most of the tested compounds did not inhibit the proliferation of mice lymphocytes, and on the contrary, some of the compounds promoted the growth of lymphocytes with viability rate up to 166.22%. In general, most of compounds inhibited the growth of HCT-116 cells in a rate higher than they did to mice lymphocytes. Therefore, these 1,4-thiazepine derivatives **4** exhibited high selective cytotoxicity to HCT 116 cells. The top three for selectively inhibiting the proliferation of HCT 116 cells but promoting the growth of mice lymphocytes are 1,4-thiazepines **4u**, **4z** and **4x** containing isoxazole skeleton with *p*-tolyl, 4-bromophenyl and thiophen-2-yl as R′ group, respectively.

These results suggest that the 1,4-thiazepine derivatives **4** not only have significant antioxidant activity, but also exhibit remarkably selective cytotoxicity to carcinoma cell line HCT 116. It has been reported that most natural products inhibiting the growth of tumor cells also possess antioxidant activity,[11] and antioxidant natural products are believed to be the pharmaceuticals able to treat or prevent oxidative stress-induced cancers.[12] The results of our study also demonstrate the correlations between the antioxidant activity and cytotoxicity.

In summary, this study has achieved the diversity-oriented synthesis of novel 1,4-thiazepine derivatives **4** embedded with carbazole, pyrazole or isoxazole motif via microwave-assisted multicomponent reactions under solvent-free condition, thus providing a green and facile access to 1,4-thiazepine derivatives with prominent features of high structural diversity, short reaction time, high yields and environmental friendliness. More importantly, these novel compounds have been subjected to the test of in vitro antioxidant and cytotoxic activities, resulting in the finding that these 1,4-thiazepine derivatives not only have significant antioxidant activity, but also exhibit remarkably selective cytotoxicity to carcinoma cell line HCT 116. Therefore, these novel 1,4-thiazepine derivatives fused with bioactive heterocyclic skeletons may find their pharmaceutical applications 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.2011.09.081.

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