Full Paper

Synthesis and *In-vitro* Antitumor Activities of Some Mannich Bases of 9-Alkyl-1,2,3,4-tetrahydrocarbazole-1-ones

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A novel series of 2-substituted aminomethyl-9-alkyl-1,2,3,4-tetrahydrocarbazole-1-ones **5a – q** was synthesized via aminomethylation of 9-alkyl-1,2,3,4-tetrahydrocarbazole-1-ones **4a – e** with hydrochlorides of the respective amines **6a – m**. The structures of these newly synthesized compounds were characterized by ^{1}H-NMR, MS, and elemental analysis. All the compounds were tested for their cytotoxic activity *in vitro* against four human tumor cell lines including human non-small lung cancer cells (A549), human gastric adenocarcinoma (SGC), human colon cancer cell (HCT116), human myeloid leukemia cells (K562), and one multi-drug resistant subline (KB-VCR). Most compounds showed moderate to potent cytotoxic activity against the tested cell lines. Preliminary mechanism research indicated that the most promising compound, 2-diethylaminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **5c**, exhibited a potential inhibitory effect against microtubule.

Keywords: 9-Alkyl-1,2,3,4-tetrahydrocarbazole-1-ones / Antitumor activity / Mannich bases / Microtubules

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Introduction

Microtubules are dynamic structures that play a crucial role in cellular division and are recognized as an important target for anticancer therapy [1]. A number of naturally occurring compounds exhibited their anticancer properties by interfering with microtubules, resulting in mitotic arrest, such as paclitaxel [2], which promotes the microtubulin polymerization, while colchicine [3], combretastatin A 4 (CA 4) [4], and vinca alkaloids [1] inhibit the microtubulin polymerization. Because the antitubulin-chemotherapy drugs had problems with toxicity and drug resistance, scientists had been actively exploring new antitubulin agents. A variety of synthetic small molecules such as BPR0L075, NSC 676693 were reported as inhibitors of tubulin polymerization [4 – 9]. Structurally, they involved various heteroaromatic cores including indole, benzothiophene [10], benzofuran [11], imidazole [12], thiazole [13], and oxadiazoline [14] moieties. A number of indole-based compounds, for example 2-aroylindoles [15], 3-aroylindoles [16], 3-aroyl-2-phenylindoles [11], 3-arylthioindoles [17], and indolyl-3-glyoxamides [18], had shown strong antiproliferative and antitubulin activity, and some of them are being developed.

1,2,3,4-Tetrahydrocarbazole-1-ones, also including a indole structure, were known for a long times and many synthesis methods were published [19, 20]. They were reported to possess many biological activities, such as antitumor activity [21], HIV integrase inhibition [22], prolonged analgesic activity [23], heat shock protein 90 (Hsp90) inhibition [24], mitogen-activated protein kinase-activated protein kinase-2 (MAPKAP-k2) inhibition [25], antibacterial and antifungal activities [26]. Moreover, they had been increasingly important intermediates in the syntheses of various biological active heterocyclic

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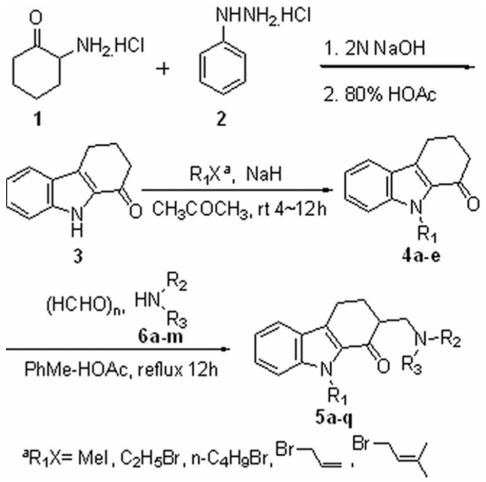


Scheme 1. Synthetic routes for the designed compounds **5a–5q** and their intermediates.

compounds because of their unique structure, such as indolo[2,3-a]carbazoles [22], furo[2,3-a]carbazoles [27], pyrimidino[4,5-a]carbazoles [28], pyrazolino[3,2,1-*j*,*k*]carbazoles [29], thieno[2,3-*a*]carbazoles [30], and so on. This provided a great impetus to search for potential pharmacologically active drugs carrying a 1,2,3,4-tetrahydrocarbazole-1-one scaffold.

Mannich bases had been reported as potential biological agents. They possess antimicrobial activity [31], anticancer activity [32], etc. Various drugs obtained from Mannich reaction had been proved to be more effective and less toxic than their parent compounds [33]. The versatile utility of the Mannich bases in pharmaceutical chemistry prompted us to prepare a series of amino methyl derivatives of 9-alkyl-1,2,3,4-tetrahydrocarbazole-1-ones. Here, we describe the synthesis and the cytotoxic and antitubulin properties of this series of compounds.

Result and discussion

Chemistry

The synthetic routes to obtain the designed compounds **5a–5q** and their intermediates are described in Scheme 1. Compound 1,2,3,4-tetrahydrocarbazole-1-one **3** was prepared by the reaction of 2-aminocyclohexanone hydrochloride **1** with phenylhydrazine hydrochloride **2** according to the described procedure [34]. It was efficiently transformed into compounds **4a–e** by treatment with NaH and suitable alkylating agents in acetone at room temperature. Then, heating the ketones **4a–e** with paraformaldehyde and the hydrochloride of the respective amines **6a–m** in a solvent system including acetic acid and toluene obtained the target compounds 2-substituted aminomethyl-9-alkyl-1,2,3,4-tetrahydrocarbazole-1-ones **5a–q** (Table 1). The structures of the newly synthesized compounds were characterized by ^{1}H-NMR, ESI-MS spectral data, and elemental analysis; they are presented in the experimental part (Section 4).

Biological studies

The synthesized 2-substituted aminomethyl-9-alkyl-1,2,3,4-tetrahydrocarbazole-1-ones **5a–5q** and the lead compound 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a** were tested for their cytotoxic activities *in vitro* against several tumor cell lines including A549, SGC, HCT116, K562, and KB-VCR. Taxol was employed as a positive control. The results are summarized in Table 1.

As shown in Table 1, most of the tested compounds showed moderate to potent cytotoxic activity against A549, SGC, K562, HCT116, and KB-VCR cells. It was noteworthy that the cytotoxic effects were more pronounced against HCT116 cell compared with the others, displayed similar or slightly lower activities (2.46–31.77 μM) in comparison with taxol (4.37 μM). Among compounds **5a–m**, which were substituted with methyl at the N-9 position, **5c** and **5m** were the most promising compounds. They exhibited similar cytotoxic activity against three or two cell lines compared to taxol. Furthermore, they showed excellent activity against A549 cell with IC_{50} values of 0.0703 and 0.7087 μM, respectively, which is 35- and 3.5-fold more potent than that of taxol (2.46 μM). The result implied that a diethylaminomethyl (**6c**) or 4-methylpiperidin methyl (**6m**) moiety was the most suitable Mannich base substituent in this series of compounds. Other Mannich base substituents led to the analogs showed strong cytotoxic activity (IC_{50} < 10 μM) against at least one cell lines except for compounds **5d**, **5e**, **5j**, **5l**, which showed only moderate activities.

Besides, a comparison between cytotoxicities of **5a** and **5o** or **5k** and **5n**, **5p**, **5q** with different substituent at the N-9 position revealed that the presence of an isoprenyl group was beneficial for the activities. The most potent compound **5p** exhibited significant antiproliferative activity against all of the tested cell lines.

At last, it is worthy to point that most of the target compounds **5a–m**, which were substituted with methyl at the N-9 position, appeared to be more potent anticancer agents than the lead compound 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a** itself. Therefore, we concluded that the Mannich bases could be used as a constructive instrument to 9-alkyl-1,2,3,4-tetrahydrocarbazole-1-ones.

To further explore whether the growth-inhibitory effect of these novel compounds was related to the interaction with the tubulin system, an *in-vitro* tubulin poly-

Table 1. *In-vitro* cytotoxic activities of compounds **5a**–**q**, **4a**, and taxol against five cell lines.

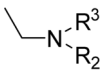
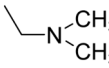
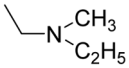
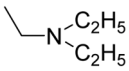
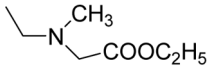
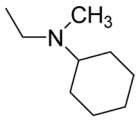
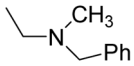
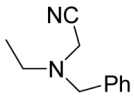
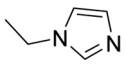
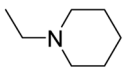
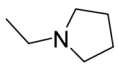
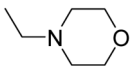
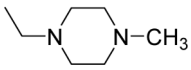
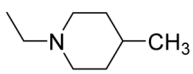
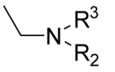
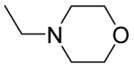
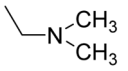
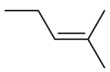
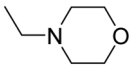
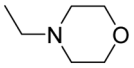
Compound	R_1		Cell line (IC_{50}, μM)[a, b]				
			A549	SGC	K562	HCT116	KB-R
5a	CH_3		110.41	24.37	17.30	2.96	117.58
5b	CH_3		8.53	115.67	22.31	11.82	NT[c]
5c	CH_3		0.0703	85.13	8.26	2.46	72.51
5d	CH_3		46.28	88.64	35.17	18.91	NT
5e	CH_3		80.72	90.09	20.93	25.58	NT
5f	CH_3		14.13	>150	23.34	2.20	74.49
5g	CH_3		>150	>150	>150	>150	NT
5h	CH_3		80.82	99.14	25.13	3.12	NT
5i	CH_3		28.89	61.66	51.42	2.33	107.67
5j	CH_3		100.39	100.46	57.34	12.84	NT
5k	CH_3		95.17	78.19	23.56	4.73	52.32
5l	CH_3		76.59	100.26	35.34	13.95	NT
5m	CH_3		0.7087	28.73	26.45	9.08	63.49

Table 1. Continued.

Compound	R_1		Cell line (IC_{50}, μM)[a, b]				
			A549	SGC	K562	HCT116	KB-R
5n	C_2H_5		73.08	85.11	13.80	31.50	73.01
5o			21.71	62.40	3.29	31.77	64.74
5p			9.29	21.70	2.56	4.55	NT
5q	n-C_4H_9		64.74	39.80	24.20	5.82	40.47
4a	CH_3	–	88.63	136.72	NT	148.01	NT
Taxol			2.46	3.34	1.16	4.37	2.28

[a] IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.
[b] Values are the mean of three experiments.
[c] NT, not tested.

merization assay was performed. The effect of the most promising compound **5c** on microtubule formation was monitored by the increase in fluorescent intensity of the reaction mixture, using tubulin destabilizer CA 4 and tubulin stabilizer taxol as comparison. The results are shown in Fig. 1. In this assay, CA 4 displayed, as expected, a depolymerized effect, and taxol displayed the opposite manner. As shown in Fig. 1, CA 4 inhibited tubulin polymerization by 50% at 3 μM, while compound **5c** inhibited tubulin polymerization by 37.5% at the same concentration. It displayed a pattern similar to that of CA 4.

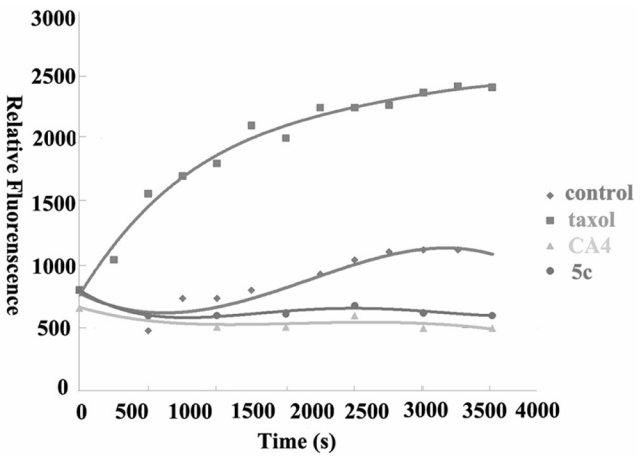


Figure 1. *In vitro* tubulin polymerization assay for compound **5c**.

Conclusion

In summary, we designed and synthesized a series of Mannich bases of 9-alkyl-1,2,3,4-tetrahydro-carbazole-1-ones, which were structurally confirmed by ^{1}H-NMR, ESI-MS, and elemental analysis and evaluated their cytotoxicity against five human cancer cell lines including a multi-drug resistant subline. The results showed that most compounds showed moderate to potent cytotoxic activity against the tested cell lines. Among them, compound **5c**, with IC_{50} values of 0.0703 μM against the A549 cell line, was 35-fold more potent than taxol. Primary mechanism research indicated it exhibited a potential inhibitory effect against microtubule. Further studies of the mechanism of this series with novel heterocyclic ketones are under way.

We thank the College of Science Zhejiang University for ^{1}H-NMR work. We are also grateful to the other staff of ZJU-ENS joint medicinal chemistry laboratory.

The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined on a Büchi B-540 apparatus (Büchi Labortechnik, Switzerland) and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AM 400 instrument (Bruker Bioscience, USA) at 400 MHz (chemical shifts are expressed as δ values relative to TMS as internal standard). Mass spectra (MS), ESI (positive) were recorded on an Esquire-LC-00075 spectrometer (Bruker). Elemental analyses were performed on an Eager 300 instrument (Thermo Fisher Scientific, USA). Reagents and solvents were purchased from common commercial suppliers and were used without further purification. The process of the reaction was monitored by thin layer chromatography (TLC).

1,2,3,4-Tetrahydrocarbazole-1-one 3 [34]

A solution of 2 N sodium hydroxide (0.48 mL, 0.98 mmol) was added dropwise to the mixture of 2-aminocyclohaxanone hydrochloride (79.3 mg, 0.53 mmol) and phenylhydrazine hydrochloride (63.6 mg, 0.44 mmol), stirred for 15 min at room temperature, and then the mixture was refluxed for 5 h followed by addition of 80% HOAc solution (3 mL). After the reaction mixture was cooled to room temperature, it was poured into aq. $NaHCO_3$ and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by silica column chromatography with petroleum ether (PE) : EtOAc (5 : 1) as eluent to give the desired 1,2,3,4-tetrahydrocarbazole-1-one.

*9-Methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a***

To a solution of **3** (185 mg, 1 mmol) in acetone (5 mL) was added NaH (180 mg, 7.5 mmol) and the reaction mixture was stirred for 30 min at ambient temperature. Then, MeI (755 mg, 5 mmol) was added into the reaction mixture and stirred for 4 h. After the completion of the reaction, the mixture was concentrated under reduced pressure. The residue was washed with water, dried and recrystallized from EtOAc. White solid (95%), m.p.: 100 – 101°C (lit [35]. 100°C). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.65 (d, *J* = 8.0 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 1H), 7.06 (t, *J* = 8.0 Hz, 1H), 4.01 (s, 3H), 3.17 (t, *J* = 6.0 Hz, 2H), 2.51 (t, *J* = 6.0 Hz, 2H), 2.25 (m, 2H). ESI-MS: m/z = 200 $[M + 1]^+$. Anal. Calcd. for $C_{13}H_{13}NO$: C, 78.36; H, 6.58; N, 7.03. Found: C, 78.57; H, 6.67; N, 7.23.

*9-Ethyl-1,2,3,4-tetrahydrocarbazole-1-one **4b***

Compound **4b** was synthesized by a similar procedure described for **4a**, using bromoethane as alkylating agent. Yellow solid (93%), m.p.: 46 – 48°C (lit [35], 49 – 50°C). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.67 (d, *J* = 8.0 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.16 (t, *J* = 8.0 Hz, 1H), 4.61 (q, *J* = 7.2 Hz, 2H), 3.15 (t, *J* = 6.0 Hz, 2H), 2.86 (t, *J* = 6.0 Hz, 2H), 2.85 (m, 2H), 1.34 (t, *J* = 7.2 Hz, 3H). ESI-MS: m/z = 214 $[M + 1]^+$. Anal. Calcd. for $C_{14}H_{15}NO$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.54; H, 6.96; N, 6.53.

*9-Butyl-1,2,3,4-tetrahydrocarbazole-1-one **4c***

Compound **4c** was synthesized by a similar procedure described for **4a**, using 1-bromobutane as alkylating agent. After the reaction, the mixture was concentrated under reduced pressure. Then, water added into the residue and extracted with EtOAc. The extracts were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The product was purified by silica column chromatography using PE / EtOAc (50 : 1) as eluent. Yellow oil (63%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.66 (d, *J* = 8.0 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 4.51 (t, *J* = 7.6 Hz, 2H), 3.05 (t, *J* = 6.0 Hz, 2H), 2.85 (t, *J* = 6.0 Hz, 2H), 2.85 (m, 2H), 1.70 (m, 2H), 1.40 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H). ESI-MS: m/z = 242 $[M + 1]^+$. Anal. Calcd. for $C_{16}H_{19}NO$: C, 79.63; H, 7.94; N, 5.80. Found: C, 79.54; H, 7.76; N, 5.57.

*9-Allyl-1,2,3,4-tetrahydrocarbazole-1-one **4d***

Compound **4d** was synthesized by a similar procedure described for **4a**, using allyl bromide as alkylating agent. After the reaction, the mixture was concentrated under reduced pressure. Then, water added into the residue and extracted with EtOAc. The extracts were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The product was purified by silica column chromatography using PE / EtOAc (50 : 1) as eluent. Yellow oil (80%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.68 (d, *J* = 8.0 Hz, 1H), 7.41 (td, *J* = 8.0, 1.2 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.18 (td, *J* = 8.0, 1.2 Hz, 1H), 6.04 (m, 1H), 5.23 (d, *J* = 3.6 Hz, 2H), 5.11 (dd, *J* = 10.0, 1.6 Hz, 1H), 4.96 (dd, *J* = 17.2, 1.6 Hz, 1H), 3.05 (t, *J* = 6.0 Hz, 2H), 2.61 (t, *J* = 6.0 Hz, 2H), 2.26 (m, 2H). ESI-MS: m/z = 226 $[M + 1]^+$. Anal. Calcd. for $C_{15}H_{15}NO$: C, 79.97; H, 6.71; N, 6.22. Found: C, 79.74; H, 7.06; N, 6.47.

*9-Isoprenyl-1,2,3,4-tetrahydrocarbazole-1-one **4e***

Compound **4e** was synthesized by a similar procedure described for **4a**, using isoprenyl bromide as alkylating agent. After the reaction, the mixture was concentrated under reduced pressure. Then water added into the residue and extracted with EtOAc. The extracts were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The product was purified by silica column chromatography using PE / EtOAc (50 : 1) as eluent. Yellow oil (90%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.57 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.18 (t, *J* = 8.0 Hz, 1H), 7.02 (t, *J* = 8.0 Hz, 1H), 5.34 (m, 3H), 3.02 (t, *J* = 6.0 Hz, 2H), 2.57 (t, *J* = 6.0 Hz, 2H), 2.14 (m, 2H), 1.79 (s, 3H), 1.72 (s, 3H). ESI-MS: m/z = 254 $[M + 1]^+$. Anal. Calcd. for $C_{17}H_{19}NO$: C, 80.60; H, 7.56; N, 5.53. Found: C, 80.74; H, 7.66; N, 5.47.

*General method for the synthesis of compounds **5***

A mixture of **4** (0.3 mmol), paraformaldehyde (1.5 mmol), and the amine hydrochloride **6** (1.5 mmol) were refluxed in a mixture of acetic acid and toluene (1 mL, 1 : 4) for 12 h. After cooling down, the mixture was neutralized with aq. Na_2CO_3, and extracted with EtOAc. The extracts were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica column chromatography using eluent mixtures of solvents in the proportions indicated for each case.

*2-Dimethylaminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **5a***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and dimethylamine hydrochloride **6a**. Purification: silica gel column chromatography using PE / EtOAc / Et_3N (50 : 20 : 1). Yellow solid (57%), m.p.: 71 – 73°C (lit [23]. 74 – 75°C). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.63 (d, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.16 (t, *J* = 8.0 Hz, 1H), 4.03 (s, 3H), 3.82 (m, 1H), 3.20 (m, 4H), 2.91 (s, 6H), 2.77 (m, 1H), 2.05 (m,

1H). ESI-MS: m/z = 257 $[M + 1]^+$. Anal. Calcd. for $C_{16}H_{20}N_2O$: C, 74.97; H, 7.86; N, 10.93. Found: C, 74.67; H, 7.77; N, 10.83.

2-Ethyl(methyl)aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5b***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and ethylmethyl- amine hydrochloride **6b**. Purification: silica gel column chromatography using PE / EtOAc / Et_3N (50 : 20 : 1). Yellow solid (74%), m.p.: 154–156°C. ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.67 (d, *J* = 8.0 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.18 (t, *J* = 8.0 Hz, 1H), 4.13 (s, 3H), 3.18 (m, 1H), 3.10 (m, 2H), 2.95 (m, 1H), 2.85 (m, 1H), 2.71 (m, 1H), 2.57 (s, 3H), 2.17 (m, 1H), 1.31 (m, 5H). ESI-MS: m/z = 271 $[M + 1]^+$. Anal. Calcd. for $C_{17}H_{22}N_2O$: C, 75.52; H, 8.20; N, 10.36. Found: C, 75.35; H, 7.97; N, 10.48.

2-Diethylaminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5c***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and diethylamine hydrochloride **6c**. Purification: silica gel column chromatography using PE / EtOAc / Et_3N (100 : 50 : 1). Yellow oil (60%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.65 (d, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.16 (t, *J* = 8.0 Hz, 1H), 4.05 (s, 3H), 3.14 (m, 3H), 2.81 (m, 6H), 2.51 (m, 1H), 2.14 (m, 1H), 1.09 (t, *J* = 7.2 Hz, 6H). ESI-MS: m/z = 285 $[M + 1]^+$. Anal. Calcd. for $C_{18}H_{24}N_2O$: C, 76.02; H, 8.51; N, 9.85. Found: C, 75.85; H, 8.23; N, 9.68.

2-(2′-Ethoxy-2′-oxo-N-methyl)ethylaminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5d***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and sarcosine ethyl ester hydrochloride **6d** (prepared according to ref. [36]). Purification: silica gel column chromatography using PE / Et_3N (40 : 1). Yellow oil (34%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.66 (d, *J* = 8.0 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.17 (t, *J* = 8.0 Hz, 1H), 4.21 (q, *J* = 7.2 Hz, 2H), 4.05 (s, 3H), 3.46 (d, *J* = 16.8 Hz, 1H), 3.36 (d, *J* = 16.8 Hz, 1H), 3.17 (m, 2H), 3.02 (m, 1H), 2.81 (m, 2H), 2.50 (s, 3H), 2.47 (m, 1H), 2.18 (m, 1H), 1.30 (t, *J* = 7.2 Hz, 3H). ESI-MS: m/z =329 $[M + 1]^+$. Anal. Calcd. for $C_{19}H_{24}N_2O_3$: C, 69.49; H, 7.37; N, 8.53. Found: C, 69.26; H, 7.46; N, 8.63.

2-Cyclohexyl(methyl)aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5e***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and *N*-methyl- cyclohexanamine hydrochloride **6e**. Purification: silica gel column chromatography using PE / EtOAc (1 : 1). Brown oil (22%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.66 (d, *J* = 8.0 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.16 (t, *J* =8.0 Hz, 1H), 4.05 (s, 3H), 3.15 (m, 1H), 3.06 (m, 1H), 2.96 (m, 1H), 2.82 (m, 1H), 2.72 (m, 1H), 2.62 (m, 1H), 2.47 (s, 3H), 2.15 (m, 1H), 2.03 (m, 1H), 1.91 (m, 4H), 1.39 (m, 6H). ESI-MS: m/z =325 $[M + 1]^+$. Anal. Calcd. for $C_{21}H_{28}N_2O$: C, 77.74; H, 8.70; N, 8.63. Found: C, 77.46; H, 8.47; N, 8.68.

2-Benzyl(methyl)aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5f***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and *N*-methylbenzyl- amine hydrochloride **6f**. Purification: silica gel column chromatography using PE / EtOAc (10 : 1). Brown oil (45%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.67 (d, *J* = 8.0 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.39 (m, 6H), 7.19 (d, *J* = 8.0 Hz, 1H), 4.08 (s, 3H), 3.74 (d, *J* = 13.2 Hz, 1H), 3.51 (d, *J* = 13.2 Hz, 1H), 3.07 (m, 1H), 2.98 (m, 3H), 2.72 (m, 1H), 2.55 (m, 1H), 2.31 (s, 3H), 2.20 (m, 1H). ESI-MS: m/z =333 $[M + 1]^+$. Anal. Calcd. for $C_{22}H_{24}N_2O$: C, 79.48; H, 7.28; N, 8.43. Found: C, 79.73; H, 7.57; N, 8.26.

2-Benzyl(cyanoethyl)aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5g***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and *N*-benzyl-2- cyanoethylamine hydrochloride **6g**. Purification: silica gel column chromatography using PE / EtOAc (10 : 1). Brown oil (50%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.66 (d, *J* = 8.0 Hz, 1H), 7.42 (m, 7H), 7.19 (t, *J* = 8.0 Hz, 1H), 4.07 (s, 3H), 3.88 (d, *J* = 13.2 Hz, 1H), 3.57 (d, *J* = 13.2 Hz, 1H), 3.11 (m, 1H), 3.00 (m, 3H), 2.83 (m, 1H), 2.77 (t, *J* = 6.8 Hz, 2H), 2.54 (m, 3H), 2.10 (m, 1H). ESI-MS: m/z =372 $[M + 1]^+$. Anal. Calcd. for $C_{24}H_{25}N_3O$: C, 77.60; H, 6.78; N, 11.31. Found: C, 77.37; H, 6.67; N, 11.68.

2-(1H-Imidazol-1-ylmethyl)-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5h***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and 1*H*-imidazole hydrochloride **6h**. Purification: silica gel column chromatography using PE / EtOAc / EtOH (10 : 10 : 1). Yellow solid (82%), m.p.: 56–58°C. ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.51 (d, *J* = 8.0 Hz, 1H), 7.45 (s, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.05 (t, *J* = 8.0 Hz, 1H), 7.00 (s, 1H), 6.95 (s, 1H), 4.30 (d, *J* = 5.6 Hz, 2H), 3.96 (s, 3H), 3.02 (m, 1H), 2.83 (m, 2H), 2.08 (m, 1H), 1.74 (m, 1H). ESI-MS: m/z = 280 $[M + 1]^+$. Anal. Calcd. for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.27; H, 6.25; N, 15.35.

2-(Piperidin-1-ylmethyl)-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5i***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and piperidine hydrochloride **6i**. Purification: silica gel column chromatography using PE / EtOAc (2 : 1). Brown oil (35%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.66 (d, *J* = 8.0 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.17 (t, *J* = 8.0 Hz, 1H), 4.05 (s, 3H), 3.15 (m, 1H), 3.01 (m, 2H), 2.89 (m, 1H), 2.61 (m, 6H), 2.15 (m, 1H), 1.70 (m, 4H), 1.51 (m, 2H). ESI-MS: m/z =297 $[M + 1]^+$. Anal. Calcd. for $C_{19}H_{24}N_2O$: C, 76.99; H, 8.16; N, 9.45. Found: C, 76.75; H, 8.35; N, 9.44.

2-(Pyrrolidin-1-ylmethyl)-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5j***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and pyrrolidine hydrochloride **6j**. Purification: silica gel column chromatography using PE / EtOAc / EtOH (10 : 10 : 1). Yellow solid (28%), m.p.: 69–70°C. ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.55 (d, *J* = 8.0 Hz, 1H), 7.34 (t, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.07 (t, *J* = 8.0 Hz, 1H), 3.92 (s, 3H), 3.70 (m, 1H), 3.41 (m, 3H), 3.13 (m, 3H), 2.98 (m, 2H), 2.09 (m, 4H), 1.94 (m, 1H), 1.15 (m, 1H). ESI-MS: m/z =283 $[M + 1]^+$. Anal. Calcd. for $C_{18}H_{22}N_2O$: C, 76.56; H, 7.85; N, 9.92. Found: C, 76.35; H, 7.83; N, 9.85.

2-Morpholinmethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5k***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and morpholine hydrochloride **6k**. Purification: silica gel column chromatography using PE / EtOAc / EtOH / Et_3N (50 : 50 : 1 : 1). Yellow solid (50%), m.p.: 80 – 82°C. ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.67 (d, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.17 (t, *J* = 8.0 Hz, 1H), 4.06 (s, 3H), 3.77 (m, 4H), 3.15 (m, 1H), 2.99 (m, 1H), 2.90 (m, 1H), 2.81 (m, 1H), 2.58 (m, 6H), 2.17 (m, 1H). ESI-MS: m/z =299 $[M + 1]^+$. Anal. Calcd. for $C_{18}H_{22}N_2O_2$: C, 74.46; H, 7.43; N, 9.39. Found: C, 74.67; H, 7.25; N, 9.53.

2-(4-Methylpiperazin-1-ylmethyl)-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5l***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and *N*-methyl- piperazine hydrochloride **6l**. Purification: silica gel column chromatography using PE / EtOAc / EtOH / Et_3N (50 : 50 : 4 : 1). Yellow solid (64%), m.p.: 53 – 55°C. ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.66 (d, *J* = 8.0 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.17 (t, *J* = 8.0 Hz, 1H), 4.05 (s, 3H), 3.14 (m, 1H), 3.00 (m, 2H), 2.81 (m, 10H), 2.45 (m, 1H), 2.39 (s, 3H), 2.13 (m, 1H). ESI-MS: m/z =312 $[M + 1]^+$. Anal. Calcd. for $C_{19}H_{25}N_3O$: C, 73.28; H, 8.09; N, 13.49. Found: C, 73.35; H, 8.24; N, 13.75.

2-(Methylpiperidin-1-ylmethyl)-9-methyl-1,2,3,4-tetrahydrocarbazole-1-one ***5m***

Reagents: 9-methyl-1,2,3,4-tetrahydrocarbazole-1-one **4a**, paraformaldehyde, and 4-methyl- piperidine hydrochloride **6m**. Purification: silica gel column chromatography using PE / Et_3N (50 : 1). Yellow solid (73%), m.p.: 48 – 50°C. ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.64 (d, *J* = 8.0 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 4.04 (s, 3H), 3.12 (m, 1H), 2.96 (m, 3H), 2.84 (m, 2H), 2.55 (m, 1H), 2.43 (m, 1H), 2.10 (m, 1H), 1.64 (m, 2H), 1.38 (m, 5H), 0.93 (t, *J* = 6.0 Hz, 3H). ESI-MS: m/z =311 $[M + 1]^+$. Anal. Calcd. for $C_{20}H_{26}N_2O$: C, 77.38; H, 8.44; N, 9.02. Found: C, 77.25; H, 8.64; N, 9.35.

2-Morpholinmethyl-9-ethyl-1,2,3,4-tetrahydrocarbazole-1-one ***5n***

Reagents: 9-ethyl-1,2,3,4-tetrahydrocarbazole-1-one **4b**, paraformaldehyde, and morpholine hydrochloride **6k**. Purification: silica gel column chromatography using PE / EtOAc / Et_3N (25 : 1). Yellow solid (26%), m.p.: 79 – 81°C. ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.67 (d, *J* = 8.0 Hz, 1H), 7.41 (m, 2H), 7.16 (td, *J* = 8.0, 1.6 Hz, 1H), 4.60 (q, *J* = 7.2 Hz, 2H), 3.77 (m, 4H), 3.15 (m, 1H), 3.00 (m, 1H), 2.91 (m, 1H), 2.82 (m, 1H), 2.58 (m, 6H), 2.16 (m, 1H), 1.36 (t, *J* = 7.2 Hz, 3H). ESI-MS: m/z =313 $[M + 1]^+$. Anal. Calcd. for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N,8.97. Found: C, 72.86; H, 7.75; N, 9.24.

2-Dimethylaminomethyl-9-allyl-1,2,3,4-tetrahydrocarbazole-1-one ***5o***

Reagents: 9-allyl-1,2,3,4-tetrahydrocarbazole-1-one **4d**, paraformaldehyde, and dimethylamine hydrochloride **6a**. Purification: silica gel column chromatography using PE /Et_3N (50 : 1). Brown oil (26%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.66 (d, *J* = 8.0 Hz, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.16 (t, *J* = 8.0 Hz, 1H), 6.01 (m, 1H), 5.20 (m, 2H), 5.08 (d, *J* = 10.4 Hz, 1H), 4.93 (d, *J* = 17.6Hz, 1H), 3.16 (m, 1H), 3.01 (m, 1H), 2.81 (m, 2H), 2.58 (m, 1H), 2.46 (m, 1H), 2.30 (s, 6H), 2.14 (m, 1H). ESI-MS: m/z = 283 $[M + 1]^+$. Anal. Calcd. for $C_{18}H_{22}N_2O$: C, 76.56; H, 7.85; N, 9.92. Found: C, 76.24; H, 7.64; N, 9.76.

2-Morpholinmethyl-9-isoprenyl-1,2,3,4-tetrahydrocarbazole-1-one ***5p***

Reagents: 9-isoprenyl-1,2,3,4-tetrahydrocarbazole-1-one **4e**, paraformaldehyde, and morpholine hydrochloride **6k**. Purification: silica gel column chromatography using PE / EtOAc (4 : 1). Yellow oil (20%). ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.67 (d, *J* = 8.0 Hz, 1H), 7.40 (m, 2H), 7.17 (t, *J* = 8.0 Hz, 1H), 5.24 (m, 3H), 3.77 (m, 4H), 3.16 (m, 1H), 3.00 (m, 1H), 2.91 (m, 1H), 2.83 (m, 1H), 2.60 (m, 6H), 2.17 (m, 1H), 1.80 (s, 3H), 1.08 (s, 3H). ESI-MS: m/z =353 $[M + 1]^+$. Anal. Calcd. for $C_{22}H_{28}N_2O_2$: C, 74.97; H, 8.01; N,7.95. Found: C, 74.78; H, 7.80; N, 7.76.

2-Morpholinmethyl-9-butyl-1,2,3,4-tetrahydrocarbazole-1-one ***5q***

Reagents: 9-butyl-1,2,3,4-tetrahydrocarbazole-1-one **4c**, paraformaldehyde, and morpholine hydrochloride **6k**. Purification: silica gel column chromatography using PE / EtOAc (5 : 1). Yellow solid (33%), m.p.: 78 – 80°C. ^{1}H-NMR ($CDCl_3$, 400 M, δ): 7.66 (d, *J* = 8.0 Hz, 1H), 7.40 (m, 2H), 7.15 (m, 1H), 4.53 (t, *J* = 7.6 Hz, 2H), 3.77 (m, 4H), 3.15 (m, 1H), 3.00 (m, 2H), 2.82 (m, 1H), 2.60 (m, 6H), 2.15 (m, 1H), 1.76 (m, 2H), 1.40 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H). ESI-MS: m/z =341 $[M + 1]^+$. Anal. Calcd. for $C_{21}H_{28}N_2O_2$: C, 74.08; H, 8.29; N,8.23. Found: C, 74.43; H, 7.98; N, 8.12.

Biological assays

The tumor cell lines (A549, SGC, HCT116, K562, KB-VCR) were obtained from Shanghai Institute of Pharmaceutical Industry, China.

Cytotoxicity assay

The cytotoxic activity *in vitro* was measured using the MTT assay [37]. MTT solution (10.0 μL/well) in RPMI-1640 (Sigma, St. Louis, MO, USA) was added after cells were treated with drug for 48 h, and cells were incubated for a further 4 h at 37°C. The purple formazan crystals were dissolved in 100.0 μL DMSO. After 5 min, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) at 570 nm. Assays were performed in triplicate in three independent experiments. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software ‚Dose-Effect Analysis with Microcomputers'. The tumor cell line panel consisted of A549, SGC, HCT116, K562, and KB-VCR. In all of these experiments, three replicate wells were used to determine each point.

Microtubule polymerization assay

In-vitro tubulin polymerization assays were conducted with reagents as described by the manufacturer (Cytoskeleton, Inc., USA). In brief, compound **5c** was incubated with purified bovine tubulin and buffer containing 20% glycerol and 1 mM GTP at 37°C, and the effect of compound **5c** on tubulin polymerization was monitored kinetically using a fluorescent plate reader.

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