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Design, synthesis, and biological evaluation of novel *N*-γ-carboline arylsulfonamides as anticancer agents

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ABSTRACT

A series of novel *N*-γ-carboline arylsulfonamide derivatives designed based on the common feature of colchicine binding site inhibitors were synthesized and evaluated for their antiproliferative activity in vitro against five human cancer cell lines. Most of the compounds showed moderate to potent cytotoxic activities against all the tested cells. Preliminary mechanism research on one of the most potent compound **6p** indicated that it was a potent tubulin polymerization inhibitor, with IC_{50} value of 3.8 μM, equivalent to that of CA-4, and arresting cell cycle in G_2/M phase.

1. Introduction

Antimitotic agents, which arrest the cell cycle at the G_2/M phase and lead to apoptotic cell death and tumor regression, have been the most successful pharmacological agents for the treatment of cancer.[1,2] Most of the antimitotics are tubulin-binding agents. So far, several small-molecule antimitotics have been developed to antagonize one of the three major binding-sites on tubulin, namely, the vinca-, the taxus-, and the colchicine binding site.[3] While the vinca alkaloids and the taxanes have well-established roles in the treatment of human cancers,[4–6] no colchicine binding site inhibitors (CSIs) are currently approved for cancer therapy. However, a large number of natural and synthetic small molecules of varied structures have been identified as CSIs over the years,[7,8] some are undergoing clinical trials for cancer therapy, such as combretastatin A-4 phosphate (CA-4P, **1**) and E7010 (**2**),[9,10] thus suggesting a high plasticity of this binding site. Quite a few attempts to describe the common pharmacophore and structural features of CSIs have been made,[11,12] which can be used for rational design of new antitubulin agents. It is reported that most CSIs bear two hydrophobic groups (ring A and B) and a *cis*-bridge between them. The volume size of ring A is often bigger than ring B. It is also reported that the existence of hydrogen-bond acceptor at ring A and hydrophobic groups or polar functional groups at ring B are beneficial for binding affinity.

2. Design

During our studies on new antitumor agents, we previously reported three series of potent cytotoxic compounds, characterized by the presence of γ-carboline connected with aromatic rings through different linkers (**3–5**, Fig. 1).[13] We supposed that these

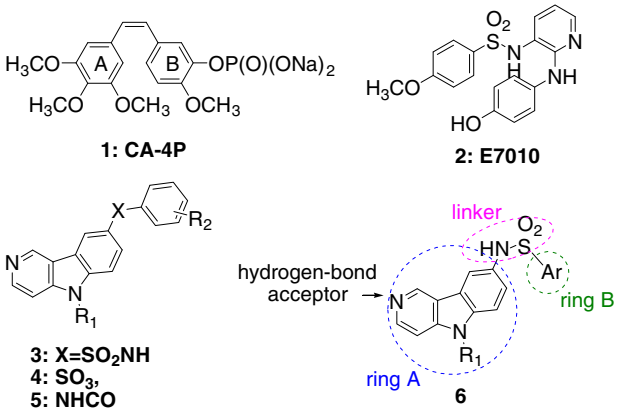

Figure 1. Structure of CA-4P, E7010, and γ-carboline derivatives.

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compounds match well with the common structure of CSIs: the planar γ-carboline acts as the hydrophobic ring A of CSIs, and the N^2 position of carboline contributes as a hydrogen-bond acceptor; the aromatic ring connected to the linker is the hydrophobic ring B of CSIs. Further mechanism study showed that these compounds exhibited antitubulin activity (data haven't been revealed yet). Enlightened by the results and for the purpose of further study on structure–activity relationships (SARs) of γ-carbolines, as well as developing more potent antitubulin agents, we reversed the linker of compounds **3** and designed a novel series of *N*-γ-carboline arylsulfonamide derivatives **6**. According to the reported feature of CSIs,[12] we also introduced hydrophobic substituents, such as halogen and methoxy group, as well as polar functional groups, such as amino group, on ring B (Fig. 1). We calculated 'cdocker-energy' of **3a–c** and **6a–c** by molecular modeling studies using the Discovery Studio 2.1/cdocker approach. The results showed that the 'cdocker-energy' of the new designed compounds **6a–c** were lower than those of the corresponding compounds **3a–c** except for compound **6b** (Table 1). This result implied that reversing the linker of compounds **3** might lead to the more effective antitubulin agents. Besides, carboline skeleton has been identified as an excellent scaffold structure with DNA intercalation.[14] Thus, we expected the resulting compounds would possess improved antitumor activities by interaction with both tubulin and DNA. Here, we report the synthesis and biological evaluation of a series of novel *N*-γ-carboline arylsulfonamides. The docking studies of the interaction will also be discussed.

3. Chemistry

The synthetic routes of *N*-γ-carboline arylsulfonamide derivatives **6a–z** were diagrammed in Scheme 1.[13] γ-Carboline **7** was prepared using our recently developed synthetic protocol.[15] Compound **7** was nitrified with concd HNO_3–H_2SO_4 to provide 6-nitro-γ-carboline **8a**, and then the N^5 of **8a** was protected as carbamate **8b** or alkylated with C_2H_5Br to give compound **8c**. Reduction the nitro group of compound **8** with Raney Ni in EtOH yielded the corresponding amino compound **9a–c**. Condensation of compound **9a** or **9c** with corresponding arylsulfonyl chlorides in the presence of pyridine in DMF at room temperature afforded the N^5 substituted products **6n–w**. Among them, the 3′-nitro group of compound **6r** could be reduced with Raney Ni to yield the 3′-amino compound **6x**, while compound **6p** was further transformed into **6y** by the N-alkylation of sulfonamide group with C_2H_5Br. The N^5 unsubstituted arysulfonamides **6a–m** were obtained through two steps. First, reaction of **9b** with various arylsulfonyl chlorides to give the N^5-*tert*-butoxycarbonyl (Boc) substituted compounds, such as **6z**. Then, N^5-Boc substituted compounds could be transformed into the target compounds **6a–m** by hydrolysis with concd HCl–EtOH.

4. Biological studies and discussion

The synthesized *N*-γ-carboline arylsulfonamides **6** were tested for their cytotoxic activities in vitro against several human cancer cell lines including human non-small lung cancer cells A549, human gastric adenocarcinoma SGC, human colon cancer cell HCT116, human breast carcinoma cell MCF-7, and human myeloid leukemia cell K562 by MTT assay for 48 h. Taxol and previously reported compounds **3a–c** was employed as the positive control. The results are summarized in Table 2.

As shown in Table 2, most of the tested compounds showed moderate to potent cytotoxic activities against all the tested cells. Ten of the twenty-six new compounds showed strong cytotoxic activity against more than three cell lines (IC_{50} <10 μM). Among them, compounds **6p** and **6t** were the most promising compounds against all the tested cell lines, with IC_{50} values of 0.47–11.29 and 0.17–6.73 μM, respectively.

The N^5-phenylsulfonyl substituted compounds **6n** and **6o** exhibited no activities against all the tested cells (IC_{50} >100 μM), except for K562 cell. N^5-Boc or N^5-ethyl substituted compounds were more potent cytotoxic activity than those of N^5 unsubstituted compounds (**6z** vs **6g**, **6p** vs **6c**, **6r** vs **6i**). These implied that introducing proper substituents at N^5 position was beneficial to the cytotoxic activity. In addition, compound **6y**, with an ethyl group at the nitrogen atom of sulfonamide group, showed significantly increased potency (fivefold) against K562 cell in comparison with **6p**. This indicated that introducing ethyl group to the linker was beneficial to cytotoxic activity. This result was similar to the SAR of our previously reported series **3**.[13]

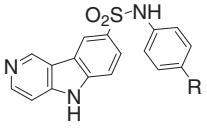
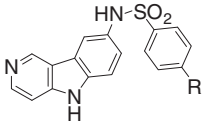
The influence of various substituents on the phenyl ring was also examined. Comparing the cytotoxic activities of compounds **6c–e** revealed that the effects of different halogen atom at *para*-position of phenyl ring was: chlorine > bromine > fluorine atom. However, two chlorine atoms substituted compound **6f** exhibited decreased activity. Besides, a comparison between compounds **6q** and **6s–v** revealed the influence of methoxy group at different position of phenyl ring. It could be concluded as: 2,4-dimethoxy > 3,4-dimethoxy > 4-methoxy > 2,4,6-trimethoxy > 2,5-dimethoxy. Moreover, replacement of the phenyl with naphthalene led compound **6m**, which retained potent cytotoxic activity against A549 and SGC cell lines, with IC_{50} values of 8.80 and 5.65 μM, respectively.

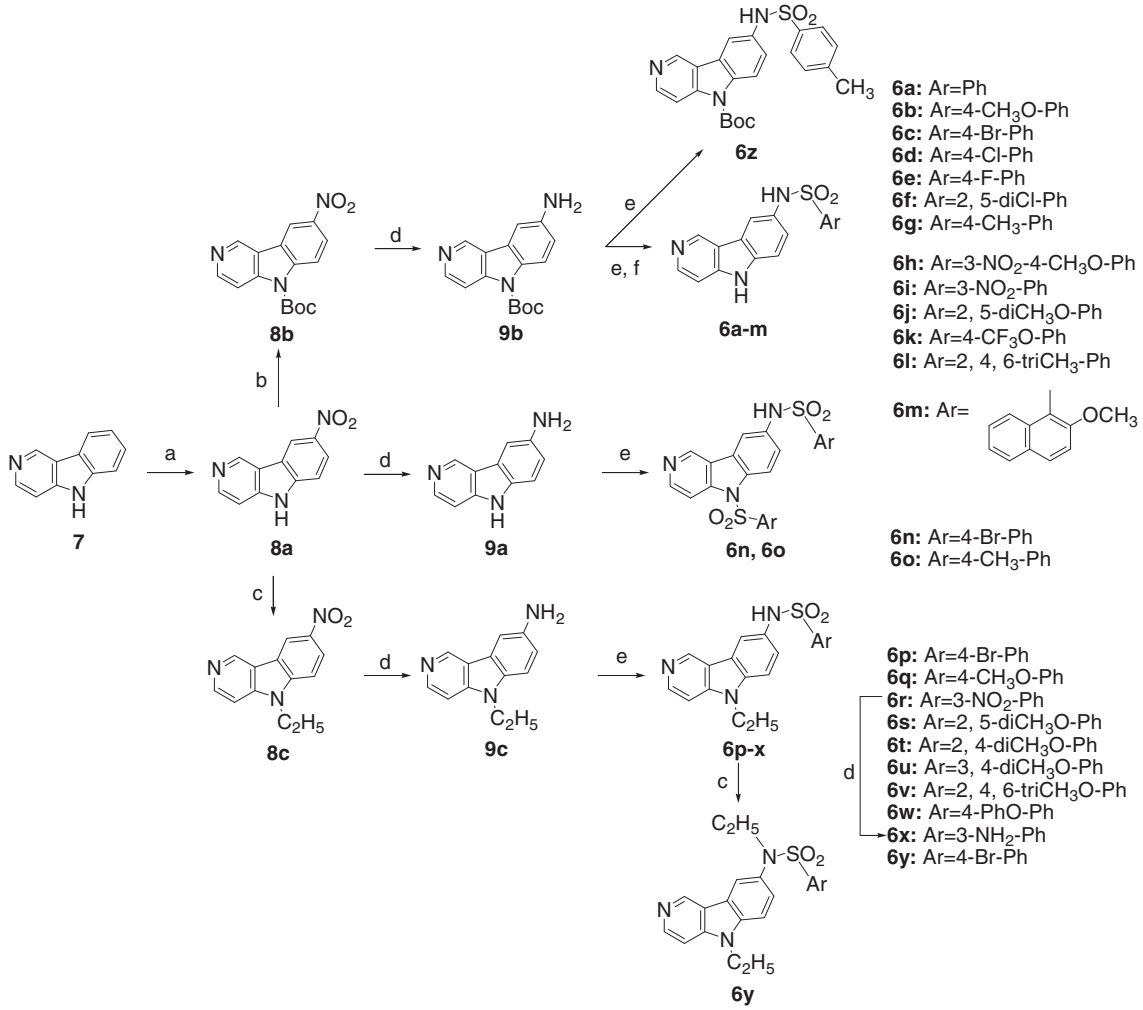
At last, it was worthy to point out that the cytotoxic activity of compounds **6a–c** were more potent than our previously synthesized compounds **3a–c** (Table 2).

Because the compounds were designed to binding to tubulin, flow cytometry experiments were carried out to determine whether the synthesized compounds could lead to cell cycle arrest at G_2/M phase. A549 cells were treated with **6p** (2 μM), one of the most potent compounds, for 48 h, and CA-4 (1 μM) was used as a positive control. Figure 2 showed that compound **6p** caused a marked increase in the percentage of cells in the G_2/M phase of the cell cycle, with a simultaneous decrease of cells in G_0/G_1 and S phase. It was in the similar trend as CA-4.

To confirm whether tubulin was the target of these compounds, five of the most promising *N*-γ-carboline arylsulfonamides **6p**, **6t–v**, and **6y** were evaluated for their inhibition of polymerization of purified tubulin in a cell-free system. CA-4 was used as a positive control. The results are shown in Figure 3. In this assay, CA-4 inhibited tubulin polymerization by 85.7% at 3 μM. All the tested compounds (**6p**, **6t–v** and **6y**) displayed a pattern similar to CA-4,

Table 1
Calculated 'cdocker-energy' of compounds **3a–c** and **6a–c**

No.	Structure	R	Cdocker-energy	No.	Structure	R	Cdocker-energy
3a		H	−14.345	**6a**		H	−18.749
3b		OCH_3	−17.466	**6b**		OCH_3	−17.149
3c		Br	−17.611	**6c**		Br	−18.656



Scheme 1. Reagents and conditions: (a) HNO_3–H_2SO_4, 0 °C to rt, 4 h; (b) $(Boc)_2O$, DMAP, dry THF, N_2, rt 24 h; (c) C_2H_5Br, NaH, dry THF–DMF, rt, 0.5 h; (d) Raney Ni, EtOH, H_2, rt, 2 h; (e) $ArSO_2Cl$, pyridine, DMF, rt, 0.5 h; (f) concd HCl–EtOH, rt, 12 h.

Table 2
In vitro cytotoxic activities of the synthesized compounds against five human cancer cell lines

Compd	Cytotoxicity (IC_{50}, μM)[a,b]					Compd	Cytotoxicity (IC_{50}, μM)				
	A549	SGC	HCT116	MCF7	K562		A549	SGC	HCT116	MCF7	K562
6a	45.52	91.29	—[c]	61.97	14.16	**6p**	1.88	1.51	11.29	3.79	0.47
6b	23.68	11.69	9.59	17.94	1.39	**6q**	15.21	15.63	9.68	3.70	3.75
6c	9.62	40.17	11.91	7.91	5.97	**6r**	10.62	8.30	6.38	1.31	7.67
6d	5.25	4.67	—	19.00	3.47	**6s**	44.26	17.59	37.70	2.62	3.01
6e	6.65	8.41	58.24	>100	1.64	**6t**	3.50	0.29	6.00	6.73	0.17
6f	60.29	48.79	—	76.22	16.70	**6u**	10.81	0.41	12.76	11.15	0.12
6g	16.83	49.35	57.73	10.85	19.62	**6v**	21.00	2.20	21.20	6.34	0.068
6h	38.44	21.15	—	30.25	28.91	**6w**	11.45	5.08	21.85	9.46	0.65
6i	38.44	24.94	—	31.71	18.31	**6x**	—	6.74	—	—	0.82
6j	17.11	55.06	3.49	15.78	5.84	**6y**	7.78	2.11	3.58	1.35	0.094
6k	7.07	6.06	27.79	11.31	2.00	**6z**	21.05	42.35	0.89	—	5.51
6l	7.69	18.96	>100	27.45	2.33	**3a**	78.39	>100	35.16	60.12	21.03
6m	8.80	5.65	16.83	18.02	—	**3b**	—	—	—	—	33.87
6n	>100	>100	>100	—	40.93	**3c**	—	—	—	—	16.43
6o	>100	>100	>100	—	35.40	Taxol	2.46	3.34	4.37	2.11	1.16

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

and they inhibited tubulin polymerization by 60.6%, 43.4%, 37.7%, 35.1%, and 30.6%, respectively, at 4 μM. The most potent antitubulin compound **6p** was further examined at concentrations from 1 to 8 μM. The IC_{50} value of its tubulin polymerization inhibition was 3.8 μM, similar to that of CA-4 (1.5 μM). Comparing the tubulin inhibition with corresponding cytotoxic activities revealed that

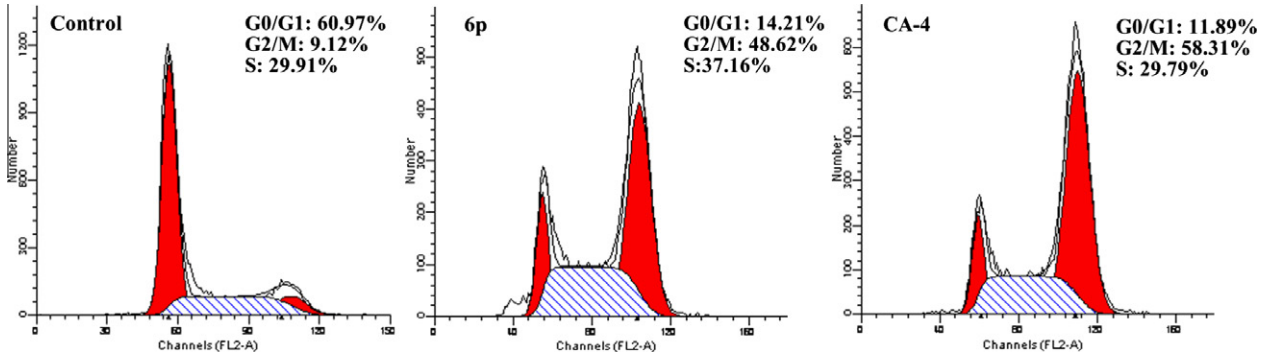


Figure 2. Effect of compound **6p** (2 μM) and CA-4 (1 μM) on the cell cycle of A549 cells.

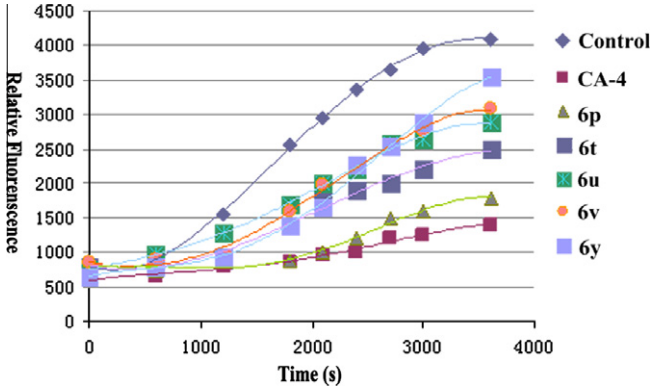


Figure 3. Effect of compounds **6p**, **6t**–**v**, and **6y** (4 μM) on tubulin assembly.

the two data were correlated except for compound **6y**, which showed increased potency (fivefold) against K562 cell with half decreased tubulin inhibition compared with **6p**. It implied that compound **6y** might act against another target.

In view of compounds possessing carboline moiety displayed DNA intercalation.[14] Compounds **6p**, **6t**, and **6y** were also evaluated by DNA interaction assay (ethidium bromide (EB) displacement) (Fig. 4). It showed that the fluorescence of the DNA-EB complex was diminished only by treatment with compound **6y**, while compounds **6p** and **6t** had no effect on it. This result indicated that most of the *N*-γ-carboline arylsulfonamides couldn't intercalate DNA. But introducing ethyl group at the nitrogen atom of sulfonamide group would improve its DNA affinity. Combined with the weak tubulin inhibition of **6y**, we supposed the mechanism of cytotoxic activity of **6y** was different from the other synthesized *N*-γ-carboline arylsulfonamides.

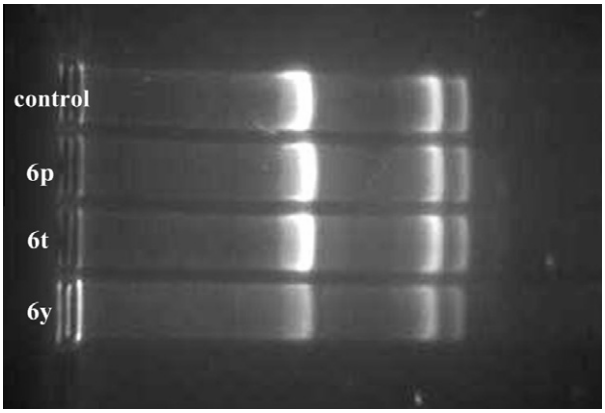


Figure 4. In vitro DNA intercalation assay for compounds **6p**, **6t** and **6y** (0.2 μM).

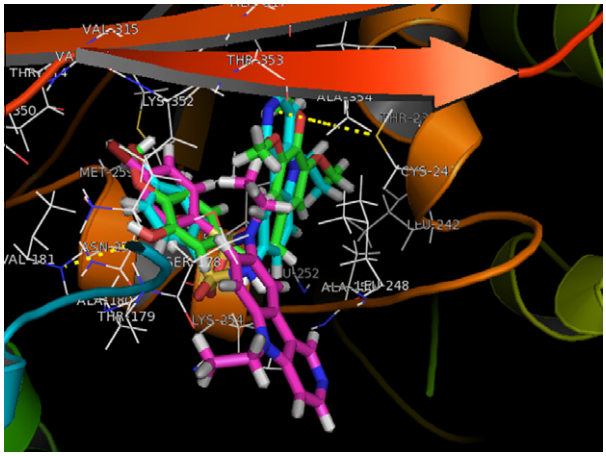


Figure 5. Proposed binding mode for CA-4 (in green), compounds **6p** (in blue) and **6y** (in mauve) in the colchicine site of tubulin.

The proposed mechanism of action was also supported by docking studies of compounds **6p** and **6y** in the colchicine site of tubulin by using the reported high-resolution crystal structure of tubulin-DAMAcolchicine complex.[16] Figure 5 showed both compounds **6p** and **6y** located in the same pocket on β-tubulin as CA-4. Among them, the docking conformation of compound **6p** well overlaps with CA-4 in the crystallized protein complex. γ-Carboline moiety and the substituted phenyl ring lay in the hydrophobic pocket between Alaβ250–Alaβ316 and Valα181–Metβ259, respectively. Introduction of ethyl group at the nitrogen atom of sulfonamide group (**6y**) would lead to the modification of the conformation as shown in Figure 5. γ-Carboline moiety shift away form the hydrophobic pocket. This result was in accordance with the observation that compound **6y** is not as effective as compound **6p** as antitubulin agent. Additionally, N^2 position of carboline formed a hydrogen-bond with Cysβ241, which always found in CSIs.

5. Conclusion

In summary, a series of novel *N*-γ-carboline arylsulfonamides were synthesized by reverse the sulfonamide linkage of the lead compounds **3**. Most of the synthesized compounds showed moderate to potent cytotoxic activities against all the tested cells, and improved activity than the lead compounds **3**. Preliminary mechanism research indicated the synthesized compounds could inhibit tubulin polymerization. The IC_{50} value of compound **6p**

was 3.8 μM, similar to that of CA-4. Moreover, it could also lead to cell cycle arrest at G_2/M phase. Introducing ethyl group at the nitrogen atom of sulfonamide group gave more cytotoxic compound **6y** with reduced antitubulin activity. Further DNA interaction assay indicated this modification would improve its DNA affinity. With all these results, further design and synthesis of potent antitumor agents are ongoing in our laboratory and the results will be reported in due course.

6. Experimental

Reagents and solvents were purchased from commercial sources. Melting points were obtained on a B-540 Büchi melting-point apparatus and are uncorrected. ^{1}H NMR spectra was recorded on a Brüker AM 400 instrument at 400 MHz (chemical shifts are expressed as δ values relative to TMS as internal standard). ESI (positive) was recorded on an Esquire-LC-00075 spectrometer. Element analyses were performed on an Eager 300 instrument.

6.1. Synthesis

6.1.1. General procedure for synthesis of *N*-γ-carboline arylsulfonamides 6

A mixture of 5-ethyl-8-amino-γ-carboline **9c** (1 mmol), arylsulfonyl chlorides (1 mmol) in DMF (5 mL) was stirred for 5 min at room temperature, pyridine (324 μL, 4 mmol) was added. Then, the mixture was stirred for an additional 0.5 h. After adding ice water (10 mL), the mixture was extracted with EtOAc (3 × 20 mL). The organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified over silica column chromatography using petroleum ether (PE)/EtOAc/EtOH (20:20:1, v/v/v) as eluent to afford N^5-ethyl products **6p–w**.

In the same manner, the N^5-phenylsulfonyl products **6n** and **6o** were synthesized by reaction of 8-amino-γ-carboline **9a** (1 mmol) with corresponding arylsulfonyl chlorides (2 mmol). N^5-Boc product (e.g., **6z**) were synthesized by reaction of 5-Boc-8-amino-γ-carboline **9b** (1 mmol) with various arylsulfonyl chloride (1 mmol). Then the N^5-Boc product (0.5 mmol) was mixed with concd HCl–EtOH (1 mL, 1:1), and stirred overnight at room temperature. After that, the mixture was filtrated. The residue was neutralized with 10% NaOH, and extracted with AcOEt (3 × 5 mL). The organic phase was washed with brine (2 × 5 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to afford N^5-unsubstituted products **6a–m**.

6.1.1.1. *N*-(5*H*-γ-Carboline-8-yl)benzenesulfonamide (6a). White solid (78%), mp: 217–219 °C. ^{1}H NMR (δ, DMSO-d_6): 11.63 (br s, 1H, NH), 9.18 (s, 1H), 8.37 (d, 1H, *J* = 5.2 Hz), 7.83 (s, 1H), 7.72 (d, 2H, *J* = 8.0 Hz), 7.51 (m, 3H), 7.39 (d, 1H, *J* = 5.2 Hz), 7.36 (d, 1H, *J* = 8.4 Hz), 7.10 (d, 1H, *J* = 8.4 Hz). ESI-MS: *m*/*z* = 324 [M+1]$^+$. Anal. Calcd for $C_{17}H_{13}N_3O_2S$: C, 63.14; H, 4.05; N, 12.99. Found: C, 63.26; H, 3.85; N, 12.88.

6.1.1.2. 4-Methoxy-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6b). White solid (81%), mp: 115–117 °C. ^{1}H NMR (δ, DMSO-d_6): 12.39 (br s, 1H, NH), 10.11 (br s, 1H, NH), 9.52 (s, 1H), 8.50 (d, 1H, *J* = 5.2 Hz), 8.06 (d, 1H, *J* = 2.0 Hz), 7.68 (m, 3H), 7.53 (d, 1H, *J* = 8.8 Hz), 7.23 (dd, 1H, *J* = 8.8, 2.0 Hz), 7.02 (d, 2H, *J* = 8.8 Hz), 3.75 (s, 3H). ESI-MS: *m*/*z* = 354 [M+1]$^+$. Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 61.18; H, 4.28; N, 11.89. Found: C, 61.24; H, 4.52; N, 12.11.

6.1.1.3. 4-Bromo-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6c). White solid (54%), mp: 183–185 °C. ^{1}H NMR (δ, DMSO-d_6): 12.59 (br s, 1H, NH), 10.38 (br s, 1H, NH), 9.58 (s, 1H), 8.52 (d, 1H, *J* = 6.4 Hz), 8.09 (s, 1H), 7.74 (m, 3H), 7.63 (d, 2H, *J* = 8.0 Hz), 7.57 (d, 1H, *J* = 8.0 Hz), 7.23 (d, 1H, *J* = 8.0 Hz). ESI-MS: *m*/*z* = 402 [M+1]$^+$. Anal. Calcd for $C_{17}H_{12}BrN_3O_2S$: C, 50.76; H, 3.01; N, 10.45. Found: C, 50.75; H, 2.95; N, 10.34.

6.1.1.4. 4-Chloro-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6d). White solid (36%), mp: 119–121 °C. ^{1}H NMR (δ, DMSO-d_6): 11.76 (br s, 1H, NH), 10.21 (br s, 1H, NH), 9.26 (s, 1H), 8.40 (d, 1H, *J* = 3.6 Hz), 7.79 (s, 1H), 7.70 (d, 2H, *J* = 8.0 Hz), 7.59 (d, 2H, *J* = 8.0 Hz), 7.44 (m, 2H), 7.12 (dd, 1H, *J* = 8.0, 1.6 Hz). ESI-MS: *m*/*z* = 358 [M+1]$^+$. Anal. Calcd for $C_{17}H_{12}ClN_3O_2S$: C, 57.06; H, 3.38; N, 11.74. Found: C, 56.94; H, 3.52; N, 11.58.

6.1.1.5. 4-Fluoro-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6e). White solid (70%), mp: 151–153 °C. ^{1}H NMR (δ, DMSO-d_6): 11.68 (br s, 1H, NH), 10.16 (br s, 1H, NH), 9.24 (s, 1H), 8.39 (d, 1H, *J* = 6.0 Hz), 7.89 (s, 1H), 7.76 (m, 2H), 7.42 (m, 2H), 7.35 (t, 2H, *J* = 8.0 Hz), 7.11 (d, 1H, *J* = 8.0 Hz). ESI-MS: *m*/*z* = 342 [M+1]$^+$. Anal. Calcd for $C_{17}H_{12}FN_3O_2S$: C, 59.81; H, 3.54; N, 12.31. Found: C, 59.87; H, 3.76; N, 12.45.

6.1.1.6. 2,5-Dichloro-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6f). White solid (43%), mp: >250 °C. ^{1}H NMR (δ, DMSO-d_6): 11.17 (br s, 1H, NH), 9.01 (s, 1H), 8.26 (d, 1H, *J* = 5.2 Hz), 7.93 (d, 1H, *J* = 2.0 Hz), 7.54 (s, 1H), 7.40 (d, 1H, *J* = 8.8 Hz), 7.34 (dd, 1H, *J* = 8.4, 2.0 Hz), 7.29 (d, 1H, *J* = 5.2 Hz), 7.16 (d, 1H, *J* = 8.4 Hz), 6.99 (d, 1H, *J* = 8.8 Hz). ESI-MS: *m*/*z* = 392 [M+1]$^+$. Anal. Calcd for $C_{17}H_{11}Cl_2N_3O_2S$: C, 52.05; H, 2.83; N, 10.71. Found: C, 51.97; H, 2.57; N, 10.43.

6.1.1.7. 4-Methyl-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6g). White solid (57%), mp: 115–117 °C. ^{1}H NMR (δ, DMSO-d_6): 11.66 (br s, 1H, NH), 9.20 (s, 1H), 8.37 (d, 1H, *J* = 5.6 Hz), 7.85 (d, 1H, *J* = 1.6 Hz, H-5), 7.60 (d, 2H, *J* = 8.0 Hz), 7.41 (d, 1H, *J* = 5.6 Hz), 7.34 (d, 1H, *J* = 8.8 Hz), 7.27 (d, 2H, *J* = 8.0 Hz), 7.12 (dd, 1H, *J* = 8.8, 1.6 Hz), 2.2 (s, 3H). ESI-MS: *m*/*z* = 338 [M+1]$^+$. Anal. Calcd for $C_{18}H_{15}N_3O_2S$: C, 64.08; H, 4.48; N, 12.45. Found: C, 64.24; H, 4.64; N, 12.14.

6.1.1.8. 4-Methoxy-3-nitro-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6h). White solid (60%), mp: 118–120 °C. ^{1}H NMR (δ, DMSO-d_6): 11.88 (br s, 1H, NH), 10.26 (br s, 1H, NH), 9.35 (s, 1H), 8.45 (d, 1H, *J* = 4.8 Hz), 8.24 (s, 1H), 8.00 (s, 1H), 7.92 (d, 1H, *J* = 8.8 Hz), 7.51 (d, 1H, *J* = 4.8 Hz), 7.50 (d, 2H, *J* = 8.8 Hz), 7.18 (d, 1H, *J* = 8.8 Hz), 3.96 (s, 3H). ESI-MS: *m*/*z* = 399 [M+1]$^+$. Anal. Calcd for $C_{16}H_{14}N_4O_5S$: C, 54.27; H, 3.54; N, 14.06. Found: C, 54.52; H, 3.35; N, 14.36.

6.1.1.9. 3-Nitro-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6i). White solid (37%), mp: 189–191 °C. ^{1}H NMR (δ, DMSO-d_6): 11.93 (br s, 1H, NH), 10.50 (br s, 1H, NH), 9.35 (s, 1H), 8.50 (s, 1H), 8.45 (m, 2H), 8.08 (d, 1H, *J* = 8.0 Hz), 7.99 (s, 1H), 7.83 (t, 1H, *J* = 8.0 Hz), 7.52 (d, 1H, *J* = 5.6 Hz), 7.49 (d, 1H, *J* = 8.0 Hz), 7.17 (dd, 1H, *J* = 8.0, 2,0 Hz). ESI-MS: *m*/*z* = 369 [M+1]$^+$. Anal. Calcd for $C_{17}H_{12}N_4O_4S$: C, 55.43; H, 3.28; N, 15.21. Found: C, 55.62; H, 3.32; N, 15.43.

6.1.1.10. 2,5-Dimethoxy-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6j). White solid (63%), mp: 187–189 °C. ^{1}H NMR (δ, DMSO-d_6): 12.77 (br s, 1H, NH), 10.03 (br s, 1H, NH), 9.63 (s, 1H), 8.52 (d, 1H, *J* = 6.0 Hz), 8.09 (s, 1H), 7.77 (d, 1H, *J* = 6.0 Hz), 7.57 (d, 1H, *J* = 8.0 Hz), 7.33 (dd, 1H, *J* = 8.0, 1.6 Hz), 7.22 (d, 1H, *J* = 2.8 Hz), 7.12 (m, 2H), 3.87 (s, 3H), 3.65 (s, 3H). ESI-MS: *m*/*z* = 384 [M+1]$^+$. Anal. Calcd for $C_{19}H_{17}N_3O_4S$: C, 59.52; H, 4.47; N, 10.96. Found: C, 59.53; H, 4.68; N, 11.02.

6.1.1.11. 4-Trifluoromethoxy-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6k). White solid (81%), mp: 246–248 °C. ^{1}H NMR (δ, DMSO-d_6): 12.07 (br s, 1H, NH), 10.30 (br s, 1H, NH), 9.37 (s, 1H), 8.44 (d, 1H, J = 4.4 Hz), 7.98 (s, 1H), 7.83 (d, 2H, J = 8.4 Hz), 7.55 (m, 4H), 7.16 (d, 1H, J = 8.4 Hz). ESI-MS: m/z = 408 [M+1]$^+$. Anal. Calcd for $C_{16}H_{12}F_3N_3O_3S$: C, 53.07; H, 2.97; N, 10.32. Found: C, 53.24; H, 2.79; N, 10.42.

6.1.1.12. 2,4,6-Trimethyl-*N*-(5*H*-γ-carboline-8-yl)benzenesulfonamide (6l). White solid (30%), mp: >250 °C. ^{1}H NMR (δ, DMSO-d_6): 11.65 (br s, 1H, NH), 9.15 (s, 1H), 8.36 (d, 1H, J = 5.2 Hz), 7.75 (s, 1H), 7.40 (d, 1H, J = 5.2 Hz), 7.30 (d, 1H, J = 8.8 Hz), 7.04 (d, 1H, J = 8.8 Hz), 6.91 (s, 2H), 2.17 (s, 9H). ESI-MS: m/z = 366 [M+1]$^+$. Anal. Calcd for $C_{20}H_{19}N_3O_2S$: C, 65.73; H, 5.24; N, 11.50. Found: C, 65.57; H, 5.36; N, 11.64.

6.1.1.13. 2-Methoxy-*N*-(5*H*-γ-carboline-8-yl)naphthalene-1-sulfonamide (6m). White solid (68%), mp: 205–207 °C. ^{1}H NMR (δ, DMSO-d_6): 11.60 (br s, 1H, NH), 9.20 (s, 1H), 8.36 (d, 1H, J = 5.6 Hz), 8.27 (d, 1H, J = 1.6 Hz), 7.97 (d, 1H, J = 9.2 Hz), 7.91 (m, 2H), 7.72 (dd, 1H, J = 8.8, 2.0 Hz), 7.38 (m, 2H), 7.32 (d, 1H, J = 8.4 Hz), 7.22 (dd, 1H, J = 8.8, 2.0 Hz), 7.12 (dd, 1H, J = 8.4, 1.6 Hz), 3.86 (s, 3H). ESI-MS: m/z = 404 [M+1]$^+$. Anal. Calcd for $C_{22}H_{17}N_3O_3S$: C, 65.49; H, 4.25; N, 10.42. Found: C, 65.36; H, 4.45; N, 10.32.

6.1.1.14. 4-Bromo-*N*-(5-(4-bromophenylsulfonyl)-5*H*-γ-carboline-8-yl)benzenesulfonamide (6n). White solid (87%), mp: >250 °C. ^{1}H NMR (δ, DMSO-d_6): 10.60 (br s, 1H, NH), 9.38 (s, 1H), 8.68 (d, 1H, J = 6.0 Hz), 8.14 (d, 1H, J = 6.0 Hz), 8.13 (d, 1H, J = 8.8 Hz), 7.95 (d, 1H, J = 1.6 Hz), 7.87 (d, 2H, J = 8.8 Hz), 7.75 (m, 4H), 7.69 (d, 2H, J = 8.8 Hz), 7.30 (dd, 1H, J = 8.8, 1.6 Hz). ESI-MS: m/z = 620 [M+1]$^+$. Anal. Calcd for $C_{23}H_{15}Br_2N_3O_4S_2$: C, 44.46; H, 2.43; N, 6.76. Found: C, 44.36; H, 2.63; N, 6.72.

6.1.1.15. 4-Methyl-*N*-(5-(4-methylphenylsulfonyl)-5*H*-γ-carboline-8-yl)benzenesulfonamide (6o). White solid (90%), mp: >250 °C. ^{1}H NMR (δ, DMSO-d_6): 10.33 (br s, 1H, NH), 9.58 (s, 1H), 8.82 (d, 1H, J = 6.4 Hz), 8.68 (d, 1H, J = 6.4 Hz), 8.17 (d, 1H, J = 8.8 Hz), 8.01 (d, 1H, J = 2.0 Hz), 7.83 (d, 2H, J = 8.0 Hz), 7.73 (d, 2H, J = 8.0 Hz), 7.61 (dd, 1H, J = 2.0, 8.8 Hz), 7.30 (d, 2H, J = 8.0 Hz), 7.23 (d, 2H, J = 8.0 Hz), 2.37 (s, 3H), 2.35 (s, 3H). ESI-MS: m/z = 492 [M+1]$^+$. Anal. Calcd for $C_{25}H_{21}N_3O_4S_2$: C, 61.08; H, 4.31; N, 8.55. Found: C, 61.36; H, 4.15; N, 8.52.

6.1.1.16. 4-Bromo-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (6p). White solid (56%), mp: 154–156 °C. ^{1}H NMR (δ, DMSO-d_6): 9.24 (s, 1H), 8.57 (d, 1H, J = 6.4 Hz), 8.04 (s, 1H), 7.86 (d, 1H, J = 6.4 Hz), 7.62 (d, 2H, J = 8.8 Hz), 7.54 (d, 2H, J = 8.8 Hz), 7.35 (m, 2H), 4.36 (q, 2H, J = 7.2 Hz), 1.47 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 430 [M+1]$^+$. Anal. Calcd for $C_{19}H_{16}BrN_3O_2S$: C, 53.03; H, 3.75; N, 9.76. Found: C, 53.24; H, 3.64; N, 9.54.

To a solution of **6p** (215 mg, 0.5 mmol) in dry THF–DMF (1 mL, 1:1) was added NaH (18 mg, 0.75 mmol) and the reaction mixture was stirred for 30 min at ambient temperature. Then C_2H_5Br (45 μL, 0.6 mmol) was added into the reaction mixture and stirred for 30 min. Then water was added into the residue and extracted with EtOAc (3 × 10 mL). The organic phase was washed with brine (2 × 10 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The product was purified by silica column chromatography using PE/EtOAc/EtOH (5:5:1, v/v/v) as eluent to afford 4-bromo-*N*-ethyl-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (**6y**) as a white solid (31%), mp: 103–105 °C. ^{1}H NMR (δ, DMSO-d_6): 9.33 (s, 1H), 8.50 (d, 1H, J = 5.6 Hz), 8.04 (d, 1H, J = 1.6 Hz), 7.82 (d, 2H, J = 8.4 Hz), 7.69 (d, 1H, J = 8.8 Hz), 7.66 (d, 1H, J = 5.6 Hz), 7.54 (d, 2H, J = 8.4 Hz), 7.15 (dd, 1H, J = 8.8, 1.6 Hz), 4.48 (q, 2H, J = 7.2 Hz), 3.71 (q, 2H, J = 7.2 Hz), 1.34 (t, 3H, J = 7.2 Hz), 1.03 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 458 [M+1]$^+$. Anal. Calcd for $C_{21}H_{20}BrN_3O_2S$: C, 55.03; H, 4.40; N, 9.17. Found: C, 54.89; H, 4.24; N, 9.15.

6.1.1.17. 4-Methoxy-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (6q). White solid (40%), mp: 122–124 °C. ^{1}H NMR (δ, DMSO-d_6): 9.24 (s, 1H), 8.45 (d, 1H, J = 6.0 Hz), 7.93 (d, 1H, J = 2.0 Hz), 7.67 (d, 2H, J = 8.8 Hz), 7.59 (d, 1H, J = 6.0 Hz), 7.56 (d, 1H, J = 8.8 Hz), 7.21 (dd, 1H, J = 2.0, 8.8 Hz), 7.01 (d, 2H, J = 8.8 Hz), 4.38 (q, 2H, J = 7.2 Hz), 3.75 (s, 3H), 1.28 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 382 [M+1]$^+$. Anal. Calcd for $C_{20}H_{19}N_3O_3S$: C, 62.97; H, 5.02; N, 11.02. Found: C, 63.14; H, 5.35; N, 11.25.

6.1.1.18. 3-Nitro-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (6r). White solid (44%), mp: 159–161 °C. ^{1}H NMR (δ, DMSO-d_6): 10.46 (br s, 1H, NH), 9.33 (s, 1H), 8.49 (d, 2H, J = 1.2 Hz), 8.44 (dd, 1H, J = 1.2, 8,8 Hz), 8.08 (d, 1H, J = 8.8 Hz), 8.00 (d, 1H, J = 2.0 Hz), 7.82 (t, 1H, J = 8.8 Hz), 7.65 (d, 1H, J = 6.0 Hz), 7.62 (d, 1H, J = 8.8 Hz), 7.21 (dd, 1H, J = 8.8, 2,0 Hz), 4.43 (q, 2H, J = 7.2 Hz), 1.29 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 397 [M+1]$^+$. Anal. Calcd for $C_{19}H_{16}N_4O_4S$: C, 57.57; H, 4.07; N, 14.13. Found: C, 57.46; H, 4.34; N, 14.22.

The nitro compound **6r** (159 mg) was dissolved in EtOH (5 mL) and Raney Ni (74 mg) was added. The reaction mixture was stirred at room temperature under H_2 for 2 h. Then, the mixture was filtered over Celite, and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography (PE/EtOAc/EtOH, 3:3:1, v/v/v), yielded pure compound 3-amino-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (**6x**) as a white solid (72%), mp: 238–240 °C. ^{1}H NMR (δ, DMSO-d_6): 9.21 (s, 1H), 8.44 (d, 1H, J = 5.6 Hz), 7.88 (s, 1H), 7.57 (d, 1H, J = 5.6 Hz), 7.53 (d, 1H, J = 8.8 Hz), 7.20 (dd, 1H, J = 1.6, 8.8 Hz), 7.08 (t, 1H, J = 8.8 Hz), 6.95 (s, 1H), 6,86 (d, 1H, J = 8.0 Hz), 6.64 (d, 1H, J = 8.0 Hz), 5.44 (br s, 2H), 4.38 (q, 2H, J = 7.6 Hz), 1.29 (t, 3H, J = 7.6 Hz). ESI-MS: m/z = 367 [M+1]$^+$. Anal. Calcd for $C_{19}H_{18}N_4O_2S$: C, 62.28; H, 4.95; N, 15.29. Found: C, 62.35; H, 4.86; N, 14.97.

6.1.1.19. 2,5-Dimethoxy-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (6s). White solid (66%), mp: 177–179 °C. ^{1}H NMR (δ, DMSO-d_6): 10.20 (br s, 1H, NH), 9.82 (s, 1H), 8.69 (d, 1H, J = 6.4 Hz), 8.22 (s, 1H), 8.19 (d, 1H, J = 6.4 Hz), 8.02 (m, 2H), 7.83 (d, 1H, J = 8.4 Hz), 7.46 (d, 1H, J = 8.0), 6.86 (s, 1H), 4.56 (q, 2H, J = 6.4 Hz), 3.84 (s, 3H), 3.66 (s, 3H), 1.34 (t, 3H, J = 6.4 Hz). ESI-MS: m/z = 412 [M+1]$^+$. Anal. Calcd for $C_{21}H_{21}N_3O_4S$: C, 61.30; H, 5.14; N, 10.21. Found: C, 61.54; H, 5.47; N, 10.12.

6.1.1.20. 2,4-Dimethoxy-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (6t). White solid (79%), mp: 77–79 °C. ^{1}H NMR (δ, DMSO-d_6): 9.69 (s, 1H, NH), 9.24 (s, 1H), 8.45 (d, 1H, J = 6.0 Hz), 7.90 (s, 1H), 7.61 (m, 2H), 7.55 (d, 1H, J = 8.8 Hz), 7.26 (dd, 1H, J = 1.2, 8.8 Hz), 6.64 (d, 1H, J = 2.0 Hz), 6.50 (dd, 1H, J = 2.0, 8.8 Hz), 4.37 (q, 2H, J = 6.8 Hz), 3.94 (s, 3H), 3.74 (s, 3H), 1.28 (t, 3H, J = 6.8 Hz). ESI-MS: m/z = 412 [M+1]$^+$. Anal. Calcd for $C_{21}H_{21}N_3O_4S$: C, 61.30; H, 5.14; N, 10.21. Found: C, 61.35; H, 4.97; N, 10.44.

6.1.1.21. 3,4-Dimethoxy-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (6u). White solid (87%), mp: 68–70 °C. ^{1}H NMR (δ, DMSO-d_6): 9.97 (s, 1H, NH), 9.27 (s, 1H), 8.46 (d, 1H, J = 6.0 Hz), 7.97 (d, 1H, J = 2.0 Hz), 7.60 (m, 2H), 7.28 (m, 2H), 7.22 (dd, 1H, J = 2.0, 8.8 Hz), 7.01 (d, 1H, J = 8.4 Hz), 4.39 (q, 2H, J = 6.8 Hz), 3.74 (s, 3H), 3.69 (s, 3H), 1.29 (t, 3H, J = 6.8 Hz). ESI-MS: m/z = 412 [M+1]$^+$. Anal. Calcd for $C_{21}H_{21}N_3O_4S$: C, 61.30; H, 5.14; N, 10.21. Found: C, 61.59; H, 4.96; N, 10.24.

6.1.1.22. 2,4,6-Trimethoxy-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (6v). White solid (68%), mp: 223–224 °C. ^{1}H NMR (δ, DMSO-d_6): 9.56 (s, 1H, NH), 9.18 (s, 1H), 8.45 (d, 1H, J = 5.6 Hz), 7.93 (s, 1H), 7.58 (m, 2H), 7.30 (dd, 1H, J = 2.0, 8.8 Hz), 6.19 (s, 2H), 4.38 (q, 2H, J = 6.8 Hz), 3.84 (s, 6H), 3.74 (s, 3H), 1.29 (t, 3H, J = 6.8 Hz). ESI-MS: m/z = 442 $[M+1]^+$. Anal. Calcd for $C_{22}H_{23}N_3O_5S$: C, 59.85; H, 5.25; N, 9.52. Found: C, 59.83; H, 5.22; N, 9.45.

6.1.1.23. 4-Phenoxy-*N*-(5-ethyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (6w). White solid (68%), mp: 100–102 °C. ^{1}H NMR (δ, DMSO-d_6): 10.10 (br s, 1H, NH), 9.27 (s, 1H), 8.47 (d, 1H, J = 4.8 Hz), 7.93 (s, 1H), 7.72 (d, 2H, J = 8.0 Hz), 7.60 (m, 2H), 7.40 (m, 2H), 7.23 (d, 2H, J = 8.0 Hz), 7.06 (m, 4H), 4.40 (q, 2H, J = 7.2 Hz), 1.30 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 444 $[M+1]^+$. Anal. Calcd for $C_{25}H_{21}N_3O_3S$: C, 67.70; H, 4.77; N, 9.47. Found: C, 67.57; H, 4.46; N, 9.74.

6.1.1.24. 4-Methyl-*N*-(5-*tert*-butoxycarbonyl-5*H*-γ-carboline-8-yl)benzenesulfonamide (6z). White solid (82%), mp: 112–114 °C. ^{1}H NMR (δ, DMSO-d_6): 9.32 (s, 1H), 8.61 (d, 1H, J = 5.6 Hz), 8.07 (d, 1H, J = 9.2 Hz), 8.05 (d, 1H, J = 5.6 Hz), 7.93 (d, 1H, J = 2.0 Hz), 7.68 (d, 2H, J = 8.0 Hz), 7.30 (d, 2H, J = 8.0 Hz), 7.28 (dd, 1H, J = 9.2, 2.0 Hz), 2.28 (s, 3H), 1.67 (s, 9H). ESI-MS: m/z = 438 $[M+1]^+$. Anal. Calcd for $C_{23}H_{23}N_3O_4S$: C, 63.14; H, 5.30; N, 9.60. Found: C, 63.33; H, 5.24; N, 9.36.

6.2. Cytotoxic assay

The tumor cell lines (A549, SGC, HCT116, MCF-7 and K562) were obtained from Shanghai Institute of Pharmaceutical Industry.

The cytotoxic activity in vitro was measured using the MTT assay.[17] MTT solution (10.0 μL/well) in RPMI-1640 (Sigma, St. Louis, MO) 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) 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, MCF-7, and K562. In all of these experiments, three replicate wells were used to determine each point.

6.3. Flow cytometry analysis[18]

For flow cytometry analysis of DNA content, A549 cells in exponential growth were treated with **6p** (2 μM) for 48 h. Cells were washed twice with PBS and fixed in 75% ethanol. The cell pellet was resuspended in 100.0 μL of PBS containing 200.0 mg/mL RNase (Amersco, Solon, OH), then incubated at 37 °C for 0.5 h. After incubation, the cells were stained with 20.0 mL/L propidium iodide (PI, Sigma, St. Louis, MO) for 15 min. The fluorescence cell was measured with FACSCalibur (Becton–Dickinson, Lincoln Park, NJ).

6.4. Microtubule polymerization assay[19]

In vitro tubulin polymerization assays were conducted with reagents as described by the manufacturer (Cytoskeleton, Inc). In brief, compounds **6p**, **6t–v**, **6y** (4 μM) and CA-4 (3 μM) were incubated with purified bovine tubulin and buffer containing 20% glycerol and 1 mM GTP at 37 °C and the effect of compounds **6p**, **6t–v**, **6y** and CA-4 on tubulin polymerization were monitored kinetically using a fluorescent plate reader.

6.5. DNA intercalation assay

Purified Lambda DNA marker (Takara) (5 μL) and EB (1.25 μL, 5 mg/mL) were treated with compounds **6p**, **6t** and **6y** (1.25 μL, 0.2 mg/mL) for 15 min, and then loaded on agarose gel. After electrophoresis, DNA-EB complex was photographed by Bio-Rad GD2000.

6.6. Molecular docking study

Tubulin-DAMAcolchicine crystal structure (PDB ID: 1SA0) was chosen as a template. The functional A, B chains were kept, polar hydrogens were added, and CHARMm force field was employed. Binding sphere (45.4407, 41.1246, 34.6219, 9) was selected from the active site using the binding site tools. For all tested compounds, hydrogens were added and CHARMm force fields were employed. Each of the compounds was minimized by Dreiding Minimize tool. CDOCKER (Discovery Studio 2.1) was used for the docking simulation. For each compound, the docking parameters were as follows: Top Hits: 25; Random Conformations: 10; Random Conformations Dynamics Steps: 1000; Grid Extension: 8.0; Random Dynamics Time Step: 0.002. Final docked conformations were scored by calculated cdocker-energy.

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