Bioorganic & Medicinal Chemistry 17 (2009) 3324–3331

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# Design, synthesis, and quantitative structure–activity relationship of cytotoxic γ-carboline derivatives

Jing Chen [a], Xiaowu Dong [a], Tao Liu [a], Jianshu Lou [b], Chaoyi Jiang [a], Wenhai Huang [a], Qiaojun He [b], Bo Yang [b], Yongzhou Hu [a,*]

[a] ZJU-ENS Joint Laboratory of Medicinal Chemistry, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, Zhejiang, China
[b] Institute of Pharmacology and Toxicology, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, Zhejiang, China

ARTICLE INFO

*Article history:*
Received 17 February 2009
Revised 19 March 2009
Accepted 20 March 2009
Available online 26 March 2009

*Keywords:*
γ-Carboline derivatives
Cytotoxicity
CoMFA
3D-QSAR

ABSTRACT

Three series of γ-carboline derivatives were designed and synthesized. All the compounds were tested for their cytotoxic activities in vitro against five human tumor cell lines (A549, SGC, HCT116, MCF-7, K562) and one multi-drug resistant subline (K562R). Most compounds showed moderate to potent cytotoxic activities against the tested cell lines. Sulfonate **11f** exhibited more potent cytotoxic activities against almost all of the tested cells in comparison with the positive control, taxol, with $IC_{50}$ values ranging from 0.15 to 4.5 μM. The structure–activity relationships were discussed and a statistically reliable QSAR model ($r^2 = 0.936$, $q^2 = 0.581$) was established by the CoMFA analysis performed using the cytotoxic data against K562 cell line as a template.



## 1. Introduction

Combretastatin A-4 (CA-4, **1**), a *cis*-stilbene natural product isolated by Pettit and co-workers in 1989 from the bark of the South African bush willow tree *Combretum caffrum*,[1,2] is one of the well-known natural tubulin-binding molecules affecting microtubule dynamics. It displays potent antitumor effect in a variety of preclinical tumor models[3–8] as well as substantial antivascular activity in tumor blood flow while causing no significant blood flow retention in normal tissues.[9–15] However, it does not show in vivo efficacy due to its low aqueous solubility.[16] Thus, several prodrugs were investigated to improve its solubility and pharmacokinetics, leading to the development of disodium phosphate analog CA-4P (**2**) which is currently under phase II and III clinical trials on advanced cancers based on the vascular shutdown mechanism of action[17] and AVE8062 (**3**) which is currently under clinical evaluation as tumor vascular targeting agent.[18,19] The promising pharmacological and clinical profiles of **2** and **3** have drawn a lot of attention for medicinal chemists to develop a variety of derivatives or analogues of CA-4 in effort to obtain better compounds.[20] Since the SAR of CA-4 derivatives demonstrated that the great importance of the 3,4,5-trimethyloxyphenyl group (ring A), most of the studies were focused on the modification of the linking group and the ring B to get more potent compounds. Besides, γ-carboline skeleton has been identified as a superior scaffold structure with potent topoisomerase inhibitory activity.[21,22] And the presence of sulfonamide, sulfonate, or amide group in many potent tubulin polymerization inhibitors is also a noteworthy point (e.g., **4**–**7**, Fig. 1).[23–30] Based on the above insights, we replaced the B ring of CA-4 with a γ-carboline moiety, and introduced sulfonamide, sulfonate, and amide group as the linker between ring A and B. We expected the resulting compounds would possess improved

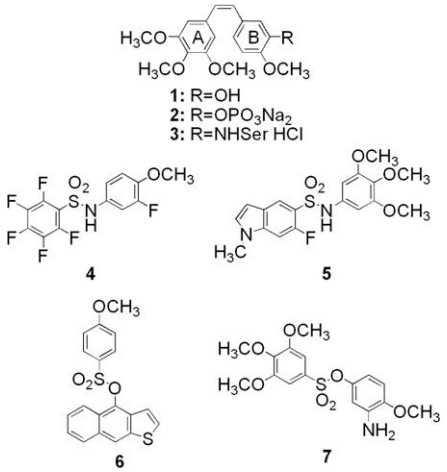
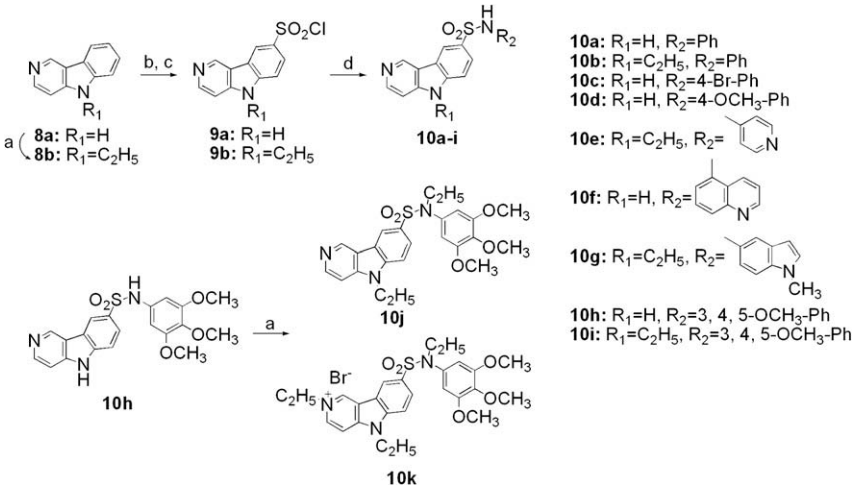



**Figure 1.** Structures of CA-4 and selected analogues of CA-4.

* Corresponding author. Tel./fax: +86 571 88208460.
*E-mail addresses:* huyz@zju.edu.cn (Y. Hu).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.03.037




**Scheme 1.** Reagents and conditions: (a) $C_2H_5Br$, $K_2CO_3$, DMF, rt 12 h; (b) $ClSO_3H$, dry $CH_3CN$, 0 °C to rt 12 h; (c) $POCl_3$, $PCl_5$, 180 °C, 4 h; (d) $R_2NH_2$, $Et_3N$, rt 0.5 h.

antitumor activities by inhibition both tubulin polymerization and topoisomerase. Herein, three series of new CA-4 analogues (**10**, **11** and **14**) were synthesized and tested for their cytotoxic activities in vitro against six human tumor cell lines. In addition, a CoMFA analysis was performed using the cytotoxic data against K562 cell line as a template to probe the QSAR of these compounds.

## 2. Results and discussion

### 2.1. Chemistry

The synthetic routes of γ-carboline sulfonamides **10a–k** were summarized in Scheme 1. γ-Carboline (**8a**) was prepared using our recently developed synthetic protocol.[31] Treatment of **8a** with $C_2H_5Br$ and $K_2CO_3$ in dimethylformamide (DMF) at room temperature gave compound **8b**.[32] The γ-carboline-6-sulfonyl chlorides **9** were prepared from **8** following the method described by Mitsumori et al.[33] Then reaction of **9** and various amines in the presence of triethylamine (TEA) in DMF at room temperature afforded the target compounds **10a–i**. In addition, compound **10h** could be further transformed into **10j** or **10k** by N-alkylation with different amount of $C_2H_5Br$ (Scheme 1).

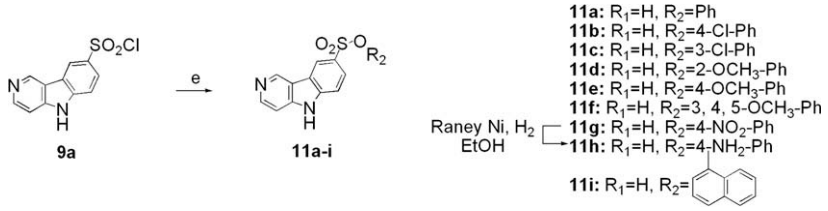
On the other hand, treatment of γ-carboline-6-sulfonyl chloride (**9a**) with various phenols in the presence of pyridine and dimethylamino pyridine (DMAP) in dry $CH_2Cl_2$ at room temperature provided γ-carboline sulfonates **11a–i** (Scheme 2).

γ-Carboline amides **14a–h** were synthesized as shown in Scheme 3. γ-Carboline (**8a**) was nitrified with concd $HNO_3$–$H_2SO_4$ to provide 6-nitro-γ-carboline (**12a**),[34] which was alkylated with $C_2H_5Br$ to give nitro compound **12b**. Reduction of compound **12b** using Raney Ni in EtOH yielded the amino compound **13**.[35] Condensation of compound **13** with corresponding acyl chlorides in *i*-$C_3H_7OH$ under reflux afforded the target compounds **14a–h**.

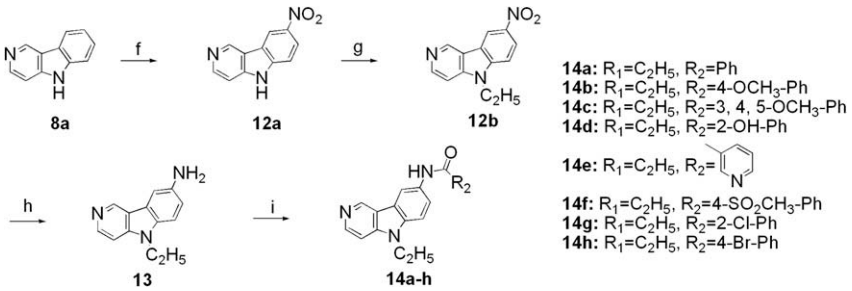
### 2.2. Biological activity and SAR

The synthesized γ-carboline derivatives **10a–k**, **11a–i** and **14a–h** 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, human myeloid leukemia cell K562 and K562R (a multidrug resistant cell) by MTT assay. Taxol was employed as the positive control. The results are summarized in Table 1.

By a scrutiny of the MTT assay results, it showed that almost all of the sulfonates showed moderate to potent cytotoxic activities against the tested cells. Compound **11f**, which bore 3,4,5-trimethyloxyphenyl group as ring A, was the most promising one among the tested compounds. It exhibited more potent cytotoxic activities against almost all of the tested cells compared with taxol, with $IC_{50}$ values ranging from 0.15 to 4.5 μM. Decreasing the number of methoxy group on ring A of compound **11f** led to compound **11d** or **11e** with lower cytotoxicities. Replacing the 3,4,5-trimethoxy group on phenyl ring with other substituents, such as chlorine atom (**11b**, **c**), nitro group (**11g**), amino group (**11h**) or introducing naphthaline (**11i**) instead of the substituted phenyl also reduced the activities. Moreover, removing all of the substituents on ring A (**11a**) diminished the cytotoxicities dramatically. Thus, it suggested the 3,4,5-trimethoxyphenyl group was essential for cytotoxicity in the sulfonate series. This was in agreement with the results reported in other literatures about CA-4 analogues.[36] But the above results were not applicable to the sulfonamide or amide series. It was found that the sulfonamides **10h**, **10i** and the amide **14c**, also with 3,4,5-trimethoxyphenyl as ring A, did not exhibited prominent activities. Introducing halogen atom instead of the 3,4,5-trimethoxy (**10c**, **14g**), decreasing the number of methoxy group (**10b**) or replacing the substituted phenyl with other aromatic heterocycles (**10g**) would retain or even improve the activities. Further more, the cytotoxicites




**Scheme 2.** Reagents and conditions: (e) $R_2OH$, Pyridine, DMAP, dry $CH_2Cl_2$, rt 12 h.

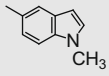



**Scheme 3.** Reagents and conditions: (f) concd $HNO_3$–$H_2SO_4$, rt 4 h (75%); (g) $C_2H_5Br$, $K_2CO_3$, DMF, rt 12 h (63%); (h) Raney Ni, $H_2$, EtOH, rt 2 h; (i) $R_2COCl$, *i*-$C_3H_7OH$, reflux 2 h.

spectrum of these two series were not as wide as that of the sulfonates. The sulfonamides were more sensitive to K562, HCT116 and MCF-7 cell lines, while the amides were more potent against K562, K562R, MCF-7 and SGC cell lines.

In addition, we also evaluated the effect of substituent at different nitrogen atom (*N*-3, *N*-9 and linker) of sulfonamides. The data in Table 1 showed that the ethylated products (**10b**, **i**) at the *N*-9 position of **10a** and **10h** displayed a drastic loss of activities against A549 and K562R cell lines. But it would not affect or even a little improved the cytotoxicities against the other cell lines. However, the N-ethylation product (**10j**) of compound **10i**, with a second ethyl substituted at the nitrogen atom of the linker, promoted

**Table 1**
In vitro cytotoxic activities of the synthesized compounds and taxol against six human cancer cell lines

| Compound | $R_1$ | $R_2$ | Cytotoxicity ($IC_{50}$, μM)[a,b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | K562 | K562R | HCT116 | MCF-7 | A549 | SGC |
| *Sulfonamides* | | | | | | | | |
| **10a** | H | Ph | 21.03 | 92.09 | 35.16 | 60.12 | 78.39 | >100 |
| **10b** | $C_2H_5$ | Ph | 20.74 | >100 | 13.74 | 0.88 | >100 | 8.00 |
| **10c** | H | 4-Br-Ph | 16.43 | NT | NT | NT | NT[c] | NT |
| **10d** | H | 4-OMe-Ph | 33.87 | NT | NT | NT | NT | NT |
| **10e** | $C_2H_5$ |  | >100 | >100 | >100 | >100 | >100 | >100 |
| **10f** | H |  | 12.71 | 18.46 | 30.82 | 23.74 | 22.81 | 95.37 |
| **10g** | $C_2H_5$ |  | 12.16 | 16.84 | 23.24 | 4.99 | 73.45 | 73.21 |
| **10h** | H | 3,4,5-OMe-Ph | 11.32 | 73.41 | 16.91 | 19.16 | 48.16 | >100 |
| **10i** | $C_2H_5$ | 3,4,5-OMe-Ph | 6.09 | >100 | 32.32 | 5.80 | >100 | >100 |
| **10j** | $C_2H_5$ | 3,4,5-OMe-Ph | 1.02 | 3.02 | 10.20 | 2.07 | 33.88 | 13.80 |
| **10k** | $C_2H_5$ | 3,4,5-OMe-Ph | >100 | >100 | >100 | >100 | >100 | >100 |
| *Sulfonates* | | | | | | | | |
| **11a** | H | Ph | 77.39 | 48.90 | 45.23 | 38.60 | >100 | >100 |
| **11b** | H | 4-Cl-Ph | 20.46 | 13.88 | 32.02 | 26.34 | 47.30 | 39.72 |
| **11c** | H | 3-Cl-Ph | 13.21 | 8.42 | 42.73 | 47.52 | 43.62 | 5.82 |
| **11d** | H | 2-OMe-Ph | 14.11 | 15.27 | 64.14 | 24.72 | 31.98 | 54.26 |
| **11e** | H | 4-OMe-Ph | 6.60 | 5.70 | 23.87 | 16.00 | 22.55 | 21.36 |
| **11f** | H | 3,4,5-OMe-Ph | 4.50 | 2.62 | 0.77 | 0.86 | NT | 0.15 |
| **11g** | H | 4-$NO_2$-Ph | 27.86 | 8.34 | 21.01 | 10.75 | 17.49 | 7.15 |
| **11h** | H | 4-$NH_2$-Ph | 18.45 | 5.95 | 20.89 | 16.56 | 10.14 | 2.12 |
| **11i** | H |  | 25.91 | 10.68 | 39.37 | 14.32 | 33.36 | 23.26 |
| *Amides* | | | | | | | | |
| **14a** | $C_2H_5$ | Ph | 26.22 | 10.62 | >100 | 11.83 | >100 | 26.54 |
| **14b** | $C_2H_5$ | 4-OMe-Ph | 11.32 | 5.15 | 50.73 | 4.92 | 72.02 | 21.34 |
| **14c** | $C_2H_5$ | 3,4,5-OMe-Ph | 13.57 | NT | NT | NT | 15.88 | 16.13 |
| **14d** | $C_2H_5$ | 2-OH-Ph | 12.74 | 22.94 | >100 | 20.85 | >100 | 19.16 |
| **14e** | $C_2H_5$ |  | 14.51 | 40.59 | 87.24 | 18.11 | 73.49 | 50.89 |
| **14f** | $C_2H_5$ | 4-$SO_2CH_3$-Ph | 22.90 | 96.58 | >100 | 22.80 | >100 | 36.04 |
| **14g** | $C_2H_5$ | 2-Cl-Ph | 8.58 | 22.93 | 2.63 | 6.52 | 37.33 | 20.78 |
| **14h** | $C_2H_5$ | 4-Br-Ph | 13.24 | 15.58 | >100 | 11.87 | >100 | 71.24 |
| **Taxol** | | | 1.16 | 5.63 | 4.37 | 2.11 | 2.46 | 3.34 |

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

the cytotoxicities against all of the tested cell lines. This result implied that the introduction of ethyl at the nitrogen atom of the linker was beneficial. But further introduction of ethyl at the *N*-3 position of γ-carboline afforded the quaternary *N*-ethylpyridinium salt **10k** with no activity.

**Table 2**
Predicted activities from CoMFA models compared with the experimental activities and the residues

| Compound | $IC_{50}$ (μM) | $pIC_{50}$ | CoMFA | |
|---|---|---|---|---|
| | | | Pred. $pIC_{50}$ | Res. |
| **10a** | 21.03 | 4.677 | 4.545 | −0.132 |
| **10b**[a] | 20.74 | 4.683 | 4.865 | 0.182 |
| **10c** | 16.43 | 4.784 | 4.688 | −0.096 |
| **10d** | 33.87 | 4.47 | 4.468 | −0.002 |
| **10f** | 12.71 | 4.896 | 4.876 | −0.02 |
| **10g**[a] | 12.16 | 4.915 | 5.182 | 0.267 |
| **10h** | 11.32 | 4.946 | 5.003 | 0.057 |
| **10i** | 6.09 | 5.215 | 5.305 | 0.09 |
| **10j** | 1.02 | 5.991 | 5.957 | −0.034 |
| **11a** | 77.39 | 4.111 | 4.372 | 0.261 |
| **11b**[a] | 20.46 | 4.689 | 4.519 | −0.17 |
| **11c** | 13.21 | 4.879 | 4.893 | 0.014 |
| **11d** | 14.11 | 4.85 | 4.754 | −0.096 |
| **11e** | 6.60 | 5.18 | 5.223 | 0.043 |
| **11f** | 4.50 | 5.347 | 5.379 | 0.032 |
| **11g** | 27.86 | 4.555 | 4.52 | −0.035 |
| **11h**[a] | 18.45 | 4.734 | 4.569 | −0.165 |
| **11i** | 25.91 | 4.587 | 4.536 | −0.051 |
| **14a** | 26.22 | 4.581 | 4.78 | 0.199 |
| **14b** | 11.32 | 4.946 | 4.909 | −0.037 |
| **14c** | 13.57 | 4.867 | 4.898 | 0.031 |
| **14d** | 12.74 | 4.895 | 4.842 | −0.053 |
| **14e** | 14.51 | 4.838 | 4.804 | −0.034 |
| **14f**[a] | 22.90 | 4.64 | 4.887 | 0.247 |
| **14g** | 8.58 | 5.067 | 5.037 | −0.03 |
| **14h** | 13.24 | 4.878 | 4.807 | −0.071 |

[a] Compounds of the testing set.

**Table 3**
Summary of CoMFA analysis

| CoMFA model | Result |
|---|---|
| $R^2$ cross-validated ($q^2$) | 0.581 |
| Number of components | 5 |
| Non cross-validated *r*2 | 0.936 |
| Standard error of estimate | 0.107 |
| *F* | 43.96 |
| Steric contribution | 71.9% |
| Electrostatic contribution | 28.1% |

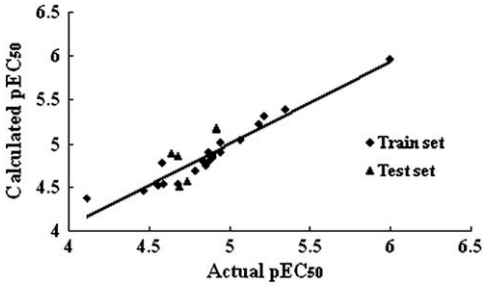



**Figure 2.** CoMFA calculated versus actual $pIC_{50}$ values.

## 2.3. CoMFA analysis

3D-QSAR methods, especially CoMFA, are widely used in drug design because they allow rapid generation of QSAR models, from which biological activity of newly designed molecules can be predicted.[37–39] In this study, 26 compounds with available $IC_{50}$ were employed for the CoMFA analysis. For 3D-QSAR analyses, 21 compounds (unasterisked molecules in Table 2) were selected as the training set for model construction, and the remaining 5 compounds (asterisked molecules in Table 2) as the testing set for model validation. The $pIC_{50}$ values ($pIC_{50} = -\log IC_{50}$) of these compounds were used as dependent variables. The CoMFA results are summarized in Table 3.

As shown in Table 3, a CoMFA model was developed with conventional correlation coefficient $r^2 = 0.936$, cross-validated coefficient $q^2 = 0.581$, the estimated $F = 43.96$, and standard error of 0.107. These statistical indexes were reasonably high, indicating that this new CoMFA model had a strong predictive ability. The data in Table 3 also showed that the contributions of steric and electrostatic field were 71.9% and 28.1%, respectively. The plots of the experimental results versus calculated values of the 21 studied compounds are shown in Figure 2. To evaluate the predictive ability of this model, we subsequently calculated the $pIC_{50}$ values of 5 compounds in the testing set. As it can be seen in Table 3 and Figure 2, the theoretical results of 5 compounds from the testing set were in good agreement with the experimental values, suggesting that the new CoMFA model was reliable.

The steric contour plot is shown in Figure 3A. It was found that a huge yellow colored contour was near the A ring of the γ-carboline derivatives, suggesting that bulky group in this area would decrease the cytotoxicity. The green region indicated that the bulky substitute near the nitrogen atom of the linker was favorable to the activity. CoMFA electrostatic contour map is shown in Figure 3B. There was a big blue contour near the ethyl substituent of the sulfonamide linker, indicating that more positive charges in this

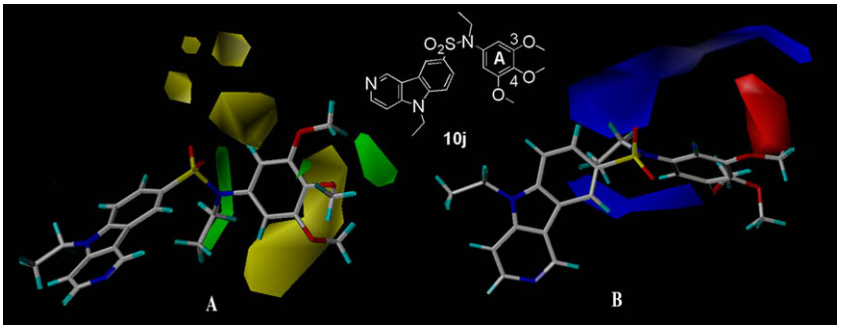



**Figure 3.** Steric and electrostatic CoMFA maps of the compound **10j** showing contributions to the inhibitory activities on the K562 cell line. (A) The favorable steric areas with more bulk are indicated by green isopleths, whereas the disfavorable steric areas are shown by yellow isopleths. (B) The favorable electrostatic areas with positive charges are indicated by blue isopleths, whereas the favorable electrostatic areas with negative charges are show by red isoplet.

region would result in enhanced activity. There was a red contour near the C-3 and C-4 of the phenyl ring, suggesting the negatively charged substituents in this area lead to an increase of cytotoxicity. This model could well explain the potent activity of compound **10j**. The ethyl group at the nitrogen atom of the sulfonamide linker was in the green and blue region. Introduction of this group changed the location of the 3,4,5-trimethoxyphenyl, and made it shift away from the yellow area. Further more, the oxygen atom of the 3-methoxy group was in the red area. Thus, the $pIC_{50}$ value of **10j** reached 5.991.

## 3. Conclusion

Three series of γ-carboline derivatives were synthesized and tested for their cytotoxic activities in vitro against six human tumor cell lines. The results demonstrated that most compounds showed moderate to potent cytotoxic activities against all the tested cell lines and sulfonate **11f** exhibited more potent cytotoxic activities in comparison with taxol, suggesting it might be the promising lead compound for future investigation. 3D-QSAR analysis using CoMFA was performed to explore comprehensive structure–activity relationships and a statistically reliable model with good predictive power ($r^2 = 0.936$, $q^2 = 0.581$) was established on the basis of the common substructure-based alignment. According to the CoMFA contours, further design, synthesis, and biological evaluation are ongoing in our laboratory and the results will be reported in due course.

## 4. Experimental

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 $\delta$ 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. All of the CoMFA calculations were performed on a SGI O2 workstation using the SYBYL 6.91 program.

### 4.1. Synthesis

#### 4.1.1. General procedure for synthesis of γ-carboline sulfonamides 10a–i

A mixture of γ-carboline-6-sulfonyl chloride **9** (1 mmol), amines (1 mmol) in DMF (5 mL) was stirred for 5 min at room temperature, TEA (210 μL, 1.5 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 (5:5:1, V/V/V) as eluent to afford **10a–i**.

**4.1.1.1. *N*-Phenyl-γ-carboline-6-sulfonamide (10a).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), aniline (92 μL, 1 mmol). White solid (54%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 12.40 (br s, 1H), 9.42 (s, 1H), 8.69 (d, 1H, $J$ = 2.0 Hz), 8.47 (d, 1H, $J$ = 5.6 Hz), 7.85(dd, 1H, $J$ = 8.8, 2.0 Hz), 7.66 (d, 1H, $J$ = 8.8 Hz), 7.52 (d, 1H, $J$ = 5.6 Hz), 7.14 (m, 4H), 6.87 (t, 1H, $J$ = 6.8). 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, 63.35; H, 4.21; N, 12.73.

**4.1.1.2. 9-Ethyl-*N*-phenyl-γ-carboline-6-sulfonamide (10b).** Reagent: 9-ethyl-γ-carboline-6-sulfonyl chloride (**9b**) (295 mg, 1 mmol), aniline (92 μL, 1 mmol). White solid (65%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.26 (br s, 1H), 9.49 (s, 1H), 8.76 (d, 1H, $J$ = 1.6 Hz), 8.56 (d, 1H, $J$ = 5.6 Hz), 7.91(dd, 1H, $J$ = 8.8, 1.6 Hz), 7.87 (d, 1H, $J$ = 8.8 Hz), 7.72 (d, 1H, $J$ = 5.6 Hz), 7.21 (m, 4H), 6.97 (t, 1H, $J$ = 7.2 Hz), 4.50 (q, 2H, $J$ = 7.2 Hz), 1.32 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 352 $[M+1]^+$. Anal. Calcd for $C_{19}H_{17}N_3O_2S$: C, 64.94; H, 4.88; N, 11.96. Found: C, 64.74; H, 4.89; N, 12.01.

**4.1.1.3. *N*-(4-Bromophenyl)-γ-carboline-6-sulfonamide (10c).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 4-bromoaniline (175 mg, 1 mmol). White solid (35%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 12.25 (br s, 1H), 10.41 (br s, 1H), 9.48 (s, 1H), 8.75 (s, 1H), 8.50 (d, 1H, $J$ = 6.0 Hz), 7.84(d, 1H, $J$ = 8.4), 7.70 (d, 1H, $J$ = 8.4 Hz), 7.56 (d, 1H, $J$ = 6.0 Hz), 7.39 (d, 2H, $J$ = 8.8 Hz), 7.09 (d, 2H, $J$ = 8.8 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, 50.45; H, 2.91; N, 10.24.

**4.1.1.4. *N*-(4-Methoxyphenyl)-γ-carboline-6-sulfonamide (10d).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 4-methoxyaniline (125 mg, 1 mmol). White solid (57%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 12.67 (br s, 1H), 9.91 (br s, 1H), 9.61 (s, 1H), 8.73 (d, 1H, $J$ = 1.6 Hz), 8.57 (d, 1H, $J$ = 6.4 Hz), 7.86 (dd, 1H, $J$ = 8.4, 1.6 Hz), 7.77 (d, 1H, $J$ = 8.4 Hz), 7.72 (d, 1H, $J$ = 6.4 Hz), 7.00 (d, 2H, $J$ = 8.8 Hz), 6.77 (d, 2H, $J$ = 8.8 Hz), 3.61 (s, 3H). ESI-MS: $m/z$ = 354 $[M+1]^+$. Anal. Calcd for $C_{18}H_{15}N_3O_3S$: C, 61.18; H, 4.28; N, 11.89. Found: C, 61.31; H, 4.26; N, 11.84.

**4.1.1.5. 9-Ethyl-*N*-(pyridine-4-yl)-γ-carboline-6-sulfonamide (10e).** Reagent: 9-ethyl-γ-carboline-6-sulfonyl chloride (**9b**) (295 mg, 1 mmol), 4-aminopyridine (94 mg, 1 mmol). White solid (51%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 9.67 (s, 1H), 8.68 (s, 1H), 8.64 (d, 1H, $J$ = 6.0 Hz), 8.11(d, 1H, $J$ = 6.0 Hz), 8.00 (d, 2H), 7.95 (dd, 1H, $J$ = 8.4, 0.8 Hz), 7.79 (d, 1H, $J$ = 8.4 Hz), 6.79 (d, 2H, $J$ = 6.0 Hz), 4.58 (q, 2H, $J$ = 7.2 Hz), 1.37 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 353 $[M+1]^+$. Anal. Calcd for $C_{18}H_{16}N_4O_2S$: C, 61.35; H, 4.58; N, 15.90. Found: C, 61.63; H, 4.36; N, 16.11.

**4.1.1.6. *N*-(Quinolin-5-yl)-γ-carboline-6-sulfonamide (10f).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 5-amino-quinoline (144 mg, 1 mmol). White solid (80%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 12.16 (br s, 1H), 9.84 (br s, 1H), 9.46 (s, 1H), 8.94 (d, 1H, $J$ = 2.0 Hz), 8.85 (dd, 1H, $J$ = 4.4, 1.6 Hz), 8.47 (d, 1H, $J$ = 5.6 Hz), 8.31 (dd, 1H, $J$ = 8.0, 1.6 Hz), 8.00 (dd, 1H, $J$ = 8.8, 2.0 Hz), 7.77 (d, 1H, $J$ = 8.0 Hz), 7.59 (d, 2H, $J$ = 8.8 Hz), 7.55 (dd, 1H, $J$ = 8.0, 4.4 Hz), 7.50 (m, 2H). ESI-MS: $m/z$ = 375 $[M+1]^+$. Anal. Calcd for $C_{20}H_{14}N_4O_2S$: C, 64.16; H, 3.77; N, 14.96. Found: C, 64.42; H, 3.86; N, 15.14.

**4.1.1.7. 9-Ethyl-*N*-(1-methyl-indol-5-yl)-γ-carboline-6-sulfonamide (10g).** Reagent: 9-ethyl-γ-carboline-6-sulfonyl chloride (**9b**) (295 mg, 1 mmol), 1-methyl-5-amino-indole (prepared as described in Ref. 40) (146 mg, 1 mmol). White solid (48%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 9.81 (br s, 1H), 9.41 (s, 1H), 8.64 (s, 1H), 8.52 (d, 1H, $J$ = 4.8 Hz), 7.83 (m, 2H), 7.68 (d, 1H, $J$ = 8.8 Hz), 7.25 (s, 1H), 7.21 (m, 2H), 6.90 (d, 1H, $J$ = 8.0 Hz), 6.26 (d, 1H, $J$ = 2.4 Hz), 4.44 (q, 2H, $J$ = 7.2 Hz), 3.64 (s, 3H), 1.29 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 405 $[M+1]^+$. Anal. Calcd for $C_{22}H_{20}N_4O_2S$: C, 65.33; H, 4.98; N, 13.85. Found: C, 65.26; H, 4.74; N, 13.79.

**4.1.1.8. *N*-(3,4,5-Trimethoxyphenyl)-γ-carboline-6-sulfonamide (10h).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 3,4,5-trimethoxyaniline (185 mg, 1 mmol). White solid (52%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 12.20 (br s, 1H), 10.06 (br s, 1H), 9.49 (s, 1H), 8.77 (d, 1H, $J$ = 1.6 Hz), 8.50 (d, 1H, $J$ = 5.6 Hz), 7.88 (dd, 1H, $J$ = 8.8, 1.6 Hz), 7.71 (d, 1H, $J$ = 8.8 Hz), 7.55 (d, 1H, $J$ = 5.6 Hz), 6.43 (s, 2H), 3.62 (s, 6H), 3.50 (s, 3H).

ESI-MS: $m/z$ = 414 $[M+1]^+$. Anal. Calcd for $C_{20}H_{19}N_3O_5S$: C, 58.10; H, 4.63; N, 10.16. Found: C, 58.14; H, 4.58; N, 10.25.

**4.1.1.9. 9-Ethyl-*N*-(3,4,5-trimethoxyphenyl)-γ-carboline-6-sulfonamide (10i).** Reagent: 9-ethyl-γ-carboline-6-sulfonyl chloride (**9b**) (295 mg, 1 mmol), 3,4,5-trimethoxyaniline (185 mg, 1 mmol). White solid (77%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.09 (br s, 1H), 9.51 (s, 1H), 8.80 (d, 1H, $J$ = 1.6 Hz), 8.57 (d, 1H, $J$ = 5.6 Hz), 7.94 (dd, 1H, $J$ = 8.8, 1.6 Hz), 7.90 (d, 1H, $J$ = 8.8 Hz), 7.73 (d, 1H, $J$ = 5.6 Hz), 6.43 (s, 2H), 4.52 (q, 2H, $J$ = 7.2 Hz), 3.62 (s, 6H), 3.30 (s, 3H), 1.33 (t, 3H, $J$ = 7.2 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, 59.67; H, 5.44; N, 9.45.

**4.1.2. *N*,9-Diethyl-*N*-(3,4,5-trimethoxyphenyl)-γ-carboline-6-sulfonamide (10j) and 3,9-diethyl-6-(*N*-ethyl-*N*-(3,4,5-trimethoxyphenyl)sulfamoyl)-γ-carboline-3-ium bromide (10k)**

To a solution of **10h** (41 mg, 0.1 mmol) in DMF (0.5 mL) was added $K_2CO_3$ (55 mg, 0.4 mmol) and the reaction mixture was stirred for 30 min at ambient temperature. Then $C_2H_5Br$ (15 μL, 0.2 mmol) was added into the reaction mixture and stirred for 4 h. After that, the mixture was concentrated under reduced pressure. Then water (10 mL) 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 **10j**. White solid (34%), mp: 172–174 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 9.54 (s, 1H), 8.70 (s, 1H), 8.58 (d, 1H, $J$ = 5.6 Hz), 7.92 (d, 1H, $J$ = 8.4 Hz), 7.76 (d, 1H, $J$ = 5.6 Hz), 7.74 (dd, 1H, $J$ = 8.4, 1.6 Hz), 6.24 (s, 2H), 4.54 (q, 2H, $J$ = 6.8 Hz), 3.64 (s, 3H), 3.60 (q, 2H, $J$ = 6.8 Hz), 3.54 (s, 6H), 1.35 (t, 3H, $J$ = 6.8 Hz), 1.02 (t, 3H, $J$ = 6.8 Hz). ESI-MS: $m/z$ = 471 $[M+1]^+$. Anal. Calcd for $C_{24}H_{27}N_3O_5S$: C, 61.39; H, 5.80; N, 8.95. Found: C, 61.35; H, 5.85; N, 9.13.

When the amount of $C_2H_5Br$ was up to 0.4 mmol, compound **10k** was afforded using a procedure similar to compound **10j**. White solid (41%), mp: 143–145 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.21 (s, 1H), 9.02 (d, 1H, $J$ = 6.8 Hz), 8.94 (d, 1H, $J$ = 8.0 Hz), 8.47 (d, 1H, $J$ = 6.8), 8.20 (d, 1H, $J$ = 8.8 Hz), 7.98 (t, 1H, $J$ = 8.8 Hz), 6.28 (s, 2H), 4.74 (m, 6H), 3.63 (s, 3H), 3.58 (s, 6H), 1.64 (t, 3H, $J$ = 7.2 Hz) 1.42 (t, 3H, $J$ = 7.2 Hz), 1.08 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 580 $[M+1]^+$. Anal. Calcd for $C_{26}H_{32}BrN_3O_5S$: C, 53.98; H, 5.58; N, 7.26. Found: C, 54.02; H, 5.35; N, 7.36.

**4.1.3. General procedure for synthesis of γ-carboline sulfonates 11a–i**

Pyridine (0.32 mL, 4 mmol) and DMAP (45 mg, 0.36 mmol) were added to a mixture of γ-carboline-6-sulfonyl chloride **9a** (1 mmol) and phenols (1 mmol) in dry $CH_2Cl_2$ (5 mL). And then, the mixture was stirred for an additional 12 h at room temperature. After that, the mixture was concentrated under vacuum. Water (10 mL) was added into the residue and 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 PE:EtOAc:EtOH (10:10:1 to 4:4:1, V/V/V) as eluent to afford **11a–i**.

**4.1.3.1. Phenyl γ-carboline-6-sulfonate (11a).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), phenol (94 mg, 1 mmol). White solid (28%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 9.54 (s, 1H), 8.87 (s, 1H), 8.52 (d, 1H, $J$ = 5.6 Hz), 7.87(d, 1H, $J$ = 8.4 Hz), 7.78 (d, 1H, $J$ = 8.4 Hz), 7.59 (d, 1H, $J$ = 5.6 Hz), 7.35 (t, 2H, $J$ = 7.2 Hz), 7.28 (t, 1H, $J$ = 7.2 Hz), 7.01 (d, 2H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 325 $[M+1]^+$. Anal. Calcd for $C_{17}H_{12}N_2O_3S$: C, 62.95; H, 3.73; N, 8.64. Found: C, 62.76; H, 3.75; N, 8.35.

**4.1.3.2. 4-Chlorophenyl γ-carboline-6-sulfonate (11b).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 4-chlorophenol (129 mg, 1 mmol). White solid (45%), mp: 222–224 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 9.53 (s, 1H), 8.86 (d, 1H, $J$ = 2.0 Hz), 8.51 (d, 1H, $J$ = 6.4 Hz), 7.84 (d, 1H, $J$ = 8.0 Hz), 7.77 (d, 1H, $J$ = 8.0 Hz), 7.58 (d, 1H, $J$ = 6.4 Hz), 7.41 (d, 2H, $J$ = 8.8 Hz), 7.04 (d, 2H, $J$ = 8.8 Hz). ESI-MS: $m/z$ = 360 $[M+1]^+$. Anal. Calcd for $C_{17}H_{11}ClN_2O_3S$: C, 56.91; H, 3.09; N, 7.81. Found: C, 57.11; H, 3.28; N, 7.68.

**4.1.3.3. 3-Chlorophenyl γ-carboline-6-sulfonate (11c).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 3-chlorophenol (129 mg, 1 mmol). White solid (45%), mp: 213–215 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 12.85 (br s, 1H) 9.56 (s, 1H), 8.92 (d, 1H, $J$ = 1.6 Hz), 8.53 (d, 1H, $J$ = 5.6 Hz), 7.90 (dd, 1H, $J$ = 8.4, 1.6 Hz), 7.81 (d, 1H, $J$ = 8.4 Hz), 7.60 (d, 1H, $J$ = 5.6 Hz), 7.37 (m, 2H), 7.20 (s, 1H), 6.96 (d, 1H, $J$ = 6.8 Hz). ESI-MS: $m/z$ = 360 $[M+1]^+$. Anal. Calcd for $C_{17}H_{11}ClN_2O_3S$: C, 56.91; H, 3.09; N, 7.81. Found: C, 56.86; H, 2.86; N, 7.96.

**4.1.3.4. 2-Methoxyphenyl γ-carboline-6-sulfonate (11d).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 2-methoxyphenol (124 mg, 1 mmol). White solid (28%), mp: 229–231 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 9.54 (s, 1H), 8.83 (s, 1H), 8.52 (d, 1H, $J$ = 6.0 Hz), 7.85 (d, 1H, $J$ = 8.8 Hz), 7.76 (d, 1H, $J$ = 8.8 Hz), 7.59 (d, 1H, $J$ = 6.0 Hz), 7.23 (t, 1H, $J$ = 8.0 Hz), 7.06 (d, 1H, $J$ = 8.0 Hz), 6.99 (d, 1H, $J$ = 8.0 Hz), 6.91 (t, 1H, $J$ = 8.0 Hz), 3.39 (s, 3H). ESI-MS: $m/z$ = 355 $[M+1]^+$. Anal. Calcd for $C_{18}H_{14}N_2O_4S$: C, 61.01; H, 3.98; N, 7.90. Found: C, 61.23; H, 4.12; N, 7.78.

**4.1.3.5. 4-Methoxyphenyl γ-carboline-6-sulfonate (11e).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 4-methoxyphenol (124 mg, 1 mmol). White solid (51%), mp:230–232 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 12.57 (br s, 1H), 9.56 (s, 1H), 8.87 (s, 1H), 8.54 (d, 1H, $J$ = 6.0 Hz), 7.86 (d, 1H, $J$ = 8.0 Hz), 7.78 (d, 1H, $J$ = 8.0 Hz), 7.59 (d, 1H, $J$ = 6.0 Hz), 6.91 (d, 2H, $J$ = 8.8 Hz), 6.85 (d, 2H, $J$ = 8.8 Hz), 3.67 (s, 3H). ESI-MS: $m/z$ = 355 $[M+1]^+$. Anal. Calcd for $C_{18}H_{14}N_2O_4S$: C, 61.01; H, 3.98; N, 7.90. Found: C, 61.14; H, 3.90; N, 7.78.

**4.1.3.6. 3,4,5-Trimethoxyphenyl γ-carboline-6-sulfonate (11f).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 3,4,5-trimethoxyphenol (180 mg, 1 mmol). White solid (43%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 9.58 (s, 1H), 8.90 (s, 1H), 8.52 (d, 1H, $J$ = 5.2 Hz), 7.93 (d, 1H, $J$ = 8.8 Hz), 7.82 (d, 1H, $J$ = 8.8 Hz), 7.59 (d, 1H, $J$ = 5.2 Hz), 6.28 (s, 2H), 3.56 (s, 3H), 3.53 (s, 6H). ESI-MS: $m/z$ = 415 $[M+1]^+$. Anal. Calcd for $C_{20}H_{18}N_2O_6S$: C, 57.96; H, 4.38; N, 6.76. Found: C, 58.08; H, 4.74; N, 6.87.

**4.1.3.7. 4-Nitrophenyl γ-carboline-6-sulfonate (11g).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 4-nitrophenol (109 mg, 1 mmol). Yellow solid (38%), mp:198–200 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 12.84 (br s, 1H), 9.57 (s, 1H), 8.96 (s, 1H), 8.54 (d, 1H, $J$ = 5.6 Hz), 8.24 (d, 2H, $J$ = 9.2 Hz), 7.93 (d, 1H, $J$ = 8.4 Hz), 7.81 (d, 1H, $J$ = 8.4 Hz), 7.60 (d, 1H, $J$ = 5.6 Hz), 7.33 (d, 2H, $J$ = 9.2 Hz). ESI-MS: $m/z$ = 370 $[M+1]^+$. Anal. Calcd for $C_{17}H_{11}N_3O_5S$: C, 55.28; H, 3.00; N, 11.38. Found: C, 55.35; H, 2.97; N, 11.47.

The nitro compound **11g** (148 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 4-aminophenyl γ-carboline-6-sulfonate **(11h)** as a white solid (98%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 12.69 (br s, 1H), 9.55 (s, 1H), 8.82 (s, 1H), 8.53 (d, 1H, $J$ = 5.6 Hz), 7.82 (d, 1H, $J$ = 8.0 Hz), 7.77

(d, 1H, $J$ = 8.0 Hz), 7.59 (d, 1H, $J$ = 5.6 Hz), 6.59 (d, 2H, $J$ = 8.4 Hz), 6.39 (d, 2H, $J$ = 8.4 Hz), 5.19 (s, 2H). ESI-MS: $m/z$ = 340 $[M+1]^+$. Anal. Calcd for $C_{17}H_{13}N_3O_3S$: C, 60.17; H, 3.86; N, 12.38. Found: C, 60.14; H, 4.02; N, 12.34.

**4.1.3.8. Naphthalen-1-yl γ-carboline-6-sulfonate (11i).** Reagent: γ-carboline-6-sulfonyl chloride (**9a**) (267 mg, 1 mmol), 1-naphthol (144 mg, 1 mmol). White solid (27%), mp: >250 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 9.53 (s, 1H), 8.96 (d, 1H, $J$ = 1.2 Hz), 8.51 (d, 1H, $J$ = 5.6 Hz), 7.94 (m, 3H), 7.88 (d, 1H, $J$ = 8.4 Hz), 7.77 (d, 1H, $J$ = 8.4 Hz),7.58 (d, 1H, $J$ = 5.6 Hz), 7.53 (m, 2H), 7.46 (t, 1H, $J$ = 8.1 Hz), 7.14 (d, 1H, $J$ = 8.4 Hz). ESI-MS: $m/z$ = 375 $[M+1]^+$. Anal. Calcd for $C_{21}H_{14}N_2O_3S$: C, 67.37; H, 3.77; N, 7.48. Found: C, 67.25; H, 3.43; N, 7.38.

### 4.1.4. General procedure for synthesis of γ-carboline amides 14a–h

A mixture of 6-amino-9-ethyl-γ-carboline (1 mmol) **13**, acyl chlorides (1 mmol) was refluxed in $i$-$C_3H_7OH$ (5 mL) for 2 h. After cooling to room temperature, the mixture was concentrated under vacuum. Then water (10 mL) was added into the residue, neutralized with aq. $Na_2CO_3$, and extracted with AcOEt (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 PE:EtOAc:EtOH (10:10:1 to 3:3:1, V/V/V) as eluent to afford **14a–h**.

**4.1.4.1. 9-Ethyl-6-benzamido-γ-carboline (14a).** Reagent: 6-amino-9-ethyl-γ-carboline (**13**) (141 mg, 1 mmol), benzoyl chloride (141 mg, 1 mmol). White solid (72%), mp: 199–201 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.63 (br s, 1H), 9.81 (s, 1H), 9.01 (d, 1H, $J$ = 0.8 Hz), 8.73 (d, 1H, $J$ = 6.8 Hz), 8.25 (d, 1H, $J$ = 6.8 Hz), 8.07 (d, 2H, $J$ = 8.0 Hz), 8.02 (m, 2H), 7.65 (m, 1H), 7.59 (m, 2H), 4.69 (q, 2H, $J$ = 7.2 Hz), 1.42 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 316 $[M+1]^+$. Anal. Calcd for $C_{20}H_{17}N_3O$: C, 76.17; H, 5.43; N, 13.32. Found: C, 76.24; H, 5.26; N, 13.11.

**4.1.4.2. 9-Ethyl-6-(4-methoxybenzamido)-γ-carboline (14b).** Reagent: 6-amino-9-ethyl-γ-carboline (**13**) (211 mg, 1 mmol), 4-methoxybenzoyl chloride (171 mg, 1 mmol). White solid (65%), mp: 153–155 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.46 (br s, 1H), 9.79 (s, 1H), 8.98 (s, 1H), 8.72 (d, 1H, $J$ = 6.4 Hz), 8.24 (d, 1H, $J$ = 6.4 Hz), 8.07 (d, 2H, $J$ = 8.8 Hz), 7.97 (m, 2H), 7.10 (d, 2H, $J$ = 8.8 Hz), 4.66 (q, 2H, $J$ = 7.2 Hz), 3.86 (s, 3H), 1.42 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 346 $[M+1]^+$. Anal. Calcd for $C_{21}H_{19}N_3O_2$: C, 73.03; H, 5.54; N, 12.17. Found: C, 73.32; H, 5.85; N, 12.42.

**4.1.4.3. 9-Ethyl-6-(3, 4, 5-trimethoxybenzamido)-γ-carboline (14c).** Reagent: 6-amino-9-ethyl-γ-carboline (**13**) (211 mg, 1 mmol), 3, 4, 5-trimethoxybenzoyl chloride (231 mg, 1 mmol). White solid (62%), mp: 117–119 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.31 (br s, 1H), 9.30 (s, 1H), 8.59 (d, 1H, $J$ = 1.2 Hz), 8.49 (d, 1H, $J$ = 5.6 Hz), 7.86 (d, 1H, $J$ = 8.8 Hz), 7.73 (d, 1H, $J$ = 8.8 Hz), 7.64 (d, 1H, $J$ = 5.6 Hz), 7.38 (s, 2H), 4.48 (q, 2H, $J$ = 7.2 Hz), 3.99 (s, 6H), 3.75 (s, 3H), 1.36 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 406 $[M+1]^+$. Anal. Calcd for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36. Found: C, 68.25; H, 5.56; N, 10.62.

**4.1.4.4. 9-Ethyl-6-(2-hydroxybenzamido)-γ-carboline (14d).** Reagent: 6-amino-9-ethyl-γ-carboline (**13**) (211 mg, 1 mmol), 2-methoxybenzoyl chloride (171 mg, 1 mmol). White solid (29%), mp: 143–145 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.64 (br s, 1H), 9.30 (s, 1H), 8.69 (s, 1H), 8.48 (d, 1H, $J$ = 4.0 Hz), 7.76 (d, 1H, $J$ = 8.4 Hz), 7.71 (d, 1H, $J$ = 8.4 Hz), 7.64 (d, 1H, $J$ = 6.4 Hz), 7.98 (d, 1H, $J$ = 8.0 Hz), 7.54 (m, 3H), 4.48 (q, 2H, $J$ = 7.2 Hz), 1.34 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 332 $[M+1]^+$. Anal. Calcd for $C_{20}H_{17}N_3O_2$: C, 72.49; H, 5.17; N, 12.68. Found: C, 72.63; H, 5.26; N, 12.56.

**4.1.4.5. 9-Ethyl-6-nicotinamido-γ-carboline (14e).** Reagent: 6-amino-9-ethyl-γ-carboline (**13**) (211 mg, 1 mmol), nicotinoyl chloride (142 mg, 1 mmol). White solid (21%), mp: 135–137 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.71 (br s, 1H), 9.30 (s, 1H), 9.20 (s, 1H), 8.78 (d, 1H, $J$ = 4.0 Hz), 8.69 (s, 1H), 8.49 (d, 1H, $J$ = 5.6 Hz), 8.42 (d, 1H, $J$ = 7.2 Hz), 7.88 (d, 1H, $J$ = 8.8 Hz), 7.73 (d, 1H, $J$ = 8.8 Hz), 7.65 (t, 1H, $J$ = 5.6 Hz), 7.61 (m, 1H), 4.49 (q, 2H, $J$ = 7.2 Hz), 1.35 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 317 $[M+1]^+$. Anal. Calcd for $C_{19}H_{16}N_4O$: C, 72.13; H, 5.10; N, 17.71. Found: C, 72.42; H, 5.22; N, 17.57.

**4.1.4.6. 9-Ethyl-6-(4-methylsulfonyl)-γ-carboline (14f).** Reagent: 6-amino-9-ethyl-γ-carboline (**13**) (211 mg, 1 mmol),4-(methylsulfonyl)benzoyl chloride (219 mg, 1 mmol). White solid (57%), mp: 110–112 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 8.80 (s, 1H), 8.13 (d, 1H, $J$ = 6.8 Hz), 7.91 (s, 1H), 7.50 (m, 5H), 7.39 (d, 1H, $J$ = 8.8 Hz), 7.32 (d, 1H, $J$ = 8.8 Hz), 4.06 (q, 2H, $J$ = 7.2 Hz), 3.02 (s, 3H), 1.19 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 394 $[M+1]^+$. Anal. Calcd for $C_{21}H_{19}N_3O_3S$: C, 64.10; H, 4.87; N, 10.68. Found: C, 64.13; H, 5.03; N, 10.67

**4.1.4.7. 9-Ethyl-6-(2-chlorobenzamido)-γ-carboline (14g).** Reagent: 6-amino-9-ethyl-γ-carboline (**13**) (211 mg, 1 mmol), 2-chlorobenzoyl chloride (175 mg, 1 mmol). White solid (46%), mp: 77–79 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.64 (br s, 1H), 9.30 (s, 1H), 8.69 (s, 1H), 8.48 (d, 1H, $J$ = 4.0 Hz), 7.76 (d, 1H, $J$ = 8.4 Hz), 7.71 (d, 1H, $J$ = 8.4 Hz), 7.64 (d, 1H, $J$ = 6.4 Hz), 7.98 (d, 1H, $J$ = 8.0 Hz), 7.54 (m, 3H), 4.48 (q, 2H, $J$ = 7.2 Hz), 1.34 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 351 $[M+1]^+$. Anal. Calcd for $C_{20}H_{16}ClN_3O$: C, 68.67; H, 4.61; N, 12.01. Found: C, 68.36; H, 4.74; N, 12.24.

**4.1.4.8. 9-Ethyl-6-(4-bromobenzamido)-γ-carboline (14h).** Reagent: 6-amino-9-ethyl-γ-carboline (**13**) (211 mg, 1 mmol), 4-bromobenzoyl chloride (220 mg, 1 mmol). White solid (90%), mp: 205–207 °C. $^1$H NMR ($\delta$, DMSO-$d_6$): 10.73 (br s, 1H), 9.81 (s, 1H), 8.98 (d, 1H, $J$ = 1.2 Hz), 8.73 (d, 1H, $J$ = 6.4 Hz), 8.25 (d, 1H, $J$ = 6.4 Hz), 8.03 (m, 4H), 7.79 (d, 2H, $J$ = 8.8 Hz), 4.68 (q, 2H, $J$ = 7.2 Hz), 1.42 (t, 3H, $J$ = 7.2 Hz). ESI-MS: $m/z$ = 394 $[M+1]^+$. Anal. Calcd for $C_{20}H_{16}BrN_3O$: C, 60.93; H, 4.09; N, 10.66. Found: C, 60.74; H, 4.25; N, 10.45.

## 4.2. Cytotoxic assay

The tumor cell lines (A549, SGC, HCT116, MCF-7, K562, K562R) were obtained from Shanghai Institute of Pharmaceutical Industry.

The cytotoxic activity in vitro was measured using the MTT assay.[41] 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, K562, and K562R. In all of these experiments, three replicate wells were used to determine each point.

## 4.3. Molecular modeling and alignment

The 3D structures of all the compounds were built and minimized by using SYBYL 6.91. The geometries of all molecules involved in this study were optimized by Powell's method using the tripos force field. Conformation analyses were also carried out using the SYBYL/Grid search module. The lowest-energy conformations were considered as the bioactive conformations. It was noticed that all the 21 molecules in the training set had the same γ-carboline skel-

eton. Therefore, compound **10j** with the most potent activity against K562 cell line was chosen as template for the structural alignment of the 26 molecules.

### 4.4. CoMFA analysis

Steric and electrostatic interactions were calculated using a $sp^3$ carbon atom as steric probe and $a + 1$ charge as electrostatic probe with tripos force field. The CoMFA grid spacing was 2.0 Å in the $x$, $y$, and $z$ directions. The minimum $r$ (column filtering) was set to 2.0 kcal/mol to improve the signal-to-noise ratio by omitting those lattice points whose energy variation was below this threshold. A cutoff of 30 kcal/mol was adopted, and the regression analysis was carried out using the full cross-validated partial least-squares (PLS) method (leave-one-out) with CoMFA standard options for scaling of variables. The final model (non-cross-validated conventional analysis) was developed with the optimum number of components equal to that yielding the highest $q^2$.

### Acknowledgement

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

### References and notes

1. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendal, D. *Experientia* **1989**, *45*, 209.
2. Young, S. L.; Chaplin, D. J. *Exp. Opin. Invest. Drugs* **2004**, *13*, 1171.
3. Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lobavanijaya, P. *Can. J. Chem.* **1982**, *60*, 1374.
4. Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200.
5. Pettit, G. R.; Cragg, G. M.; Singh, S. B. *J. Nat. Prod.* **1987**, *50*, 386.
6. Lin, C. M.; Ho, H. H.; Pettit, G. R. *Biochemistry* **1989**, *28*, 7753.
7. Pettit, G. R.; Singh, S. B. *Can. J. Chem.* **1987**, *65*, 2390.
8. Pettit, G. R.; Temple, C. J.; Narayanan, V. L.; Varma, R.; Simpson, M. J.; Boyd, M. R.; Rener, G. A.; Bansal, N. *Anti-cancer Drug Des.* **1995**, *10*, 299.
9. Thorpe, P. E.; Chaplin, D. J.; Backley, D. C. *Cancer Res.* **2003**, *63*, 1144.
10. Liekens, S.; Clercq, E. D.; Neyts, J. *J. Biochem. Pharmacol.* **2001**, *61*, 253.
11. Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, *57*, 1829.
12. Tozer, G. M.; Kanthou, C.; Parkins, C. S.; Hili, S. A. *Int. J. Exp. Pathol.* **2002**, *83*, 21.
13. Pettit, G. R.; Lippert, J. W.; Herald, D. L.; Hamel, E.; Pettit, R. K. *J. Nat. Prod.* **2000**, *63*, 969.
14. Chaplin, D. J.; Pettit, G. R.; Parkins, C. S.; Hill, S. A. *Br. J. Cancer* **1996**, *27*, 586.
15. Chaplin, D. J.; Pettit, G. R.; Hill, S. A. *Anticancer Res.* **1999**, *19*, 189.
16. Ohsumi, K.; Nakagava, R.; Fukuda, Y.; Hatanaka, T.; Morinaga, Y.; Niheri, U.; Ohishi, K.; Suga, Y.; Akiyama, Y.; Tsuji, T. *J. Med. Chem.* **1998**, *41*, 3022.
17. Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 3033.
18. Tozer, G. M.; Kantholl, C.; Baguley, B. C. *Nat. Rev. Cancer* **2005**, *5*, 423.
19. Kelland, L. R. *Curr. Cancer Ther. Rev.* **2005**, *1*, 1.
20. Chaudhary, A.; Pandeya, S. N.; Kumar, P.; Sharma, P. P.; Gupta, S.; Soni, N.; Verma, K. K.; Bhardwaj, G. *Mini Rev. Med. Chem.* **2007**, *7*, 1186.
21. Acramone, F.; Penco, S. In *Antitumor Natural Products*; Takeuchi, T., Nitta, K., Tanaka, N., Eds.; Japan Scientific Press: Tokyo, 1989; p 1.
22. Funayama, Y.; Nishio, K.; Wakabayashi, K.; Nagao, M.; Shimoi, K.; Ohira, T.; Hasegawa, S.; Saijo, N. *Mutat. Res.* **1996**, *349*, 183.
23. Yoshino, H.; Ueda, N.; Niijima, J.; Sugumi, H.; Kotake, Y.; Koyanagi, N.; Yoshimatsu, K.; Asada, M.; Watanabe, T.; Nagasu, T. *J. Med. Chem.* **1992**, *35*, 2496.
24. Yoshimatsu, K.; Yamaguchi, A.; Yoshino, H.; Koyanagi, N.; Kitoh, K. *Cancer Res.* **1997**, *57*, 3208.
25. Koyanagi, N.; Nagasu, T.; Fujita, F.; Watanabe, T.; Tsukahara, K.; Funahashi, Y.; Fujita, M.; Taguchi, T.; Yoshino, H.; Kitoh, K. *Cancer Res.* **1994**, *54*, 1702.
26. Shan, B.; Medina, J. C.; Santha, E.; Frankmoelle, W. P.; Chou, T.-C.; Learned, R. M.; Narbut, M. R.; Stott, D.; Wu, P.; Jaen, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5686.
27. Medina, J. C. WO 9936391, 1999.
28. Prinz, H. *Exp. Rev. Anticancer Ther.* **2002**, *2*, 695.
29. Zuse, A.; Schmidt, P.; Baasner, S.; Böhm, K. J.; Müller, K.; Gerlach, M.; Günther, E. G.; Unger, E.; Prinz, H. *J. Med. Chem.* **2007**, *50*, 6059.
30. Gwaltney, S. L.; Imade, H. M.; Barr, K. J.; Li, Q.; Gehrke, L.; Credo, R. B.; Warner, R. B.; Lee, J. Y.; Kovar, P.; Wang, J.; Nukkala, M. A.; Zielinski, N. A.; Frost, D.; Ng, S.-C.; Sham, H. L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 871.
31. Chen, J.; Chen, W. L.; Hu, Y. *Synlett* **2008**, 77.
32. Nguyen, C. H.; Jean, M. L.; Francois, L.; Marie, C. B.; Emile, B. *J. Med. Chem.* **1990**, *33*, 1519.
33. Mitsumori, S.; Tsuri, T.; Honma, T.; Hiramatsu, Y.; Okada, T.; Hashizume, H.; Inagaki, M.; Arimura, A.; Yasui, K.; Asanuma, F.; Kishino, J.; Ohtani, M. *J. Med. Chem.* **2003**, *46*, 2436.
34. Lee, C. -S.; Ohta, T.; Shudo, K.; Okamoto, T. *Heterocycles* **1981**, *16*, 1081.
35. Yan, X.; Li, X.; Luo, X.; Sun, J.; Xu, Z. CN 1660771, 2005.
36. Tron, G. C.; Pagliai, F.; Del Grosso, E.; Genazzani, A. A.; Sorba, G. *J. Med. Chem.* **2005**, *48*, 3260.
37. Yang, G. F.; Huang, X. Q. *Curr. Pharm. Des.* **2006**, *12*, 4601.
38. Cramer, R. D.; Patterson, D. E.; Bunce, J. D. *J. Am. Chem. Soc.* **1988**, *110*, 5959.
39. Fatemi, M. H.; Gharaghani, S. *Bioorg. Med. Chem.* **2007**, *15*, 7746.
40. Ferlin, M. G.; Chiarelotto, G.; Dall'Acqua, S.; Maciocco, E.; Mascia, M. P.; Pisu, M. G.; Biggio, G. *Bioorg. Med. Chem.* **2005**, *13*, 3531.
41. Carmichael, J.; DeGraff, W. G.; Gadzar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.