# Norcantharidin analogues — Synthesis and evaluation of growth inhibition in a panel of selected tumor-cell lines

**Liping Deng, Li Shen, Jing Zhang, Bo Yang, Qiaojun He, and Yongzhou Hu**

**Abstract:** A series of norcantharidin (NCTD) analogues have been synthesized by [3+2]1,3-dipolar cycloaddition reaction of norcantharidin derivatives of substituted aromatic amines with four nitrile oximes. All analogues have been screened for their antiproliferative activity in vitro against a panel of tumor cell lines: KB, SGC-7901, HL60, Bel-7402, HO-8910, and ECA109, producing $IC_{50}$ values from 0.36 μmol/L to >100 μmol/L. Compound **9d** showed potency for the treatment of hepatoma, with $IC_{50}$ value to Bel-7402 cell line comparable to that of norcantharidin.

*Key words:* norcantharidin analogues, isoxazoline, growth inhibition.

**Résumé :** On a réalisé la synthèse d'une série d'analogues de la norcantharidine en procédant à une réaction de cycloaddition 1,3-dipolaire [3+2] de dérivés de la norcantharidine d'amines aromatiques substituées avec quatre oximes de nitriles. On a évalué l'activité comme inhibiteur de croissance in vitro de chacun des analogues contre un ensemble de souches de cellules tumorales: KB, SGC-7901, HL60, Bel-7402, HO-8910 et ECA109 pour lesquelles les valeurs de $IC_{50}$ varient de 0,36 μmol/L à plus de 100 μmol/L. Le composé **9d** présente un grand potentiel pour le traitement de l'hépatome, avec une valeur de $IC_{50}$ qui, par comparaison avec celle des cellules Bel-7402, est comparable à celle de la norcantharidine.

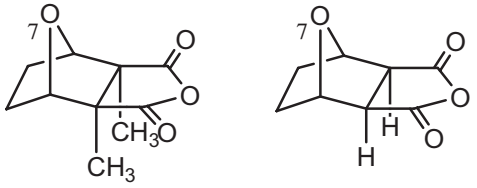
*Mots-clés :* analogues de la norcantharidine, isoxazoline, inhibition de la croissance.

[Traduit par la Rédaction]

## Introduction

Traditional Chinese medicine is a rich source of potential anticancer agents. In particular, cantharidin (CAN; exo, exo-2,3-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid anhydride; Fig. 1) is the active ingredient of Mylabris, which has been used in China as a medicinal agent for the treatment of cancer, particularly hepatoma (1). Although cantharidin is cytotoxic to cancer cells and stimulatory on the bone marrow, the renal toxicity of this drug has limited its application (2). A series of bioactive analogues have been synthesized in an attempt to increase the utility and to reduce the toxicity of cantharidin. Norcantharidin (NCTD; Fig. 1), the demethylated analogue of cantharidin, has been used to treat human cancers in China since 1984 (2). NCTD appeared to improve the awkward side of cantharidin, making the drug safer in application. It was recently found to be capable of inducing apoptosis in human cervical, tongue, ginival, mucoepidermoid carcinoma, adenocystic carcinoma, neuroblastoma, bone, leukaemia, ovarian, and colon cancer cell lines (3–5); however, the main application so far is treating hepatoma. We have referred to all the known cantharidin SAR data; briefly, no modification of the bicyclo[2.2.1] skeleton is permissible, the 7-oxa bridge is required to maintain activity, and the presence of a double bond (5,6-ene) has little effect on activity (6–10). Replacement of the O-atom (anhydride) with N (as N–H and N–R, where R = aryl or thiazolyl) allows the development of a new series of anhydride-modified cantharidin analogues; some are more potent than CAN or display potency similar to NCTD (11, 12). On the other hand, it is well-known that isoxazoline derivatives possess a wide range of pharmacological activities (13). These observations prompted us to design a new series of norcantharidin analogues by combining isoxazoline with norcantharidin derivatives in one single molecule to improve their antiproliferative activities and to carry out a structure–activity relationship study. In this paper, we describe the synthetic methodology towards the development of a series of norcantharidin analogues and their antiproliferative activities in vitro against various human tumor-cell lines.

**Fig. 1.** Chemical structures of Cantharidin and Norcantharidin.




Cantharidin Norcantharidin

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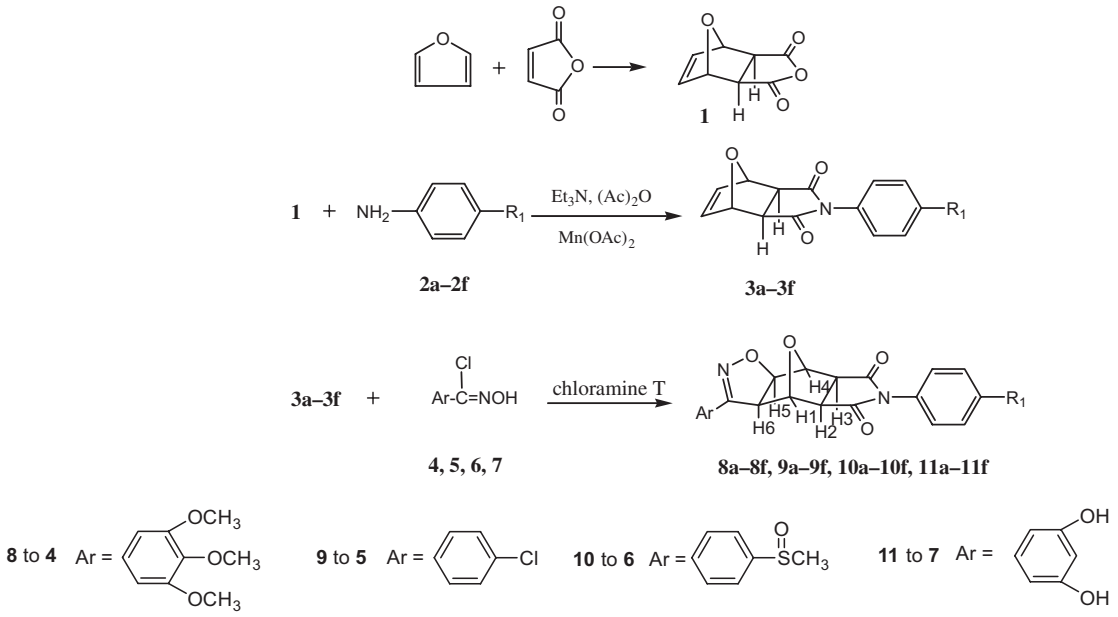
**Scheme 1.** Synthetic route of novel norcantharidin analogues **8a–8f**, **9a–9f**, **10a–10f**, and **11a–11f**.




**2a, 3a, 8a–11a** $R_1$ = H; **2b, 3b, 8b–11b** $R_1$ = F; **2c, 3c, 8c–11c** $R_1$ = Cl;
**2d, 3d, 8d–11d** $R_1$ = Br; **2e, 3e, 8e–11e** $R_1$ = $CH_3$; **2f, 3f, 8f–11f** $R_1$ = $OCH_3$;

## Results and discussion

### Synthesis

Norcantharidin analogues were prepared as shown in Scheme 1. Treatment of furan with maleic anhydride resulted in 5,6-dehydronorcantharidin **1**, then compound **1** reacted with substituted phenylamine **2a–2f** to give compounds **3a–3f** according to the published procedure (16). [3+2] Cycloaddition of **3a–3f** with four nitrile oximes **4–7** in the presence of chloramine T yielded target compounds **8a–8f**, **9a–9f**, **10a–10f**, and **11a–11f**. All compounds were tested as racemic mixtures.

To identify the configuration of the norcantharidin analogues (**8a–8f**, **9a–9f**, **10a–10f**, and **11a–11f**), we have studied selective $^1$H–$^1$H COSY spectra and NOESY spectra of the compounds. $^1$H–$^1$H COSY spectra showed cross peaks between H-2 and H-3 and H-5 and H-6. NOESY spectra showed cross peaks between H-2 and H-6 and H-3 and H-5. The Diels–Alder adduct 5,6-dehydronorcantharidin of furan with maleic anhydride have the exo configuration exclusively; the endo isomer has never been reported (14, 15), which, combined with $^1$H–$^1$H COSY and NOESY spectra data, give us a reasonable exo-adduct configuration, as we have proved before similarly (16, 17).

### Biological activity

The norcantharidin analogues **8a–8f**, **9a–9f**, **10a–10f**, and **11a–11f** were screened for their antiproliferative activity against a panel of six tumor-cell lines: KB, SGC7901, HL60, Bel7402, HO8910 and ECA109. CAN and NCTD were included as internal standards. $IC_{50}$ values of different compounds are summarized in Table 1.

As shown in Table 1, the synthesized compounds displayed growth inhibition with $IC_{50}$ values ranging from 0.36 μmol/L to >100 μmol/L. Most norcantharidin analogues were antiproliferative against HL60 cell line after 72 h treatment and were inactive to the KB and SGC7901 cell lines. Some norcantharidin analogues exhibited higher growth inhibition than NCTD, e.g., **9c** was more potent than NCTD against HL60 cell line, and **11c** was more potent than NCTD against HL60 and HO8910 cell lines. Also, compound **9d** displayed potency against Bel-7402 cell line comparable to that of NCTD, suggesting that the halogeno group in the aryl moiety ($R_1$) might be crucial for activity. It is also noteworthy that the presence of the methoxyl (such as **8f**, **9f**, **10f**, and **11f**) appeared to have an unfavorable effect.

The apoptosis-inducing activity of NCTD and its analogues was dose- and time-dependent; for instance, the antiproliferative activity of compound **9d** and cantharidin against Bel-7402 cell line are shown in Fig. 2.

## Conclusion

From the data presented, subtle modifications of norcantharidin's skeleton permitted the development of a new series of analogues with alterations in their antiproliferative activity against a panel of tumor-cell lines. The lead compounds cantharidin and norcantharidin are somewhat more potent against tumor-cell lines, respectively. Of the synthesized analogues, the notable exception being **9c**, which showed both good potency and selectivity for the HL60 cell line, and compound **9d** displayed moderate potency for the treatment of hepatoma with $IC_{50}$ values to Bel7402 cell line comparable to that of norcantharidin. We are currently working towards the development of more po-

**Table 1.** Antiproliferative activity of the skeletally modified norcantharidin analogues (**8a–8f**, **9a–9f**, **10a–10f**, and **11a–11f**).

| | $IC_{50}$ (μmol/L)[a, b] | | | | | |
|---|---|---|---|---|---|---|
| Compound | KB | SGC7901 | HL60 | Bel7402 | HO8910 | ECA109 |
| CAN | 21.9 | 58.2 | 0.1 | 1 | 1.28 | 0.38 |
| NCTD | 32.2 | 79 | 5 | 24 | >100 | 47.4 |
| **8a** | >100 | >100 | >100 | >100 | >100 | >100 |
| **8b** | >100 | >100 | >100 | >100 | >100 | >100 |
| **8c** | >100 | >100 | >100 | >100 | >100 | >100 |
| **8d** | >100 | >100 | 7.2 | >100 | >100 | >100 |
| **8e** | >100 | >100 | 60.6 | >100 | >100 | >100 |
| **8f** | >100 | >100 | >100 | >100 | >100 | >100 |
| **9a** | >100 | >100 | 51.2 | >100 | >100 | >100 |
| **9b** | >100 | >100 | 74.4 | >100 | >100 | >100 |
| **9c** | >100 | >100 | 0.36 | >100 | >100 | >100 |
| **9d** | >100 | >100 | 1.9 | 37.7 | >100 | 88.3 |
| **9e** | >100 | >100 | 3.4 | 62.4 | >100 | 87.5 |
| **9f** | >100 | >100 | >100 | >100 | >100 | >100 |
| **10a** | >100 | >100 | >100 | >100 | >100 | >100 |
| **10b** | >100 | >100 | 4.9 | 69.6 | >100 | >100 |
| **10c** | >100 | >100 | 4.8 | >100 | >100 | >100 |
| **10d** | >100 | >100 | 20 | >100 | >100 | >100 |
| **10e** | >100 | >100 | >100 | >100 | >100 | >100 |
| **10f** | >100 | >100 | >100 | >100 | >100 | >100 |
| **11a** | >100 | >100 | 1.6 | >100 | >100 | >100 |
| **11b** | >100 | >100 | 92.3 | >100 | >100 | >100 |
| **11c** | >100 | >100 | 1.6 | >100 | 16.5 | >100 |
| **11d** | >100 | >100 | 17.8 | >100 | >100 | >100 |
| **11e** | >100 | >100 | 13 | >100 | >100 | >100 |
| **11f** | >100 | >100 | >100 | >100 | >100 | >100 |

[a]The $IC_{50}$ values represent the concentration that results in a 50% decrease in cell growth after 72 h incubation.
[b]The $IC_{50}$ values were the mean values of three repeated experiments.

tent and selective analogues of this nature and will report the outcome of these studies in due course.

## Experimental section

### General

All reagents were of commercial quality and were used as received (Aldrich). Solvents were dried and purified using standard techniques. Reactions were monitored by TLC on aluminum plates coated with silica gel with fluorescent indicator (Merck 60 F254). Melting points were obtained on a B-540 Büchi melting-point apparatus and are uncorrected. $^1$H NMR and NOESY spectra were recorded on a Brucker AM-400 MHz spectrometer with $SiMe_4$ as the internal standard in DMSO-$d_6$. Mass Spectra were recorded with a HP5989B analyzer. Elemental analyses were performed on an EA-1110 instrument.

### General procedure for the preparation of the 5,6-dehydronorcantharidinisoxazoline adducts (8a–8f, 9a–9f, 10a–10f, 11a–11f)

To a solution of 3-acetyl-7-oxa-bicyclo[2.2.1]hept-5-ene-2-carboxylic acid phenylamide **3a** (1 mmol) and **4** (1 mmol) in ethanol (20 mL) was added chloramine T (1.2 mmol), and the reaction mixture was refluxed in ethanol for 4–7 h. Then, it was cooled to room temperature, water (10 mL) was added to the reaction mixture, and it was extracted with dichloromethane (30 mL). The extract was dried over anhyd. sodium sulfate, concentrated in vacuum, and the residue was recrystallized from acetone to give the compound **8a**. The synthesis of compounds **8b–8f, 9a–9f, 10a–10f, 11a–11f** were performed using the same method.

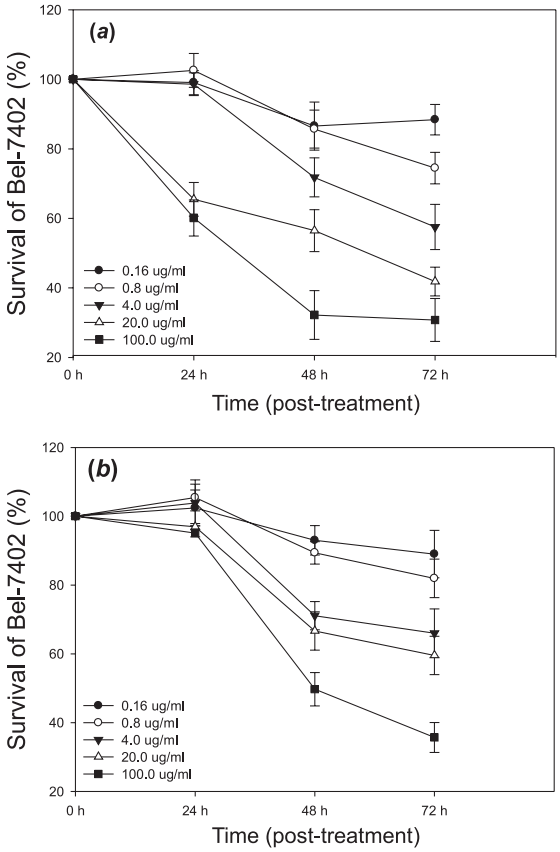
#### *exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-6-phenyl-3-(3,4,5-trimethoxyphenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (8a)*

Compound **8a** was obtained as a colorless solid: yield 94%; mp 284 °C. IR ν (KBr, $cm^{-1}$): 3062 and 3027 (ArH), 1778, 1721 (C=O), 1598 (C=N), 1240 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.83–7.19 (m, 7H, Ar–H), 5.24 (d, $J$ = 7.9 Hz, 1H, H-5), 4.98 (s, 1H, H-4), 4.75 (s, 1H, H-1), 4.52 (d, $J$ = 7.9 Hz, 1H, H-6), 3.77 (s, 9H, 3 × $OCH_3$), 3.59 (d, $J$ = 7.9 Hz, 1H, H-3), 3.38 (d, $J$ = 7.9 Hz, 1H, H-2). MS $m/z$: 450 ($M^+$). Anal. calcd. for $C_{24}H_{22}N_2O_7$: C 63.99, H4.92, N 6.22; found: C 63.80, H 4.99, N 6.18.

#### *exo,exo-6-(4-fluorophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(3,4,5-trimethoxyphenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (8b)*

Compound **8b** was obtained as a colorless solid: yield 81%; mp 300 °C (Dec.). IR ν (KBr, $cm^{-1}$): 3121 and 3010

**Fig. 2.** The antiproliferative activity of (*a*) cantharidin and (*b*) **9d** in Bel-7402 cell line. Cells were treated with various concentrations of cantharidin and **9d** for 24 h, 48 h, and 72 h. The percent of Bel-7402 cell line at each time point (post-treatment) is observed by MTT assay and expressed as the mean *D* of three independent experiments. Antiproliferative activity induced by cantharidin and **9d** is dose- and time-dependent in Bel-7402 cell line.




(ArH), 1777, 1718 (C=O), 1570 (C=N), 1224 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.83–7.25 (m, 6H, Ar–H), 5.23 (d, *J* = 7.9 Hz, 1H, H-5), 4.98 (s, 1H, H-4), 4.76 (s, 1H, H-1), 4.51 (d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.77 (s, 9H, 3 × $OCH_3$), 3.38(d, *J* = 7.9 Hz, 1H, H-2). MS *m*/*z*: 468 ($M^+$). Anal. calcd. for $C_{24}H_{21}FN_2O_7$: C 61.54, H 4.52, N 5.98; found: C 61.60, H 4.55, N 5.95.

### *exo,exo-6-(4-Chlorophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(3,4,5-trimethoxyphenyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (8c)*

Compound **8c** was obtained as a colorless solid: yield 83%; mp 279 °C. IR ν (KBr, $cm^{-1}$): 3072 and 3029 (ArH), 1777, 1716 (C=O), 1571 (C=N), 1248 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.83–7.18 (m, 6H, Ar–H), 5.24 (d, *J* = 7.9 Hz, 1H, H-5), 4.98 (s, 1H, H-4), 4.76 (s, 1H, H-1), 4.51 (d, *J* = 7.9 Hz, 1H, H-6), 3.60 (d, *J* = 7.9 Hz, 1H, H-3), 3.77 (s, 9H, 3 × $OCH_3$), 3.38 (d, *J* = 7.9 Hz, 1H, H-2). MS *m*/*z*: 484 ($M^+$), 486 ($M^+$ + 2). Anal. calcd. for $C_{24}H_{21}ClN_2O_7$: C 59.45, H4.37, N 5.78; found: C 59.51, H 4.38, N 5.75.

### *exo,exo-6-(4-Bromophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(3,4,5-trimethoxyphenyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (8d)*

Compound **8d** was obtained as a colorless solid: yield 91%; mp 280 °C. IR ν (KBr, $cm^{-1}$): 3056 and 3043 (ArH), 1780, 1721(C=O), 1567 (C=N), 1243 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.83–7.25 (m, 6H, Ar–H), 5.24 (d, *J* = 7.9 Hz, 1H, H-5), 4.98 (s, 1H, H-4), 4.75 (s, 1H, H-1), 4.51(d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.77 (s, 9H, 3 × $OCH_3$), 3.38 (d, *J* = 7.9 Hz, 1H, H-2). MS *m*/*z*: 528 ($M^+$), 530 (M + 2). Anal. calcd. for $C_{24}H_{21}BrN_2O_7$: C 54.46, H 4.00, N 5.29; found: C 54.48, H 4.03, N 5.25.

### *exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(3,4,5-trimethoxyphenyl)-6-(**p**-tolyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (8e)*

Compound **8e** was obtained as a colorless solid: yield 90%; mp 261 °C. IR ν (KBr, $cm^{-1}$): 3072 and 3060 (ArH), 1781, 1721(C=O), 1569 (C=N), 1286 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.83–7.15 (m, 6H, Ar–H), 5.23 (d, *J* = 7.9 Hz, 1H, H-5), 4.97 (s, 1H, H-4), 4.75 (s, 1H, H-1), 4.51 (d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.35 (d, *J* = 7.9 Hz, 1H, H-2), 3.77 (s, 9H, 3 × $OCH_3$), 2.33 (–$CH_3$). MS *m*/*z*: 464 ($M^+$). Anal. calcd. for $C_{25}H_{24}N_2O_7$: C 64.65, H 5.21, N 6.03; found: C 64.67, H 5.22, N 6.00.

### *exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-6-(4-methoxyphenyl)-3-(3,4,5-trimethoxypheny)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (8f)*

Compound **8f** was obtained as a colorless solid: yield 91%; mp 279 °C. IR ν (KBr, $cm^{-1}$): 3051 and 3027 (ArH), 1778, 1714 (C=O), 1568 (C=N), 1250 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 8.37–7.49 (m, 6H, Ar–H), 5.25 (d, *J* = 7.9 Hz, 1H, H-5), 5.02 (s, 1H, H-4), 4.80 (s, 1H, H-1), 4.53 (d, *J* = 7.9 Hz, 1H, H-6), 3.66 (d, *J* = 7.9 Hz, 1H, H-3), 3.44 (d, *J* = 7.9 Hz, 1H, H-2), 3.77 (s, 9H, 3 × $OCH_3$), 3.83 (s, 3H, –$OCH_3$). MS *m*/*z*: 480 ($M^+$). Anal. calcd. for $C_{25}H_{24}N_2O_8$: C 62.49, H 5.03, N 5.83; found: C 62.51, H 5.02, N 5.81.

### *exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-6-phenyl-3-(4-chlorophenyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (9a)*

Compound **9a** was obtained as a colorless solid: yield 95%; mp 300 °C (Dec.). IR ν (KBr, $cm^{-1}$): 3046 and 3008 (ArH), 1788, 1721 (C=O), 1567 (C=N), 1258 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.74–7.06 (m, 9H, Ar–H), 5.18(d, *J* = 7.9 Hz, 1H, H-5), 4.95 (s, 1H, H-4), 4.73 (s, 1H, H-1), 4.48 (d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.36 (d, *J* = 7.9 Hz, 1H, H-2). MS *m*/*z*: 394 ($M^+$). Anal. calcd. for $C_{21}H_{15}ClN_2O_4$: C 63.89, H 3.83, N 7.10; found: C 63.90, H 3.84, N 7.06.

### *exo,exo-6-(4-Fluorophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-chlorophenyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (9b)*

Compound **9b** was obtained as a colorless solid: yield 82%; mp 310 °C (Dec.). IR ν (KBr, $cm^{-1}$): 3055 and 3017 (ArH), 1780, 1721 (C=O), 1543 (C=N), 1252 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.75–7.06 (m, 8H, Ar–H), 5.18 (d, *J* = 7.9 Hz, 1H, H-5), 4.95 (s, 1H, H-4), 4.74 (s, 1H, H-1), 4.47(d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.37(d, *J* = 7.9 Hz, 1H, H-2). MS *m*/*z*: 412 ($M^+$). Anal. calcd. for $C_{21}H_{14}ClFN_2O_4$: C 61.10, H 3.42, N 6.79; found: C 61.12, H 3.45, N 6.75.

***exo,exo-6-(4-Chlorophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-chlorophenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (9c)***

Compound **9c** was obtained as a colorless solid: yield 84%; mp 309 °C (Dec.). IR ν (KBr, $cm^{-1}$): 3053 and 3027 (ArH), 1778, 1713 (C=O), 1550 (C=N), 1280 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.75–7.06 (m, 8H, Ar–H), 5.18 (d, *J* = 7.9 Hz, 1H, H-5), 4.95 (s, 1H, H-4), 4.74 (s, 1H, H-1), 4.47 (d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.37(d, *J* = 7.9 Hz, 1H, H-2). MS *m/z*: 428 ($M^+$). Anal. calcd. for $C_{21}H_{14}Cl_2N_2O_4$: C 58.76, H 3.29, N 6.53; found: C 58.78, H 3.30, N 6.49.

***exo,exo-6-(4-Bromophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-chlorophenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (9d)***

Compound **9d** was obtained as a colorless solid: yield 70%; mp 325 °C (Dec.). IR ν (KBr, $cm^{-1}$): 3053 and 3029 (ArH), 1778, 1714 (C=O), 1528 (C=N), 1233 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.76–7.02 (m, 8H, Ar–H), 5.17 (d, *J* = 7.9 Hz, 1H, H-5), 4.95 (s, 1H, H-4), 4.74 (s, 1H, H-1), 4.47 (d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.37 (d, *J* = 7.9 Hz, 1H, H-2). MS *m/z*: 471($M^+$), 473 ($M^+$ + 2). Anal. calcd. for $C_{21}H_{14}ClBrN_2O_4$: C 53.25, H 2.98, N 5.91; found: C 53.27, H 2.32, N 5.89.

***exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-chlorophenyl)-6-(*p*-tolyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (9e)***

Compound **9e** was obtained as a colorless solid: yield 68%; mp 342 °C (Dec.). IR ν (KBr, $cm^{-1}$): 3058 and 3034 (ArH), 1787, 1723 (C=O), 1514 (C=N), 1289 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.77–7.02 (m, 8H, Ar–H), 5.17 (d, *J* = 7.9 Hz, 1H, H-5), 4.93 (s, 1H, H-4), 4.72 (s, 1H, H-1), 4.46 (d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.37 (d, *J* = 7.9 Hz, 1H, H-2), 2.33 (–$CH_3$). MS *m/z*: 408 ($M^+$). Anal. calcd. for $C_{22}H_{17}ClN_2O_4$: C 64.63, H 4.19, N 6.85; found: C 64.65, H 4.23, N 6.85.

***exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-chlorophenyl)-6-(4-methoxyphenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (9f)***

Compound **9f** was obtained as a colorless solid: yield 58%; mp 315 °C(Dec.). IR ν (KBr, $cm^{-1}$): 3057 and 3021 (ArH), 1786, 1718 (C=O), 1516 (C=N), 1258 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.86–7.22 (m, 8H, Ar–H), 5.19 (d, *J* = 7.9 Hz, 1H, H-5), 4.72 (s, 1H, H-1), 4.46 (d, *J* = 7.9 Hz, 1H, H-6), 3.56 (d, *J* = 7.9 Hz, 1H, H-3), 3.33 (d, *J* = 7.9 Hz, 1H, H-2), 3.83 (s, 3H, –$OCH_3$). MS *m/z*: 424 ($M^+$). Anal. calcd. for $C_{22}H_{17}ClN_2O_5$: C 62.20, H 4.03, N 6.59; found: C 62.23, H 4.03, N 6.58.

***exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-6-phenyl-3-(4-methylsulfinylphenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (10a)***

Compound **10a** was obtained as a colorless solid: yield 95%; mp 310 °C (Dec.). IR ν (KBr, $cm^{-1}$): 3062, 3028 (ArH), 1778, 1722 (C=O), 1597 (C=N), 1239 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.83–7.20 (m, 9H, Ar–H), 5.24 (d, *J* = 7.9 Hz, 1H, H-5), 4.98 (s, 1H, H-4), 4.75 (s, 1H, H-1), 4.52 (d, *J* = 7.2 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.38 (d, *J* = 7.9 Hz, 1H, H-2), 3.27 (s, 3H, –$SOCH_3$). MS *m/z*: 422 ($M^+$). Anal. calcd. for $C_{22}H_{18}N_2O_5S$: C 62.55, H 4.29, N 6.63; found: C 62.56, H 4.30, N 6.61.

***exo,exo-6-(4-Fluorophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-methylsulfinylphenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (10b)***

Compound **10b** was obtained as a colorless solid: yield 81%; mp 177 °C. IR ν (KBr, $cm^{-1}$): 3121 and 3011 (ArH), 1778, 1718 (C=O), 1570 (C=N), 1224 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.83–7.25 (m, 8H, Ar–H), 5.23 (d, *J* = 7.9 Hz, 1H, H-5), 4.97(s, 1H, H-4), 4.76 (s, 1H, H-1), 4.51 (d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.38 (d, *J* = 7.9 Hz, 1H, H-2), 3.27 (s, 3H, –$SOCH_3$). MS *m/z*: 440 ($M^+$). Anal. calcd. for $C_{22}H_{17}FN_2O_5S$: C 59.99, H 3.89, N 6.36; found: C 59.98, H 3.91, N 6.35.

***exo,exo-6-(4-Chlorophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-methylsulfinylphenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (10c)***

Compound **10c** was obtained as a colorless solid: yield 83%; mp 152 °C. IR ν (KBr, $cm^{-1}$): 3072 and 3030 (ArH), 1774, 1716 (C=O), 1571 (C=N), 1239 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.83–7.23 (m, 8H, Ar–H), 5.24 (d, *J* = 7.9 Hz, 1H, H-5), 4.98 (s, 1H, H-4), 4.77 (s, 1H, H-1), 4.51 (d, *J* = 7.9 Hz, 1H, H-6), 3.60 (d, *J* = 7.9 Hz, 1H, H-3), 3.38 (d, *J* = 7.9 Hz, 1H, H-2), 3.27 (s, 3H, –$SOCH_3$). MS (70 eV) *m/z*: 456 ($M^+$), 458 ($M^+$ + 2), 460 ($M^+$ + 4). Anal. calcd. for $C_{22}H_{17}ClN_2O_5S$: C 57.83, H 3.75, N 6.13; found: C 57.84, H 3.77, N 6.12.

***exo,exo-6-(4-Bromophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-methylsulfinylphenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (10d)***

Compound **10d** was obtained as a colorless solid: yield 91%; mp 244 °C. IR ν (KBr, $cm^{-1}$): 3063, 3041 (ArH), 1779, 1721 (C=O), 1567 (C=N), 1240 (C–O–C). $^1$HNMR (DMSO-$d_6$) δ: 7.83–7.25 (m, 8H, Ar–H), 5.24 (d, *J* = 7.9 Hz, 1H, H-5), 4.98 (s, 1H, H-4), 4.76 (s, 1H, H-1), 4.51 (d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.38 (d, *J* = 7.9 Hz, 1H, H-2), 3.27 (s, 3H, –$SOCH_3$). MS *m/z*: 500 ($M^+$), 502 ($M^+$ + 2), 503 ($M^+$ + 3), 504 ($M^+$ + 4). Anal. calcd. for $C_{22}H_{17}BrN_2O_5S$: C 52.70, H 3.42, N 5.59; found: C 52.71, H 3.44, N 5.56.

***exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-methylsulfinylphenyl)-6-(*p*-tolyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (10e)***

Compound **10e** was obtained as a colorless solid: yield 71%; mp 341 °C (Dec.). IR ν (KBr, $cm^{-1}$): 3069, 3035 (ArH), 1781, 1721 (C=O), 1568 (C=N), 1256 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.83–7.19 (m, 6H, Ar–H), 5.23 (d, *J* = 7.9 Hz, 1H, H-5), 4.97 (s, 1H, H-4), 4.75 (s, 1H, H-1), 4.51 (d, *J* = 7.9 Hz, 1H, H-6), 3.59 (d, *J* = 7.9 Hz, 1H, H-3), 3.35 (d, *J* = 7.9 Hz, 1H, H-2), 3.27 (s, 3H, –$SOCH_3$), 2.33 (–$CH_3$). MS *m/z*: 436 ($M^+$), 437 ($M^+$ + 1). Anal. calcd. for $C_{23}H_{20}N_2O_5S$: C 63.29, H 4.62, N 6.42; found: C 63.28, H 4.64, N 6.41.

***exo,exo-4, 8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-6-(4-methoxyphenyl)-3-(4-methylsulfinylphenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole (10f)***

Compound **10f** was obtained as a colorless solid: yield

67%; mp 304 °C (Dec.). IR ν (KBr, $cm^{-1}$): 3051, 3027 (ArH), 1778, 1717 (C=O), 1568 (C=N), 1246 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 8.37–7.49 (m, 6H, Ar–H), 5.25 (d, $J$ = 7.9 Hz, 1H, H-5), 5.01 (s, 1H, H-4), 4.79 (s, 1H, H-1), 4.53 (d, $J$ = 7.9 Hz, 1H, H-6), 3.66 (d, $J$ = 7.9 Hz, 1H, H-3), 3.43 (d, $J$ = 7.9 Hz, 1H, H-2), 3.83 (s, 3H, –$OCH_3$), 3.27 (s, 3H, –$SOCH_3$). MS *m/z*: 452 ($M^+$), 453 ($M^+$ + 1). Anal. calcd. for $C_{23}H_{20}N_2O_6S$: C 61.05, H 4.46, N 6.19; found: C 61.03, H 4.46, N 6.18.

### *exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-6-phenyl-3-(1,3-diolphenyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (11a)*

Compound **11a** was obtained as a colorless solid: yield 95%; mp 183 °C. IR ν (KBr, $cm^{-1}$): 3047, 3018 (ArH), 1782, 1723 (C=O), 1564 (C=N), 1248 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.74–7.07 (m, 8H, Ar–H), 5.25 (s, 2H, OH), 5.18 (d, $J$ = 7.9 Hz, 1H, H-5), 4.96 (s, 1H, H-4), 4.73 (s, 1H, H-1), 4.48 (d, $J$ = 7.9 Hz, 1H, H-6), 3.59 (d, $J$ = 7.9 Hz, 1H, H-3), 3.35 (d, $J$ = 7.9 Hz, 1H, H-2). MS *m/z*: 392 ($M^+$). Anal. calcd. for $C_{21}H_{16}N_2O_6$: C 64.28, H 4.11, N 7.14; found: C 64.27, H 4.13, N 7.13.

### *exo,exo-6-(4-fluorophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(1,3-diolphenyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (11b)*

Compound **11b** was obtained as a colorless solid: yield 82%; mp 176 °C. IR ν (KBr, $cm^{-1}$): 3054, 3016 (ArH), 1780, 1720 (C=O), 1544 (C=N), 1252 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.75–7.06 (m, 7H, Ar–H), 5.25 (s, 2H, OH), 5.18 (d, $J$ = 7.9 Hz, 1H, H-5), 4.9 (s, 1H, H-4), 4.74 (s, 1H, H-1), 4.47 (d, $J$ = 7.9 Hz, 1H, H-6), 3.59 (d, $J$ = 7.9 Hz, 1H, H-3), 3.37 (d, $J$ = 7.9 Hz, 1H, H-2). MS *m/z*: 410 ($M^+$). Anal. calcd. for $C_{21}H_{15}FN_2O_6$: C 61.47, H 3.68, N 6.83; found: C 61.46, H 3.69, N 6.81.

### *exo,exo-6-(4-Chlorophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(1,3-diolphenyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (11c)*

Compound **11c** was obtained as a colorless solid: yield 84%; mp 191 °C. IR ν (KBr, $cm^{-1}$): 3053, 3027 (ArH), 1775, 1715 (C=O), 1550 (C=N), 1266 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.75–7.06 (m, 7H, Ar–H), 5.25 (s, 2H, OH), 5.18 (d, $J$ = 7.2 Hz, 1H, H-5), 4.95 (s, 1H, H-4), 4.73 (s,1H, H-1), 4.47 (d, $J$ = 7.9 Hz, 1H, H-6), 3.59 (d, $J$ = 7.9 Hz, 1H, H-3), 3.37 (d, $J$ = 7.9 Hz, 1H, H-2). MS *m/z*: 426 ($M^+$), 428 ($M^+$ + 2). Anal. calcd. for $C_{21}H_{15}ClN_2O_6$: C 59.10, H 3.54, N 6.56; found: C 59.11, H 3.55, N 6.53.

### *exo,exo-6-(4-Bromophenyl)-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(1,3-diolphenyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (11d)*

Compound **11d** was obtained as a colorless solid: yield 70%; mp 199 °C. IR ν (KBr, $cm^{-1}$): 3053, 3028 (ArH), 1778, 1714 (C=O), 1538 (C=N), 1244 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.76–7.02 (m, 7H, Ar–H), 5.25 (s, 2H, OH), 5.17 (d, $J$ = 7.2 Hz, 1H, H-5), 4.96 (s, 1H, H-4), 4.74 (s, 1H, H-1), 4.47 (d, $J$ = 7.9 Hz, 1H, H-6), 3.59 (d, $J$ = 7.9 Hz, 1H, H-3), 3.37 (d, $J$ = 7.9 Hz, 1H, H-2). MS *m/z*: 470 ($M^+$), 472 ($M^+$ + 2), 474 ($M^+$ + 4). Anal. calcd. for $C_{21}H_{15}BrN_2O_6$: C 53.52, H 3.21, N 5.94; found: C 53.52, H 3.23, N 5.92.

### *exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(1,3-diolphenyl)-6-(**p**-tolyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (11e)*

Compound **11e** was obtained as a colorless solid: yield 68%; mp 176 °C. IR ν (KBr, $cm^{-1}$): 3058, 3034 (ArH), 1780, 1723 (C=O), 1544 (C=N), 1282 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.77–7.02 (m, 7H, Ar–H), 5.25 (s, 2H, OH), 5.17 (d, $J$ = 7.9 Hz, 1H, H-5), 4.94 (s, 1H, H-4), 4.72 (s, 1H, H-1), 4.46 (d, $J$ = 7.9 Hz, 1H, H-6), 3.59 (d, $J$ = 7.9 Hz, 1H, H-3), 3.37 (d, $J$ = 7.9 Hz, 1H, H-2), 2.33 (–$CH_3$). MS *m/z*: 406 ($M^+$), 408 ($M^+$ + 2). Anal. calcd. for $C_{22}H_{18}N_2O_6$: C 65.02, H 4.46, N 6.89; found: C 65.01, H 4.47, N 6.87.

### *exo,exo-4,8-Epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(1,3-diolphenyl)-6-(4-methoxyphenyl)-pyrrolo[3,4-**f**]-1,2-benzisoxazole (11f)*

Compound **11f** was obtained as a colorless solid: yield 58%; mp 185 °C. IR ν (KBr, $cm^{-1}$): 3057, 3021 (ArH), 1786, 1719(C=O), 1536 (C=N), 1238 (C–O–C). $^1$H NMR (DMSO-$d_6$) δ: 7.86–7.22 (m, 7H, Ar–H), 5.25 (s, 2H, OH), 5.25 (d, $J$ = 7.9 Hz, 1H, H-5), 4.97 (s, 1H, H-4), 4.73 (s, 1H, H-1), 4.46 (d, $J$ = 7.9 Hz, 1H, H-6), 3.83 (s, 3H, –$OCH_3$), 3.56 (d, $J$ = 7.9 Hz, 1H, H-3), 3.33 (d, $J$ = 7.9 Hz, 1H, H-2). MS *m/z*: 422 ($M^+$), 424 ($M^+$ + 2). Anal. calcd. for $C_{22}H_{18}N_2O_7$: C 62.56, H 4.30, N 6.63; found: C 62.55, H 4.32, N 6.62.

### Cell culture and antiproliferative assays

Stock solutions of drugs were prepared as follows (and stored at –20 °C): Cantharidin (Biomol, USA) as a 12 mmol/L solution in dimethylsulphoxide (DMSO); norcantharidin and cantharidin analogues as 10 mmol/L solutions in phosphate buffered saline. All cell lines were cultured at 37 °C in 5% $CO_2$.

Antiproliferative activity of different compounds was determined by MTT method. Cells in logarithmic growth were plated in triplicate in 100 μL medium at a density of 3 000–5 000 cells/well in 96-well plates. When the cells were in logarithmic growth, 100 μL medium with or without the test compounds were added to each well. After 72 h, MTT solution (5.0 mg/mL) was added (10.0 μL/well), and plates 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 at 570 nm. Concentration of drug that inhibited 50% of cells ($IC_{50}$) was calculated using the software of Dose–Effect analysis with Microcomputers.

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