# Cytotoxic Acridone and Indoloquinazoline Alkaloids from *Zanthoxylum poggei*

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## Abstract

Two new alkaloids, poggeicridone (**1**) and 2-methoxy-7,8- dehydroruteacarpine (**6**), together with nine known compounds, were isolated from the dichloromethane (DCM) extract of the bark of *Zanthoxylum poggei* (Engl.) P. G. Waterman. The structures of all compounds were determined by comprehensive spectroscopic analyses (1D and 2D NMR and EI- and ESI-MS)). Compounds **5-9** exhibited strong suppressive effects on the phagocytosis response upon activation with serum opsonized zymosan in the *in vitro* oxidative burst studies using whole blood. The $IC_{50}$ values were in the range of 12.0-25.9 μM. These compounds displayed a moderate level of cytotoxic activity against the human Caucasian prostate adenocarcinoma cell line PC-3, with $IC_{50}$ values of 15.8 and 22.1 μM (the $IC_{50}$ value of the positive control standard doxorubicin was $IC_{50}$ 0.9 μM). All isolated compounds were also tested against plant pathogenic bacteria, fungi and oomycetes using the paper disk agar diffusion assay, resulting in no significant activities (MICs > 1 mg/mL).


**Keywords:** *Zanthoxylum pogeei*; Rutaceae; acridone; indoloquinazoline alkaloids; oxidative burst inhibition; antimicrobial; cytotoxic activities.

### 1. Introduction

As part of our continuing research for bioactive molecules from Cameroonian plants, *Zanthoxylum poggei* (Engl.) P. G. Waterman (Rutaceae) (Syn. *Fagara poggei* Engl.) was studied as there has been no report on any phytochemical study on this species available to date. *Z. poggei* is a liane or scandent shrub of the African rainforests in countries including Cameroon and Congo. In a traditional medicine setting, it is used for the treatment of tumours, swellings, inflammation, malaria and gonorrhoea, as well as to prepare poisonous arrows (Irvine, 1961; Raponda-Walker and Sillans, 1961); the root bark of this plant is also used as a toothbrush (Kerharo and Adam, 1971). Previous phytochemical investigations on other species of the *Zanthoxylum* revealed the presence of acridones, amides, aporphines, bezophenanthridines, coumarins, fatty acids, flavonoids, indoloquinazolines, lignans, phenolic compounds, steroids and triterpenoids, (Waterman, 1976; Tringali et al., 2001; Mbaze et al., 2007; Mbaze et al., 2009; Wansi et al., 2009; Wouatsa et al., 2013, Wang et al., 2014) with some of these compounds exhibiting potent antibacterial, fungicidal and cytotoxic activity, as well as oxidative burst and $\alpha$-glucosidase inhibition and toxic properties (Tringali et al., 2001;

Ogwal-Okeng et al., 2003; Mbaze et al., 2007; Mbaze et al., 2009; Wansi et al., 2009; Wouatsa et al., 2013). In this report, we describe the isolation and structural elucidation of a novel dimer acridone-acridone alkaloid with C-O-C linkage, poggeicridone (**1**), and a new indoloquinazoline alkaloid, 2-methoxy-7,8-dehydroruteacarpine (**6**), together with the oxidative burst inhibition, cytotoxic evaluation and antimicrobial properties of isolated compounds.

## 2. Results and discussion

The stem bark of *Z. poggei* was extracted with $CH_2Cl_2$ and subjected to bioassay-guided fractionation based on its inhibitory activity against the oxidative burst of whole blood (90%). This extract was subjected to column chromatography (silica gel) and preparative TLC to afford two new and eleven known compounds (Fig. 1). By comparison with the reported data, the known compounds were identified as citracridone I (**2**), citracridone III (**3**), 5-hydroxynoracronycine (**4**), 5-methoxynoracronycine (**5**), 2-hydroxyruteacarpine (**7**), 2-methoxyruteacarpine (**8**), 5,8,13,14-tetrahydro-2-methoxy-14-méthyl-5-oxo-7H-indolo[2',3':3,4]pyrido[2,1-b]quinazolin-6-ium chloride (**9**), lupeol and *β*-sitosterol (Ezugwu et al., 2003; Wansi et al., 2008, Happi et al., 2011; Fomani et al., 2016), whilst the new compounds were identified by comprehensive spectroscopic analyses.

Compound **1** was obtained as a yellow amorphous powder, showing a positive reaction with $FeCl_3$, indicating its phenolic nature. The pseudomoelcular ion was found to be $C_{39}H_{34}O_8N_2Na$ by HR-ESIMS ($[M+Na]^+$ at *m/z* 681.2209, calcd 681.2213). The UV spectrum showed a highly conjugated system with absorption bands characteristic of acridone-type alkaloids at $\lambda_{max}$ 265, 295, 350 and 390 nm (Wu and Chen, 2000). The IR bands at $\upsilon_{max}$ 3900, 3450 and 1640, 1631 $cm^{-1}$ inferred the presence of an OH and conjugated carbonyl groups.

The $^1H$ NMR spectrum (Table 1) exhibited two deshielded protons at $\delta_H$ 14.51 (1H, *s*) and 14.55 (1H, *s*) exchangeable with $D_2O$ suggesting the presence of two acridone nuclei in **1** (Takemura et al., 1998). In the aromatic region, an ABC system of three aromatic protons at $\delta_H$ 7.83 (dd, *J* = 7.5, 1.3 Hz); 7.24 (t, *J* = 7.5 Hz); 7.33 (dd, *J* = 7.5, 1.3 Hz) could be attributed to H-8', H-7' and H-6', respectively. An AB system of two aromatic protons at $\delta_H$ 7.97 (d, *J* = 8.8 Hz); 7.01 (d, *J* = 8.8 Hz) were assigned to H-8 and H-7, respectively. These assignments were determined through the lower-field signal of H-8 and H-8', which was deshielded by the adjacent carbonyl group of the acridone moiety (Wansi et al., 2006). Furthermore, the $^1H$ NMR spectrum showed two *N*-methyl group at $\delta_H$ 3.80 and 3.87, two aromatic protons as singlet at

$\delta_H$ 6.12 (H-2) and 6.13 (H-2'), one methoxy group at $\delta_H$ 3.90 and the presence of two 2,2-dimethylpyran ring at $\delta_H$ 6.76 (1H, d, *J* = 9.7 Hz, H-11), 5.70 (1H, d, *J* = 9.7 Hz, H-12), 1,52 ($CH_3$-14) and 1,48 ($CH_3$-15) and at $\delta_H$ 6.74 (1H, d, *J* = 9.8 Hz, H-11'), 5.68 (1H, d, *J* = 9.8 Hz, H-12'), 1,52 ($CH_3$-14') and 1,47 ($CH_3$-15'). Thus, compound **1** was assumed to be a bis-alkaloid of two acridone moieties. This inference was confirmed by the $^{13}C$ NMR data (Table 1), displaying characteristic signals of citracridone I and hydroxynoracronycine (Happi et al., 2011), and also by 2D NMR analyses.

The orientation of the two dimethylchroman rings was precisely determined by the HMBC analysis. In the HMBC spectrum, proton H-11 ($\delta_H$ 6.76) showed correlation with the carbon signals at C-4a ($\delta_C$ 147.6), C-3 ($\delta_C$ 161.2), C-4 ($\delta_C$ 102.5) and C-13 ($\delta_C$ 76.5) and on the other hand, H-11' ($\delta_H$ 6.74) exhibited correlations to the carbon signals at C-3' ($\delta_C$ 160.9), C-4a' ($\delta_C$ 147.9), C-4' ($\delta_C$ 102.3) and C-13' ($\delta_C$ 76.5). These findings confirmed that the two dimethylchromene rings were fused in an angular fashion to the acridone's nucleus at positions C-3/C-4 and C-3'/C-4'. Therefore, establishing the linkage between the two nuclei was required. In the HMBC spectrum, the $^4J$ correlation observed between proton H-6' ($\delta_H$ 7.33) and carbon C-5 ($\delta_C$ 137.1) supported the position of the linkage between C5-O-C5'. The presence of C-O-C linkage was further supported by the EIMS, which showed two important ion fragments at *m/z* 363 (5-hydroxy-6-methoxynoracronycine, $C_{20}H_{28}NO_5$) and at *m/z* 347 (5-hydroxynoracronycine, $C_{19}H_{23}NO_5$) resulting in the breaking of the C-O-C linkage and the fixation of one molecule of $H_2O$. From the above spectroscopic data, the structure of compound **1** was determined as a *bis*-acaridone, named, poggeicridone.

It should be noted that poggeicridone is very unstable and easily decomposed, this may explain why only three dimers linked by C-O-C have been isolated from *Tephrosia tepicana* (Standl.) Standl. (Leguminosae) (Gomez-Garibay et al., 1997), from *Swertia chirata* Buch. Ham. (Gentianaceae) (Mandal and Chatterjee, 1987) and from *Beilschmiedia zenkeri* Engl. (Lauraceae) (Lenta et al., 2009).

Compound **6** was obtained as a yellow amorphous powder. It gave a positive reaction with $FeCl_3$, indicating its phenolic nature. The molecular composition was found to be $C_{19}H_{14}N_3O_2$ by HR-EI-MS ($[M]^+$ at *m/z* 315.1001, calcd 315.1004). The UV spectrum showed a highly conjugated system with absorption bands characteristic of indolopyridoquinazoline-type alkaloids (Ezugwu et al., 2003; Wansi et al., 2008). Its IR spectrum showed the presence of a

NH and a carbonyl at 3384 and 1656 $cm^{-1}$, respectively. The $^{1}H$ NMR spectrum (Table 2) of compound **6** exhibited four aromatic protons corresponding to an *ortho*-disubstituted ring A at $\delta_H$ 7.27 (t, *J* = 7.9 Hz, H-10), 7.47 (t, *J* = 8.2 Hz, H-11), 7.59 (d, *J* = 8.2 Hz, H-12), and 7.96 (d, *J* = 7.9 Hz, H-9), together with three aromatic protons for an ABC system of ring E at $\delta_H$ 6.98 (d, *J* = 1.3 Hz, H-1), 7.01 (dd, *J* = 8.3, 1.3 Hz, H-3), and 8.28 (d, *J* = 8.3 Hz, H-4) (Wansi et al., 2012). This inference was supported by the $^{13}C$ NMR data (Table 2), which showed characteristic signals of an indoloquinazoline alkaloid of ring A at $\delta_C$ 120.7 (C-9), 121.0 (C-10), 127.4 (C-11), and 112.5 (C-12), as well as for rings D and E at $\delta_C$ 105.1 (C-3), 116.0 (C-1), 110.0 (C-4a), 129.2 (C-4), 141.0 (C-13b), 149.7 (C-14a), 158.5 (C-5), and 165.3 (C-2) (Wansi et al., 2012). Furthermore, the $^{1}H$ NMR spectrum of compound **6** showed an AB system of two olefinic CH resonances at $\delta_H$ 7.50 (d, *J* = 7.6 Hz, H-8) and 8.65 (d, *J* = 7.6 Hz, H-7), and a methoxy group at $\delta_H$ 4.30 (brs). This assignment was furthermore supported by the $^{13}C$ NMR spectrum, which showed additional two olefinic signals of C-7 and C-8 at $\delta_C$ 107.7 and 118.3 respectively. The complete assignments of the indoloquinazoline skeleton and its substitution pattern were based on COSY, HMQC, and HMBC experiments. In the HMBC spectrum, correlations between the aromatic low-field signal of H-4 ($\delta_H$ 8.28) with C-5 ($\delta_C$ 158.8) and C-2 ($\delta_C$ 165.3) and between proton H-1 ($\delta_H$ 6.98) with C-3 ($\delta_C$ 105.1), C-4a ($\delta_C$ 110.0), C-14a ($\delta_C$ 149.7) and C-2 ($\delta_C$ 165.3) indicated the position of a methoxy group at C-2. From the above spectroscopic data, the structure of compound **6** was determined as 2-methoxy-7,8-dehydroruteacarpine.

Tests of pure compounds **2-9** on disc diffusion agar against bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*), fungi (*Mucor miehei* and *Candida albicans*), and plant pathogen oomycetes (*Aphanomyces cochlioides*, *Pythium ultimum* and *Rhizoctonia solani*) resulted in absent or low activities.

As compounds **2-4** have previously been reported with oxidative burst inhibition and cytotoxicity properties (Happi et al., 2011), only compounds **5-9** were screened over a wide range of concentrations (3.1-50 $\mu g \cdot mL^{-1}$) for their immunomodulatory potential. These compound were shown to possess inhibitory activity upon activation with serum opsonized zymosan, which was tested *in vitro* for oxidative burst studies of whole blood. All compounds showed a strong suppressive effect on phagocyte oxidative burst of the whole blood in a dose-dependent manner with $IC_{50}$ ranging from 12.0 to 25.9 μM, compared to the control ibuprofen with $IC_{50}$ = 11.2 μM (table 3). The results of the chemiluminescence assay suggest that compounds **5-9** represent effective naturally occurring molecules that prevent zymosan-

induced oxidative burst with high efficiency. Further investigation could define pharmacological activities connected with oxidative stress, like inflammation and other pathologic conditions. In order to check whether the oxidative burst inhibition of those compounds is related to their cytotoxicity, we carry out cytotoxicity tests. Interestingly, those compounds (**5**-**9**) displayed moderate cytotoxic activity against the human Caucasian prostate adenocarcinoma cell PC-3 line with $IC_{50}$ 15.8 and 22.1 µM compared to standard doxorubicine with $IC_{50}$ = 0.9 µM (table 4).

The genus *Zanthoxylum* belong to the subfamily Rutoideae, tribe Zanthoxyleae (Takhtajan, 2009), this subfamily rarely produces acridones alkaloids. To our knowledge, the acridones alkaloids were reported only in four species (*Z. zanthoxyloides*, *Z. leprieuri*, *Z. macrophylla* and *Z. bungeanum*) (Tringali et al., 2001; Wouatsa et al., 2013; Wang et al., 2014). Nonetheless several acridone alkaloids were isolated from the genera *Ruta* and *Melicope* belonging to the subfamily Rutoideae (Kuzovkina et al., 2004; Wang et al., 2014). Strong oxidative burst inhibition and moderate *in vitro* cytotoxic activity against the human Caucasian prostate adenocarcinoma cell line PC-3 of some constituents have been observed, which may contribute to the traditional use.

## 3. Experimental

### *3.1. General experimental procedures*

Ultraviolet spectra were recorded on a Hitachi UV 3200 spectrophotometer in MeOH. Infrared spectra were recorded on a JASCO 302-A spectrophotometer. ESI-HR mass spectra were recorded on a Bruker FTICR 4.7 T mass spectrometer. EI-MS were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for EI-HR-MS. The $^{1}H$- and $^{13}C$-NMR spectra were recorded at 500 MHz and 125 MHz respectively on Bruker DRX 500 NMR spectrometers. Methyl, methylene and methine carbons were distinguished by DEPT experiments. Homonuclear $^{1}H$ connectivities were determined by using the COSY experiment. One-bond $^{1}H$-$^{13}C$ connectivities were determined with HMQC gradient pulse factor selection. Two- and three-bond $^{1}H$-$^{13}C$ connectivities were determined by HMBC experiments. Chemical shifts are reported in δ (ppm) using TMS as internal standard and coupling constants (J) were measured in Hz. Column chromatography was carried out on silica gel (70-230 mesh, Merck). TLC was performed on Merck precoated silica gel 60 $F_{254}$ aluminium foil, and spots were detected using ceric sulphate spray reagent. The purity of the

compounds was investigated by means of $^{1}$H-NMR and ESI-MS. The degree of purity of the positive control compounds was ⩾ 98 %, while that of the isolated compounds was > 95 %. Doxorubicin was purchased from Sigma-Aldrich (Germany), nystatin was purchased from Maneesh Pharmaceutic Pvt. Ltd., Govandi, Mumbai 400043 (India) and gentamicin from Jinling Pharmaceutic (Group) corp., Zhejang Tieng Feng Pharmaceutic Factory, No. 11 Chezhan Road, Huzhou city, Zhejang, China. The Caucasian prostate adenocarcinoma cell line PC-3 was purchased from the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, USA. All reagents used were of analytical grade.

*3.2. Plant material*

The fresh stem bark of *Z. poggei* were collected from Batouri, East region, Cameroon and identified by Mr Nana Victor of the National Herbarium, Yaoundé, Cameroon, where a voucher specimen was deposited under ref. 1970 SRF/CAM.

*3.3. Extraction and isolation*

The air-dried and powdered stem bark (1.5 kg) of *Z. poggei* was Soxhlet-extracted using DCM as a solvent. After evaporation under reduced pressure, 26.5 g of crude extract was obtained. This extract was purified by column chromatography over silica gel 60 (230-400 mesh, 6.5 × 50 cm) and preparative TLC using a gradient system of *n*-hexane, $CH_2Cl_2$, ethyl acetate, and MeOH. A total of 95 sub-fractions (ca. 100 mL each) were collected and pooled on the basis of TLC analysis leading to four main series (A -D).

Fraction A [6.5 g, combination of sub-fractions 1-28 (100–2700 mL), eluted with a mixture of *n*-hexane-DCM (3:1)] was chromatographed over silica gel 60C column (2.5 × 30.0 cm) with an *n*-hexane-DCM gradient. 18 sub-fractions of ca. 100 mL each were collected and combined based on TLC profiles. Sub-fractions 1-13 (100-1300 mL) were further chromatographed on silica gel 60H (2.5 × 30.0 cm) with a mixture of *n*-hexane-DCM (4:1) for elution to yield lupeol (15.5 mg).

Fraction B [5.3 g, constituted by sub-fractions 29-50 (1000-2100 mL), eluted with a mixture of *n*-hexane-DCM (3:2)] was chromatographed over a silica gel 60C column (2.5 × 30.0 cm) with *n*-hexane-DCM gradient. 22 sub-fractions of ca. 100 mL each were collected and combined based on TLC comparison. Sub-fractions 1-10 (100-900 mL) were further chromatographed over silica gel 60H (2.5 × 30.0 cm) with a mixture of an *n*-hexane-$CH_2Cl_2$ (3:1) to yield *β*-sitosterol (11.5 mg).

Fraction C [9.0 g, combination of sub-fractions 51-70 (100-1900 mL), eluted with *n*-hexane-DCM (1:1) to 100% DCM] was chromatographed on a silica gel 60C column (2.5 × 30.0 cm) with an *n*-hexane-DCM gradient. A total of 32 fractions of ca. 100 mL each were collected and combined based on TLC. Sub-fractions 1-20 sub-fractions were chromatographed over silica gel 60H (2.5 × 30.0 cm) with a mixture of an *n*-hexane-DCM (1:1) and 100% DCM to yield Poggeicridone (**1**) (9.1 mg), citracridone I (**2**) (15.5 mg), and 2-methoxy-7,8-dehydroruteacarpine (**6**) (13.4 mg). Sub-fractions 21-32 sub-fractions were further chromatographed over silica gel 60H (2.5 × 30.0 cm) with 100% DCM to yield 5-hydroxynoracronycine (**4**) (24.2 mg), 5-methoxynoracronycine (**5**) (5.5 mg) and 2-methoxyruteacarpine (**8**) (16.5 mg).

Similarly, fraction D [5.6 g, constituted by sub-fractions 71-95 (100-2400 mL), eluted with 100% DCM and a mixture of DCM-MeOH (9.5:0.5) gradients] was chromatographed over silica gel 60C column (2.5 × 30.0 cm) with 100% $DCM_2$ to gradients. As a result, 20 sub-fractions of ca. 100 mL each were collected and combined on the basis of TLC to yield citracridone III (**3**) (6.6 mg), 2-hydroxyruteacarpine (**7**) (12.4), 5,8,13,14-tetrahydro-2-methoxy-14-méthyl-5-oxo-7H-indolo[2',3':3,4]pyrido[2,1-b]quinazolin-6-ium chloride (**9**) (7.8).

*3.4. Poggeicridone (1)*

Yellow amorphous powder (Aceton); $R_f$ = 0.50, silica gel 60 $F_{254}$, DCM; UV (MeOH) $\lambda_{max}$ (log ε) 260 (3.42), 290 (3.14), 335 (4.00), 345 (4.40) nm; IR (KBr) $\upsilon_{max}$ 3100, 2950, 1639, 1600, 1500, 1390, 1012, 750 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 1; EI-MS (%) *m/z* 363 ($C_{20}H_{28}NO_5$, 100), 347 ($C_{19}H_{23}NO_5$, 76), 310 (36), 289 (25), 263 (14), 243 (20), 215 (15), 178 (45), 149 (26); (+)-ESI-MS (%) *m/z* 681 ($[M+Na]^+$, 100); HR-ESIMS $[M+Na]^+$ *m/z* 681.2209 (calcd for $C_{39}H_{34}O_8N_2Na$, 681.2213).

*3.5. 2-methoxy-7,8-dehydroruteacarpine (6)*

Yellow amorphous powder ($CHCl_3$-MeOH); $R_f$ = 0.69, silica gel 60 $F_{254}$, $CH_2Cl_2$/MeOH (9.5/0.5); UV (MeOH) $\lambda_{max}$ (log ε) 210 (4.63), 282 (4.53), 290 (5.70), 326 (4.54), 337 (4.45), 366 (4.60), 390 (4.50) nm; IR (KBr) $\upsilon_{max}$ 3380, 3000, 2832, 1780, 1663, 1656, 1595, 1390, 1217, 1015, 754 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 2; EI-MS (%) *m/z* 315 ($[M]^+$, 100), 297 (28), 257 (42), 242 (45), 228 (75), 156 (32), 199 (25), 173 (12), 68 (42); HR-EIMS $[M]^+$ *m/z* 315.1001 (calcd for $C_{19}H_{14}N_3O_2$, 315.1004).

*3.6. Biological activities*

*3.6.1. Paper disk agar diffusion assay*

Agar diffusion test plates with the bacteria *B. subtilis* and *Escherichia coli* (on peptone agar), *S. aureus* (Bacto nutrient agar) and the fungi *M. miehei* and *C. albicans* (Sabouraud agar) as test strains were performed as previously described (Maskey et al., 2002). For the plant pathogen oomycetes *A. cochlioides*, *P. ultimum* and *R. solani*, squares of 0.5 cm x 0.5 cm were cut with a microbiological hook from the growth margins of mycelial mats grown on PDA plates, inoculated onto the centers of fresh plates and cultivated for 24 h at 28 °C to initiate radial growth. Compounds **2**–**9** were dissolved in DCM/MeOH (9:1) and paper disks (Ø 9 mm) were impregnated with 40- mg each, dried for 1 h under sterile conditions and arranged evenly on the premade agar test plates, while for oomycete plates, the disks were placed around the mycelia squares at a distance of 30 mm. Bacteria and fungi plates were kept in an incubator at 37 °C for 15 h, oomycetes at 28 °C for 48 h. The diameter of inhibition zones (mm) was measured directly or calculated from the radius. Nystatin was used as positive control for fungi and gentamycin for bacteria. *B. subtilis*, *E. coli*, *S. aureus* and *C. albicans* are clinical isolates received from the Center Pasteur du Cameroun, Yaounde, Cameroon and were carefully purified. *Mucor miehei* and the plant pathogen oomycetes *Aphanomyces cochlioides*, *Pythium ultimum*, and *Rhizoctonia solani* originate from the Institute of Organic and Biomolecular Chemistry, Georg-August University Göttingen.

*3.6.2. Microdilution broth assay*

The MICs of compound were measured by the microdilution broth susceptibility assay (CLSI/NCCLS, 2008). The inocula of bacterial and fungal strains were prepared from 12 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The compounds were dissolved in 10% DMSO and serial twofold diluted in 96-well microtiter plates in duplicate, using BHI broth for bacterial and Sabouraud dextrose broth for fungal tests. Standardized inocula of test strains were added and incubated at 37 °C for 24 h on a rotary shaker at 200 rpm. The MIC is read as the lowest concentration that inhibits test strain growth.

*3.6.3. Chemiluminescence assay for determination of immunomodulatory activity:*

A luminol-enhanced chemiluminescence assay was performed as previously described (Helfand et al., 1982). Whole blood (diluted 1:200) and neutrophils ($1 \times 10^7$) suspended in Hank's balance salt solution with calcium and magnesium ($HBSS^{++}$) were incubated with 50

μL of each test compound at concentrations of 3.1-50 μg.mL$^{-1}$ for 30 min. To each well, 50 μL (20 mg.mL$^{-1}$) of zymosan (Sigma Chemical Co.), 50 μL (7 × $10^5$ M) of luminol (G-9382; Sigma Chemical Co.), and then HBSS$^{++}$ were added to adjust the final volume to 0.2 ml. HBSS$^{++}$ was used as a control. Luminescence peaks are recorded with a luminometer (Luminoskan RS Lab system) and expressed as median inhibitory concentrations ($IC_{50}$) ± standard error of mean, with ibuprofen as the standard ($IC_{50}$ = 11.2 ± 1.8 μM).

*3.6.4. Cytotoxicity assay*

Cytotoxic activities of the compound were evaluated against the human Caucasian prostate adenocarcinoma cell line PC3 by the MTT method as reported by Zhao et al., (2008). Freshly trypsinized cell suspensions were seeded into 96-well microtiter plates at densities of 1 × $10^4$ cells per well, and test compounds were added from DMSO-diluted stock. After 3 days, the attached cells were incubated with MTT and subsequently solubilized in DMSO. The absorbance at 550 nm was measured by using a microplate reader. The $IC_{50}$ is the concentration of agent that reduced cell growth under experimental conditions by 50%, with Doxorubicin as the positive control ($IC_{50}$ = 0.9 μM).

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Table **1**. $^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR assignments for (**1**) in Aceton-$d_6$ at 15°C[a]

| Attribution | 1 | |
|---|---|---|
| | $^{13}C$ | $^{1}H$ [m, *J* (Hz)] |
| 1 | 164.6 | 14.51 (s) |
| 2 | 97.5 | 6.13 (s) |
| 3 | 161.2 | - |
| 4 | 102.5 | - |
| 4a | 147.6 | - |
| 5 | 137.1 | - |
| 6 | 156.1 | - |
| 7 | 113.0 | 7.01 (d, 8.8) |
| 8 | 122.2 | 7.97 (d, 8.8) |
| 8a | 119.9 | - |
| 9 | 181.3 | - |
| 9a | 106.8 | - |
| 10a | 142.6 | - |
| 11 | 122.3 | 6.76 (d, 9.7) |
| 12 | 124.9 | 5.70 (d, 9.7) |
| 13 | 76.5 | - |
| 14 | 28.5 | 1.52 (s) |
| 15 | 26.5 | 1.47 (s) |
| *N*-$CH_3$ | 48.2 | 3.80 (s) |
| O-$CH_3$ | 59.6 | 3.92 (s) |
| 1’ | 163.9 | 14.44 (s) |
| 2’ | 97.2 | 6.12 (s) |
| 3’ | 160.9 | - |
| 4’ | 102.3 | - |
| 4a’ | 147.9 | - |
| 5’ | 148.3 | - |
| 6’ | 120.6 | 7.33 (d, 7.5) |
| 7’’ | 124.3 | 7.24 (dd, 7.8 ; 7.5) |
| 8’ | 117.6 | 7.83 (dd, 7.5; 1.5) |
| 8a’ | 124.9 | - |
| 9’ | 181.9 | - |
| 9a’ | 106.5 | - |
| 10a’ | 147.9 | - |
| 11’ | 122.3 | 6.74 (d, 9.7) |
| 12’ | 123.9 | 5.68 (d, 9.7) |
| 13’ | 76.5 | - |
| 14’ | 28.5 | 1.51 (s) |
| 15’ | 26.4 | 1.48 (s) |
| *N*-$CH_3$ | 48.1 | 3.86 (s) |

[a]Assignments were based on HMQC, HMBC, COSY and NOESY experiment

Table **2**. $^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR assignments for (**6**) in $CDCl_3$/MeOD[a]

| **Attribution** | **6** | |
|---|---|---|
| | **$^{13}C$** | **$^{1}H$ [m, *J* (Hz)]** |
| 1 | 116.0 | 6.98 (d, *J* = 1.3) |
| 2 | 165.3 | - |
| 3 | 105.1 | 7. 01 (dd, *J* = 8.3; 1.3) |
| 4 | 129.2 | 8.28 (d, *J* = 8.3) |
| 4a | 110.0 | - |
| 5 | 158.8 | - |
| 7 | 118.3 | 8.65 (d, *J* = 7.6) |
| 8 | 107.7 | 7.50 (d, *J* = 7.6) |
| 8a | 121.7 | - |
| 9a | 123.7 | - |
| 9 | 120.7 | 7.96 (d, *J* = 7.9) |
| 10 | 121.0 | 7.27 (t, *J* = 7.9) |
| 11 | 127.4 | 7.47 (t, *J* = 8.2) |
| 12 | 112.5 | 7.59 (d, *J* = 8.2) |
| 12a | 139.8 | - |
| 13a | 129.3 | - |
| 13b | 141.7 | - |
| 14a | 149.7 | - |
| OMe | 55.5 | 4.30 (s) |

[a]Assignments were based on HMQC, HMBC, COSY and NOESY experiment

Table **3.** Effect of compounds **5-9** on oxidative burst of whole blood.

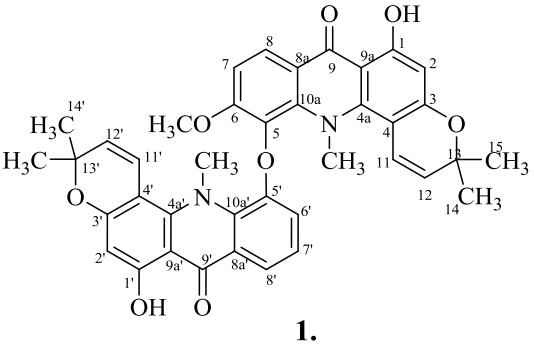
| Compounds | $IC_{50}$ ± S.E.M.(μM) |
|---|---|
| 5 | 13.5 ± 3.5 |
| 6 | 21.6 ± 2.5 |
| 7 | 24.5 ± 3.6 |
| 8 | 25.9 ± 1.5 |
| 9 | 12.5 ± 0.4 |
| Ibuprofen[a] | 0.9 ± 0.1 |

[a]standard used in the assay

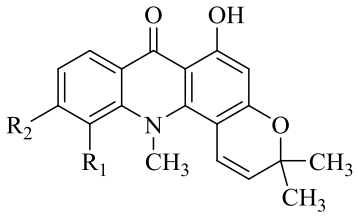
Table **4.** Cytotoxicity against human prostate adenocarcinoma cell line PC-3.

| Compounds | $IC_{50}$ ± S.E.M.(μM) |
|---|---|
| 5 | 16.5 ± 1.4 |
| 6 | 22.1 ± 2.5 |
| 7 | 17.4 ± 2.0 |
| 8 | 19.6 ± 3.0 |
| 9 | 15.8 ± 1.5 |
| doxorubicin[a] | 0.9 ± 0.1 |

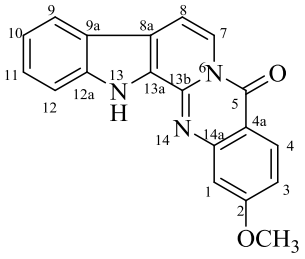
[a]standard used in the assay



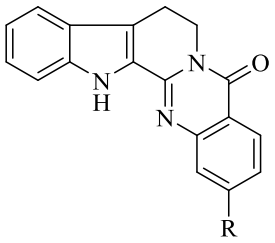
**1.**



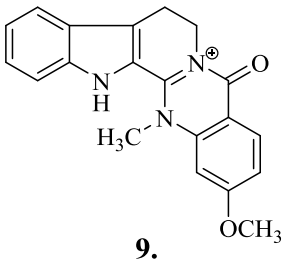
**2.** $R_1 = OCH_3$; $R_2 = OH$
**3.** $R_1 = R_2 = OH$
**4.** $R_1 = OH$; $R_2 = H$
**5.** $R_1 = OCH_3$; $R_2 = H$



**6.**



**7.** R = OH
**8.** R = $OCH_3$



**9.**

**Figure 1** Structures of compounds **1-9.**