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Antimalarial activity and cytotoxicity of zerumbone derivatives

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ABSTRACT: Zerumbone, a sesquiterpene isolated from the rhizome of *Zingiber zerumbet*, shows strong antimalarial and cytotoxic activities. A series of zerumbone derivatives were synthesized and evaluated for in vitro antimalarial activity against *Plasmodium falciparum* and cytotoxicity against human cancer cell lines (NCI-H187, KB and BC) as well as normal cells (Vero cells). Three of the compounds showed potent antimalarial activity with IC_{50} values of 32.8, 13.7, and 9.8 μM. They were less toxic against Vero cells.

KEYWORDS: *Zingiber zerumbet*, sesquiterpene, cyanozerumbone, *Plasmodium falciparum*

INTRODUCTION

As part of our on-going research programme on biologically active substances from Thai bioresources [1–4], we have intensively screened biological activities of extracts and pure compounds from plants. Among these, zerumbone isolated from *Zingiber zerumbet* Smith has been studied intensively for potential use in anti-inflammatory, chemopreventive, and chemotherapeutic strategies [5]. This compound suppresses tumour promoter [6], inhibits the growth of a human leukaemia cell line [7,8], exhibits antiproliferative [9], antihyperglycaemic [10], antioxidant, anti-microbial [11], anti-platelet activating factor [12], anti-inflammatory [13] and anti-HIV activities [14].

Zerumbone, the major component in the rhizome of *Z. zerumbet*, contains three double bonds; an isolated one at C6 and two double bonds at C2 and C10 which are part of a cross conjugated dienone system [15]. It was reported that the C2 double bond appears least hindered due to being furthest from the *gem* dimethyl substituents at C9 [16]. Kitayama and co-workers reported the structural transformation of this compound and the regio- and stereochemistry of its derivatives [17,18]. In our previous work, we synthesized zerumbone derivatives and evaluated cytotoxicity against cholangiocarcinoma cell lines [19] and studied for bioreductive agent [20]. In addition, the other activities such as antimalarial and cytotoxicity against NCI-H187 (human small cell lung cancer), BC (Breast cancer cells) and KB (human epidermoid carcinoma), and normal (Vero cells) cell lines were interested. Preliminary bioactivity screening in our laboratory revealed that zerumbone exhibits strong antimalarial activity against *P. falciparum* with an IC_{50} value of 11.8 μM. It also shows strong cytotoxicity against cancer cells, NCI-H187, BC and KB, as well as against the normal cells, Vero cells, with IC_{50} values ranging from 11–33 μM. This means that this compound is unsuitable not only as an antimalarial but also as an anticancer agent. This has led to a limitation for further clinical studies or biopharmaceutical uses.

The chemical structure and biological activities of this compound prompt us to modify the chemical structure by simple reaction and evaluate bioactivities especially antimalarial and cytotoxicity of zerumbone derivatives. This compound was designed to examine the role of different substitutes such as hydroxyl, amine, epoxy, and cyano groups. It is hoped that continued research will lead to development of a new lead compound from zerumbone as antimalarial and anticancer agents.

MATERIALS AND METHODS

General

NMR spectra were recorded on a Varian Mercury plus spectrometer operating at 400 MHz (^{1}H) and at 100 MHz (^{13}C). IR spectra were recorded as KBr disks using a Perkin Elmer Spectrum One FT-IR

spectrophotometer. Mass spectra were determined on a Micromass Q-TOF 2 hybrid quadrupole time-of-flight mass spectrometer with a Z-spray ES source (Micromass, Manchester, UK). Melting points were determined on a SANYO Gallenkamp melting point apparatus and were uncorrected. Thin layer chromatography (TLC) was carried out on MERCK silica gel 60 F_{254} TLC aluminium sheet. Column chromatography was done with silica gel 0.063–0.200 mm or less than 0.063 mm. Preparative layer chromatography (PLC) was carried out on glass supported silica gel plates using silica gel 60 PF_{254} for preparative layer chromatography. All solvents were routinely distilled prior to use.

Plant sample and extract preparation

Dried rhizomes of *Z. zerumbet* (4.0 kg) were ground into powder and then extracted with EtOAc (3×5 l) at room temperature. Removal of solvent under reduced pressure gave crude EtOAc extract (32.8 g) which was further subjected to column chromatography on silica gel 60 (500 g) and subsequently eluted with three solvents (hexane, EtOAc and MeOH) by gradually increasing polarity of elution solvents system. The eluents were collected and monitored by TLC resulting in 15 fractions (F_1–F_{15}). The solid in F_6 was separated by filtration, recrystallized from CH_2Cl_2-hexane to afford zerumbone (**1**, 15.0 g).

PREPARATION OF ZERUMBONE DERIVATIVES

Preparation of novel compound 8

To a solution of compound **2** (60 mg, 0.26 mmol) in MeCN (1.0 ml) was added the solution of KCN (665 mg, 10.21 mmol) in H_2O (1.0 ml), the reaction mixture was stirred for 5 days at room temperature. The reaction mixture was quenched with H_2O (10 ml) and extracted with EtOAc (3 times). The organic layer was combined, washed with water, brine and dried with anhydrous Na_2SO_4 and evaporated to dryness. Purification of crude oil by PLC (10% MeOH:EtOAc) gave compound **8**.

(±)-[6*E*]-3-Amino-10-cyano-2,6,9,9-tetramethyl-cycloundeca-6-enone (8). Yield 38 mg, 56%; mp 158–160 °C; IR $\nu_{\max}$ 3421, 2966, 2236, 1716 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.07 (1H, dd, J = 10.8, 3.6 Hz, H-7), 3.37–3.43 (1H, m, H-11), 3.22 (1H, t, J = 4.1 Hz, H-10), 3.08–3.12 (1H, m, H-3), 2.60 (1H, dq, J = 6.7, 2.4 Hz, H-2), 2.38 (1H, dd, J = 3.6, 3.6 Hz, H-11), 2.07 (1H, dd, J = 10.9, 3.8 Hz, H-8), 1.95–2.02 (1H, m, H-5), 1.87–1.91 (1H, m, H-5), 1.75–1.85 (1H, m, H-8), 1.57–1.66 (1H, m, H-4), 1.36 (3H, s, CH_3 on C6), 1.15–1.23 (1H, m, H-4), 1.17 (3H, s, CH_3 on C9), 1.09 (3H, s, CH_3 on C9), 0.96 (3H, d, J = 6.7 Hz, CH_3 on C2); ^{13}C NMR ($CDCl_3$) δ 208.8 (C1), 140.8 (C6), 126.2 (CN at C10), 125.0 (C7), 57.8 (C3), 55.2 (C2), 45.4 (C11), 45.1 (C8), 42.4 (C5), 39.7 (C9), 37.1 (C10), 35.7 (CH_3 on C9), 32.9 (C4), 25.1 (CH_3 on C9), 19.8 (CH_3 on C6), 11.9 (CH_3 on C2); HRMS m/z calcd mass for $C_{32}H_{53}N_3O_2$ 512.3100 $[M+H]^+$, found 512.3100.

BIOASSAY

Antimalarial assay: Antimalarial activity was performed against *P. falciparum* (K1, multidrug resistant strain) which was cultured continuously according to the method of Trager and Jensen[21]. Quantitative assessment of antimalarial activity in vitro was determined by means of the microdilution radioisotope technique based on the method described by Desjardins[22]. The inhibitory concentration was that which causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H] hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin.

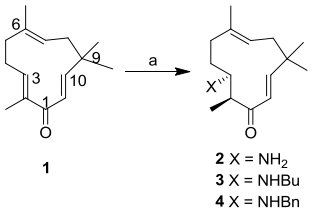
Cytotoxicity assay: Cytotoxicity assay against human epidermoid carcinoma (KB), breast cancer cell (BC), and human small cell lung cancer (NCI-H187) cell lines were performed employing Resazurin Microplate Assay (REMA)[23] while cytotoxicity assay against Vero cells (African green monkey kidney) was performed by Green Fluorescent Protein based assay[24]. Ellipticine was included as a reference substance.

RESULTS AND DISCUSSION

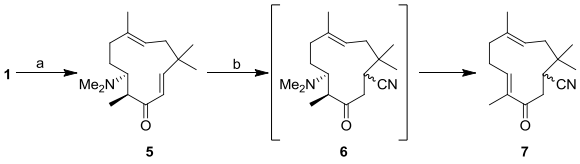
Chemistry

Zerumbone (**1**) was reacted with ammonia, *n*-butylamine, and benzylamine at room temperature to give a single diastereomer of conjugate addition products **2**–**4**, respectively, (Scheme 1). This conjugated addition occurred at C3 while C10 position was more hindered due to *gem* dimethyl group at C9. The relative configuration at C2 and C3 were obtained as *trans* configuration adducts which were confirmed by X-ray diffraction[25].

In an attempt to introduce any nucleophile to the more stable C10 double bond, **1** was treated with dimethylamine to obtain **5**. Without any purification, **5** was treated with KCN to afford **7** in 30% yield in two steps (Scheme 2)[25]. It was reported that dimethyl amine was easily eliminated to generate double bond. Cyanation of amine **2** with KCN at room temperature gave conjugate addition of cyanide ion at C10 in 56%



Scheme 1 Reagents and conditions: (a) NH_3 or $BuNH_2$ or $BnNH_2$, MeCN, rt, 5 days, 56% (**2**), 93% (**3**), 68% (**4**).



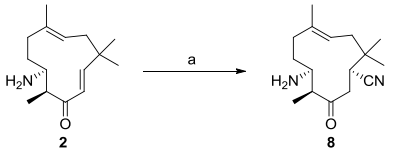
Scheme 2 Reagents and conditions: (a) Me_2NH, MeCN, rt, 5 days; (b) KCN, MeCN-H_2O, 15 °C, 2 days, 30% in two steps.

yield (Scheme 3). The relative stereochemistry at C2 and C3 was not changed in this condition while the relative stereochemistry between cyano group at C10 and amino group at C3 are relatively *cis* configuration as shown in the X-ray crystallographic structure (Fig. 1).

Zerumbone was reduced with lithium aluminium hydride to afford racemic zerumbol (**9**) in high yield[26]. Further acetylation of **9** with acetic anhydride in pyridine furnished a crystalline **10** in 74% yield. Treatment of **9** with *m*CPBA in EtOAc as the solvent provided a single diastereomer of **11** as relatively *trans* configuration (Scheme 4)[26]. The epoxidation did not occur at the C10-C11 double bond due to the high steric hindrance of *gem* dimethyl substituents at the C9 position.

Biological activity

Zerumbone and its derivatives were assayed for in vitro antimalarial activity against *P. falciparum*[20,21] and cytotoxicity against human cancer cell lines, NCI-H187, KB and BC, as well as the normal cells, Vero cells[23,24]. Zerumbone (**1**) exhibited strong antimalarial activity and showed strong cytotoxicity against all



Scheme 3 Reagents and conditions: (a) 40 eq KCN, MeCN-H_2O, rt, 5 days, 56%.

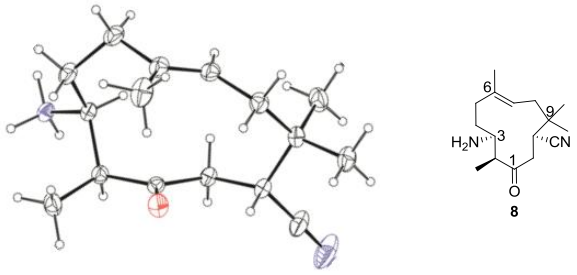
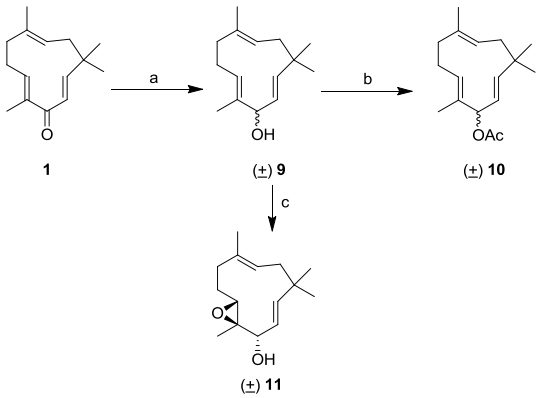


Fig. 1 X-ray crystallographic structure of compound **8**.



Scheme 4 Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C, 1 h, 88%; (b) Ac_2O, pyridine, reflux, 30 min, 74%; (c) *m*CPBA, EtOAc, rt, 24 h, 15%.

cancer cell lines. Unfortunately, this compound was also strongly toxic to Vero cells (IC_{50} = 19.9 μM) (Table 1). These are the problems of drug discovery. Therefore, zerumbone derivatives were designed and synthesized to evaluate their activities. It is anticipated that this work will find potent compounds for antimalarial and anticancer agents which will be less toxic to normal cells.

Amines **2** (IC_{50} = 5.6 μM) and **4** (IC_{50} = 7.0 μM) showed strong antimalarial activity while **3** (IC_{50} = 20.9 μM) showed moderate activity. These amine derivatives exhibited strong to moderate cytotoxicity against all cell lines with IC_{50} values ranging from 8–80 μM. Particularly, cytotoxicity against normal cell line (Vero cells) was found to have IC_{50} values ranging from 26–47 μM. The results show that amines **2**, **3**, and **4** are not suitable to develop as antimalarial or anticancer agents. Nitril derivatives **7** and **8** showed strong and moderate activity for antimalarial and cytotoxicity, however they exhibited cytotoxic against Vero cells.

These results seem to suggest that amino or cyano derivatives are unsuitable for antimalarial and anticancer agents as these derivatives cause toxicity to

Table 1 Antimalarial activity and cytotoxicity properties of the tested compounds.

Compound	Antimalarial	Cytotoxicity; IC_{50} (μM)			
	IC_{50} (μM)	NCI-H187	BC	KB	Vero cell
1	11.8	11.1	20.2	33.5	19.9
2	5.6	17.0	66.8	45.3	31.7
3	20.9	8.6	12.5	17.0	26.9
4	7.0	33.3	79.9	35.8	46.8
7	7.5	9.0	35.4	20.7	25.3
8	10.5	41.8	35.8	15.6	58.7
9	32.8	NR	NR	NR	NR
10	13.7	NR	NR	NR	NR
11	9.8	NR	NR	NR	NR
Dihydroarte-misinin	3.7 nM	–	–	–	–
Ellipticine	–	1.8	2.2	1.5	3.8

Data shown are from triplicate experiments.
NR = no response at $>$ 100 μM.

normal cells. From the previous report, carbonyl group may play an important role for cytotoxicity[5]. In our opinion, if this group were reduced to alcohol, the cytotoxicity might be decreased. Accordingly, the alcohol derivatives **9**–**11** were synthesized and tested for the activities.

Compounds **9**, **10**, and **11** exhibited strong to moderate antimalarial activity with IC_{50} values ranging from 9–32 μM. Fortunately, they showed rather weak cytotoxicity to Vero cells and cancer cells. These observations indicate that these derivatives are potent for development as antimalarial agents. It is interesting to note that alcohol or alcohol derivatives may play an important role in antimalarial activity and non-toxic against all cell lines.

In conclusion, eight zerumbone derivatives were synthesized by simple reactions. All compounds were tested as antimalarial against *P. falciparum* and for cytotoxicity against cancer cells (NCI-H187, BC and KB cell lines) and normal cells (Vero cells). Among tested compounds, alcohol derivatives **9**, **10**, and **11** showed high potent antimalarial activity while they were almost inactive against Vero cells. These results encourage the synthesis of these analogues to improve the antimalarial properties.

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