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Preliminary communication

New antimalarial and cytotoxic 4-nerolidylcatechol derivatives

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ABSTRACT

4-Nerolidylcatechol (**1**) was isolated from cultivated *Pothomorphe peltata* root on a multigram scale using straight-forward solvent extraction-column chromatography. New semi-synthetic derivatives of **1** were prepared and tested in vitro against multidrug-resistant *Plasmodium falciparum* K1 strain. Mono-*O*-methyl, mono-*O*-benzyl, *O*,*O*-dibenzyl and *O*,*O*-dibenzoyl derivatives **2**–**8** exhibited IC_{50} in the 0.67–22.52 μM range. Mono-*O*-methyl ethers **6** and **7** inhibited the in vitro growth of human tumor cell lines HCT-8 (colon carcinoma), SF-295 (central nervous system), LH-60 (human myeloblastic leukemia) and MDA/MB-435 (melanoma). In general, derivatives **2**–**8** are more stable to light, air and pH at ambient temperatures than their labile, natural precursor **1**. These derivatives provide leads for the development of a novel class of antimalarial drugs with enhanced chemical and pharmacological properties.

1. Introduction

Pothomorphe spp. (caapeba, pariparoba) are traditionally used plants for the treatment of malaria in the form of teas [1]. In vitro and in vivo antimalarial activities of *Pothomorphe* spp. extracts have been reported in Refs. [2–6] and we recently showed that 4-nerolidylcatechol (**1**), isolated from dried, ground *Pothomorphe peltata* roots, presents significant in vitro cytotoxic [7] and antiplasmodial activities [8].

Compound **1** is labile under ordinary laboratory conditions or in (alkaline) solution due a priori to the presence of highly reactive catechol hydroxyls and the aromatic ring activating quaternary carbon of the nerolidyl side chain. Early experience showed that compound **1** underwent *O*,*O*-diacetylation in good yield. The resulting diacetate exhibited greater stability and modulation of in vitro cytotoxic activity towards tumor cell lines as compared to **1** [7]. The only other *O*-substituted derivatives of **1** known in the literature came as a result of an 8-step racemic synthesis of *O*,*O*-dimethyl-4-nerolidylcatechol as part of studies on structural elucidation of **1** [9]. There is no information available on the biological activity of the latter racemic mixture. On this basis and pursuing our interest in the discovery of new anticancer and antimalarial agents, we have recently focused our attention on semi-synthetic mono- and di-*O*-benzyl, *O*-benzoyl and *O*-methyl 4-nerolidylcatechol derivatives, prepared in simple one- or two-step procedures starting from **1**, as promising cytotoxic and antimalarial agents with greater inherent stability than their natural precursor. In the present study, new semi-synthetic derivatives **2**–**8** were synthesized and their in vitro antimalarial activity towards the human malaria parasite *Plasmodium falciparum* K1 strain, as well as in vitro cytotoxic activity towards 4 human cell lines, were investigated. Also, structurally analogous catechol (**9**) and nerolidol (**10**) were tested to provide further insight into what may be important carbon skeleton/connectivity features related to antimalarial and cytotoxic activities.

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2. Chemistry

4-Nerolidylcatechol (**1**) was isolated on a multigram scale from dried, ground roots of *P. peltata* (cultivated at Embrapa Amazonia Ocidental in Manaus, Amazonas State, Brazil) using straightforward solvent extraction-column chromatography steps which have been previously described [8]. In general, the 4-nerolidylcatechol derivatives prepared (patent solicited 12/2007, PCT/BR2007, 0003375) took advantage of the reactive catechol hydroxyl groups present in **1** yielding mono- and di-*O*-derivatized compounds depending upon the specific reactions employed. The structures of derivatives were established based on spectroscopic analyses. Structurally, these *O*-substituted derivatives offer a simple range of steric, functional and electronic differences around the aromatic nucleus which in comparison to **1**, catechol and nerolidol provide insight into qualitative structure–activity requirements for both in vitro anti-plasmodial as well as in vitro cytotoxic effects.

3. Results and discussion

Pure 4-nerolidylcatechol (**1**) is labile in ambient light, air, room temperatures and in solution under mild alkaline conditions. Within a few minutes to hours **1** in CD_3OD/K_2CO_3 is converted to a mixture of higher and lower polarity products having vinylic and allylic hydrogens corresponding to internal olefin groups of the terpenyl side chain, but no aromatic or terminal olefin hydrogens, according to TLC and NMR analysis (A.M. Pohlit, A.C.S. Pinto, unpublished results). This is evidence for the oxidation of the catechol nucleus of **1** under alkaline conditions. There is also evidence that oxidation of **1** may preferentially involve single electron transfer and/or formation of radical intermediates. In this vein, a quantitative study on the antiradical capacity of several natural catechols and polyphenols revealed that **1** anomalously underwent oxidation involving only one hydroxyl group (presumably through loss of $1H^+ + 1e^-$ per molecule of **1**) whereas polyphenols in general were oxidized proportionally to the number (n) of hydroxyl and alkyloxyl substituent groups (loss of $nH^+ + ne^-$ per molecule) present on the aromatic nucleus [10]. This result is also reproducible in other common systems used for quantitative evaluation of antioxidant behavior, such as $ABTS^+$ where rapid oxidation kinetics and a TEAC of 0.50 are observed in aerobic and anaerobic conditions, whereas other catechols exhibit the expected TEAC value of 1 (D. Rettori, A.M. Pohlit, Pinto et al., unpublished data). No conclusive mechanistic interpretation is available for these results at the moment, but rapid formation of radical intermediates might be a feasible explanation for these data and the general lability of **1** under a number of different conditions.

New mono- and di-*O*-substituted 4-nerolidylcatechol derivatives **2**–**8** exhibit greater stability in general than 4-nerolidylcatechol, as would be expected, given the lower number (or absence) of phenol groups in these derivatives. Despite the inert atmosphere used during reactions, decomposition of 4-nerolidylcatechol (discussed above) appears to be a factor under the mildly alkaline conditions used in benzylation, benzoylation and methylation (with methyl iodide), as well as methylation with diazomethane, leading to relatively low yields.

The antimalarial activity of semi-synthetic derivatives **2**–**8** (Fig. 1) was determined as the percentage reduction of parasite growth versus untreated controls (Table 1). The concentration of each derivative required for reduction of parasite growth by 50% was expressed as median inhibition concentrations (IC_{50}) after probit analysis. The cytotoxic activity of each compound was determined as the percentage inhibition of tumor cell growth caused by each derivative relative to the inhibition presented by doxorubicin-treated controls (Table 2).

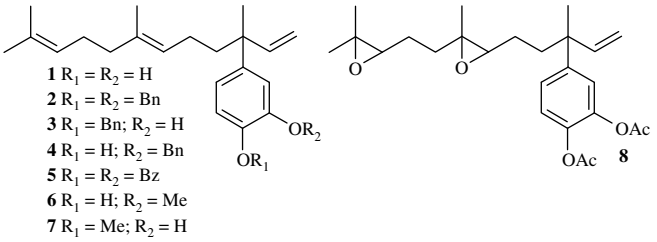

Fig. 1. Structures of 4-nerolidylcatechol (**1**), semi-synthetic derivatives **2**–**8** and structural analogues, catechol (**9**) and nerolidol (**10**).

Four of the seven 4-nerolidylcatechol derivatives exhibited significant in vitro anti-plasmodial activities ($IC_{50} < 2$ μg/mL). Dibenzoyl derivative **5** was the most active antimalarial derivative and presented an IC_{50} comparable to that of chloroquine and natural precursor **1**. Monobenzyl derivatives **3** and **4**, and diepoxy diacetyl mixture **8** exhibited important inhibition ($IC_{50} = 2.8$–4.0 μM) while monomethyl derivative **7** was only partially active and its structural isomer **6** was inactive.

In previous work, 4-nerolidylcatechol (**1**) was shown to exhibit cytotoxic potential in five tumor cell lines with IC_{50} ranging from 6 to 13 μg/mL [7]. Monomethyl derivative **6** presented good cytotoxic potential (IC_{50}, 14.4–20.7 μg/mL) in human colon carcinoma, central nervous system and melanoma tumor cell lines while its structural isomer **7** inhibited human leukemic cells. Dibenzyl, monobenzyl and dibenzoyl derivatives **2**, **3** and **5**, respectively, were essentially inactive towards all tumor cell lines. Among derivatives, cytotoxic potential was associated with small *O*-substituent groups, such as CH_3 and CH_3CO. This is further corroborated by the significant in vitro inhibition exhibited by another derivative, *O*,*O*-diacetyl-4-nerolidylcatechol, to human leukemia cell strains HL-60 and CEM [IC_{50}, 6.17 (4.74–8.03) and 6.22 (4.83–8.02) μg/mL, respectively] [7]. The inherent cytotoxicity (IC_{50}, 1.08–13.54 μg/mL) exhibited by catechol (**9**) in three tumor cell lines is noteworthy as is the general absence of cytotoxicity

Table 1
In vitro IC_{50} values of 4-nerolidylcatechol (**1**), its semi-synthetic derivatives **2**–**8** and compounds **9** and **10** to the K-1 strain of *Plasmodium falciparum*.

Compounds	*P. falciparum*	
	Mean IC_{50} values	
	μg/mL	μM
1	0.21[a] (0.19–0.4)	0.67[a] (0.61–0.73)
2	11.14 (8.85–13.43)	22.52 (17.92–27.12)
3	1.56 (1.48–1.64)	3.86 (3.78–3.94)
4	1.15 (0.95–1.35)	2.84 (2.44–3.24)
5	0.35 (0.3–0.4)	0.67 (0.58–0.76)
6	I[b]	–
7	PA[b]	–
8	1.70 (1.56–1.84)	3.95 (3.63–4.27)
9	8.88 (5.88–11.88)	80.65 (53.45–107.85)
10	PA[b]	–
Chloroquine diphosphate	0.46 (0.44–0.48)	0.89 (0.86–0.92)
Quinine salt	0.004 (0.002–0.006)	0.012 (0.006–0.018)

[a] Ref. [8].
[b] I – inactive (<50% inhibition) or PA – partially active (50–79% inhibition) at 50 and 2.5 μg/mL.

Table 2
In vitro IC_{50} values to the relative activities in 4 human tumor cell lines of 4-nerolidylcatechol (**1**) and semi-synthetic derivatives **2**–**8** and compounds **9** and **10**.

Compounds	Inhibition of tumor cell lines (μg/mL)			
	HL-60	HCT-8	SF-295	MDA/MB-435
1	6.17[a] (4.74–8.03)	12.17[a] (8.38–17.67)	–	–
2	I	I	I	–
3	I	I	I	–
4	12.42 (10.41–15.62)	20.60 (17.70–23.99)	18.22 (16.64–19.95)	18.57 (15.92–21.67)
5	–	I	I	I
6	14.40 (10.85–17.34)	14.53 (11.87–17.77)	20.70 (15.57–27.51)	>25
7	18.84 (13.63–26.06)	>25	>25	>25
8	17.41 (9.82–30.86)	17.47 (10.63–28.73)	22.85 (13.45–38.82)	>25
9	1.08 (0.84–1.39)	13.54 (10.53–17.41)	12.92 (10.76–15.50)	>25
10	–	I	I	I
Doxorubicin	0.02 (0.01–0.02)	0.04 (0.03–0.05)	0.25	

I – inactive; – not tested.
[a] Ref. [7].

observed for nerolidol (**10**). Based on these results, the catechol moiety would appear to be relevant to the observed cytotoxicity in 4-nerolidylcatechol (**1**) and derivatives, while the nerolidyl side chain would appear not to be a necessary structural element for the observed cytotoxicity. In this light, it is interesting that synthetic 4-salicylamidophenylalkyl catechols inhibit growth of murine colon tumor cells in vitro, as well as 5-, 12- and 15-lipoxygenase in intact cells and rabbit reticulocyte 15-lipoxygenase [11].

Nerolidol causes total inhibition of *P. falciparum* trophozoite development at the schizont stage [12] and in vitro IC_{50} values in the range 120–760 nM [13,14] have been reported for nerolidol towards *P. falciparum*. Terpenoid compounds like nerolidol, farnesol and linalol have been shown to strongly inhibit the biosynthesis of both dolichol and the isoprenic side chain of ubiquinones, as well as the isoprenylation of proteins in the intraerythrocytic stages of *P. falciparum* in a specific manner, which does not affect overall protein biosynthesis [14]. It is assumed that the observed inhibition of *P. falciparum* by 4-nerolidylcatechol (**1**) and derivatives **2**–**8** is related to a similar mechanism involving the nerolidyl side chain eventhough in the present study, nerolidol (**10**) presented only partial antimalarial activity (Table 1). Catechol (**9**) and nerolidol, which exhibit carbon skeletal elements present in substances **1**–**8**, are, on a molar basis, much less active in vitro against *P. falciparum* than **1**, which leads to the supposition that the combined terpenyl side chain and aromatic ring system of the nerolidylcatechyl skeleton is important to the antimalarial activity of this class of compound. In this vein, it is noteworthy that synthetic (mono- and di-*O*-substituted) 4-allylcatechol derivatives (which share a 4-(propen-3-yl)catechol subskeleton in common with compounds **1**–**8**) have antimalarial properties [15]. Also, it is interesting that catechol siderophores (ironchelators) such as dicatecholates with long aliphatic chains (i.e. FR160) have similar in vitro IC_{50} values to those observed for 4-nerolidylcatechol (**1**) and its derivatives **2**–**8** in chloroquine resistant *P. falciparum* infected-erythrocytes [16].

4. Conclusion

Several semi-synthetic 4-nerolidylcatechol derivatives were identified herein with significant antimalarial and cytotoxic potential. Syntheses of new derivatives are underway in light of the qualitative structure–activity relationships suggested herein.

5. Experimental

5.1. Chemistry

^1H and ^{13}C NMR spectra were recorded on a Varian (500 MHz) spectrometer using $CDCl_3$ as solvent. Accurate mass (+)-ESI-tof-MS spectra were recorded on a Bruker-Daltronics UltrOTof Mass Spectrometer by direct infusion of sample dissolved in suitable solvents into the ion source. Column chromatography was performed on silica using Kiesegel 60 (230–400 mesh, Merck). All reagents used in the present work were of analytical grade.

5.1.1. Preparation of O,O-dibenzyl-4-nerolidylcatechol (*2*) and mono-O-benzyl derivatives *3* and *4*

Semi-synthetic derivatives **2**–**4** (Fig. 1) were prepared from **1** (401.0 mg, 1.3 mmol) by treatment with $C_6H_5CH_2Br$ (432.0 mg, 2.5 mmol, 2 equiv), K_2CO_3 (705.4 mg, 5.1 mmol, 4 equiv), KI (22.3 mg, 0.1 mmol), and DMF (2.4 mL) for 20–30 min under N_2 (adapted from [17,18]). The reaction was stirred at room temperature for 18 h and then filtered. The filtrate was extracted with $CHCl_3$ (3×5 mL). The combined extracts were washed with 1% NaOH (5 mL) and water (5 mL), dried with $MgSO_4$, filtered and concentrated using rotary evaporation. The product was purified by flash column chromatography flash (using a 95:5 to 1:1 hexanes:AcOEt gradient), yielding a mixture of benzylated derivatives **2** (slightly viscous, yellowish-clear oil, 163.8 mg, 26.0%), **3** (viscous, clear oil, 46.1 mg, 7.3%) and **4** (viscous, clear oil, 40.6 mg, 6.4%) which were separated by preparative TLC (hexanes:$CHCl_3$, 1:4). **2**: ^1H NMR (500 MHz, $CDCl_3$): δ 7.43–7.45 (m, 2H, OCH_2Ph), 7.33–7.37 (m, 4H, OCH_2Ph), 7.27–7.31(m, 4H, OCH_2Ph), 6.91 (m, 1H, Ar-H_3), 6.32 (dd, $J = 8.5$, 2.0 Hz, 1H, Ar-H_5) 6.87 (m, 1H, Ar-H_6), 5.96 (dd, 1H, $J = 17.7$, 10.5 Hz), 5.14 (s, 2H, OCH_2Ph), 5.13 (s, 2H, OCH_2Ph), 5.10 (m, 1H), 5.08 (m, 1H), 5.05 (dd, 1H, $J = 10.5$, 1.5 Hz), 4.99 (dd, 1H, $J = 17.7$, 1.5 Hz), 2.05 (m, 2H), 1.95 (m, 2H), 1.77 (m, 2H), 1.68 (s, 3H), 1.66 (m, 2H), 1.60 (s, 3H), 1.49 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 148.3, 147.2, 147.0, 140.9, 137.5, 134.8, 131.3, 128.4, 128.3, 127.7, 127.6, 127.5, 127.3, 124.5, 124.3, 119.7, 115.1, 114.5, 111.5, 71.7 (OCH_2Ph), 71.4 (OCH_2Ph), 43.9, 41.1, 39.7, 26.7, 25.7, 24.9, 23.1, 17.7, 15.9. *m/z* 517.2958 $[M + Na]^+$ ($C_{35}H_{42}NaO_2^+$, $m_{calc} = 517.3077$). R_f 0.36 (hexanes:Et_2O, 95:5). **3**: ^1H NMR (500 MHz, $CDCl_3$): δ 7.38–7.40 (m, 2H, OCH_2Ph), 7.41–7.42 (m, 1H, OCH_2Ph), 7.34–7.37 (m, 2H, OCH_2Ph), 6.94 (d, 1H, $J = 3.0$ Hz, Ar-H_3), 6.85 (d, 1H, $J = 8.5$ Hz, Ar-H_6), 6.77 (dd, $J = 8.5$, 2.5 Hz, 1H, Ar-H_5), 6.00 (dd, 1H, $J = 10.7$, 17.4 Hz), 5.58 (s, 1H, OH), 5.12 (m, 1H), 5.08 (s, 2H, OCH_2Ph), 5.07 (m, 1H), 5.05 (dd, 1H, $J = 6.0$, 1.2 Hz), 5.03 (dd, 1H, $J = 17.4$, 1.2 Hz), 2.04 (m, 2H), 1.95 (m, 2H), 1.84 (m, 2H), 1.76 (m, 2H), 1.68 (s, 3H), 1.60 (s, 3H), 1.52 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (125 MHz, CDCl3): δ 147.0, 145.3, 143.8, 141.4, 136.5, 134.9, 131.3, 127.8, 128.7, 128.3, 124.5, 124.4, 113.5, 118.0, 111.6, 111.4, 71.2 (OCH_2Ph), 43.8, 41.2, 39.7, 26.7, 25.7, 24.9, 23.2, 17.7, 15.9. *m/z* 405.2789 $[M + H]^+$ ($C_{28}H_{37}O_2^+$, $m_{calc} = 405.2788$), R_f 0.36 (hexanes:Et_2O, 9:1) **4**: ^1H NMR (500 MHz, $CDCl_3$): δ 7.41–7.42 (m, 1H, OCH_2Ph), 7.38–7.40 (m, 2H, OCH_2Ph), 7.34–7.36 (m, 2H, OCH_2Ph), 6.89 (d, 1H, $J = 2.2$ Hz, Ar-H_3), 6.87 (d, 1H, $J = 8.2$ Hz, Ar-H_6), 6.83 (dd, $J = 8.2$, 2.2 Hz, 1H, Ar-H_5), 5.99 (dd, 1H, $J = 17.6$, 11.7 Hz), 5.51 (s, 1H, OH), 5.13 (m, 1H), 5.08 (s, 2H, OCH_2Ph), 5.07 (m, 1H), 5.05 (dd, 1H, $J = 11.7$, 1.3 Hz), 5.02 (dd, 1H, $J = 17.6$, 1.3 Hz), 2.05 (m, 2H), 1.96 (m, 2H), 1.83 (m, 2H), 1.70 (m, 2H), 1.68 (s, 3H), 1.60 (s, 3H), 1.52 (s, 3H), 1.34 (s, 3H). *m/z* 405.2787 $[M + H]^+$ ($C_{28}H_{37}O_2^+$, $m_{calc} = 405.2788$), R_f 0.30 (hexanes:Et_2O, 9:1).

5.1.2. Preparation of O,O-dibenzoyl-4-nerolidylcatechol (*5*)

Compound **1** (300.1 mg, 1 mmol) was dissolved in dry C_5H_5N (2.0 mL) or Et_3N under N_2 atmosphere and stirring, followed by dropwise addition of C_6H_5COCl (337.5 μL, 2.9 mmol, 3 equiv). The solution was maintained at 80 °C with stirring for 1 h. Then the reaction was stirred and allowed to come to room temperature over

48 h. Cold H_2O (3 mL) was added to the reaction mixture which was then extracted with $CHCl_3$ (3 × 5 mL) and water (2 × 5 mL), in an alternate fashion. The $CHCl_3$ phase was washed with HCl 0.1 N, diluted $NaHCO_3$, water (5 mL) and saturated NaCl and then dried with anhydrous Na_2SO_4. The product was purified by column chromatography flash (hexanes:AcOEt, 97:3), yielding dibenzoylated derivative **5** (viscous, yellowish-clear oil, 73.6 mg, 14.7%), R_f 0.33 (hexanes:acetone, 9:1) ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04–8.09 (m, 4H, COOPh), 7.52–7.56 (m, 4H, COOPh), 7.36–7.40 (m, 2H, COOPh), 7.32–7.35 (m, 2H, Ar-H_3, Ar-H_6), 7.31 (dd, J = 8.5, 2.0 Hz, 1H, Ar-H_5), 6.06 (m, 1H, J = 17.2, 10.2 Hz), 5.17 (m, 1H), 5.14 (dd, 1H, J = 10.2, 1.0 Hz), 5.11 (dd, 1H, J = 17.2, 1.0 Hz), 5.08 (m, 1H), 2.06 (m, 2H), 1.96 (m, 2H), 1.86 (m, 2H), 1.84 (m, 2H), 1.68 (s, 3H), 1.60 (s, 3H), 1.55 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.3 (C═O), 164.2 (C═O), 146.7, 146.1, 142.0, 140.3, 135.2, 133.5, 131.3, 130.1, 129.0, 128.9, 124.8, 128.4, 124.4, 124.2, 122.8, 121.9, 112.5, 44.3, 41.1, 39.7, 26.7, 25.7, 25.0, 23.2, 17.7, 15.9. m/z 545.2632 $[M + Na]^+$ ($C_{35}H_{38}NaO_4^+$, m_{calc} = 545.2662).

5.1.3. Preparation of 1 and 2-O-methyl-4-nerolidylcatechols (*6* and *7*)

Semi-synthetic derivatives **6** and **7** (Fig. 1) were prepared from **1** by two methods, the first by treatment of **1** (410.0 mg, 1.3 mmol) with CH_2N_2 (10 mL) of an Et_2O solution prepared by decomposition/distillation of Diazald (5.5 g) in Et_2O (30 mL) at room temperature for 30 min. The solvent was removed by rotary evaporation and products were purified by column chromatography (gradient of hexanes:acetone (9:1 to 1:1)) to yield monomethyl derivatives **6** (viscous, transparent oil, 33.0 mg, 7.7%) and **7** (viscous, transparent oil, 36.0 mg, 8.4%) separated by preparative TLC (hexanes:acetone, 9:1). The second method was adapted from Ref. [19]: to a solution of **1** (50 mg, 0.2 mmol) in $(CH_3)_2CO$ (5 mL) was added K_2CO_3 (76.9 mg, 0.5 mmol, 3.5 equiv) and CH_3I (25 μL, 8.1 mmol, 2.5 equiv). The reaction mixture was heated under reflux for 2 h. The mixture was concentrated and extracted three times with equal volumes of $CHCl_3$. After concentration, the combined extracts were purified by flash column chromatography (gradient of hexanes:acetone, 9:1 to 1:1) to yield monomethyl derivative mixture (5.3 mg, 9.7%). **6**, R_f 0.39 (hexanes:$CHCl_3$, 2:3): ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.85 (d, 1H, J = 1.5 Hz, Ar-H_3), 6.83 (d, 1H, J = 7.2 Hz, Ar-H_6), 6.81 (dd, J = 7.2, 1.5 Hz, 1H, Ar-H_5), 6.01 (dd, 1H, J = 17.7, 9.5 Hz), 5.46 (s, OH), 5.11 (m, 1H), 5.09 (m, 1H), 5.06 (dd, 1H, J = 9.5, 1.0 Hz), 5.04 (dd, 1H, J = 17.7, 1.0 Hz), 3.87 (s, 3H, OMe), 2.04 (m, 2H), 1.95 (m, 2H), 1.86 (m, 2H), 1.76 (m, 2H), 1.67 (s, 3H), 1.59 (s, 3H), 1.52 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 147.4, 146.3, 143.8, 139.8, 135.2, 131.5, 124.8, 124.6, 119.6, 114.0, 111.7, 109.8, 56.1 (OMe), 44.3, 41.4, 39.9, 26.9, 25.9, 25.3, 23.5, 17.9, 16.1. m/z 329.2476 $[M + H]^+$ ($C_{22}H_{33}O_2^+$, m_{calc} = 329.2475). **7**, R_f 0.45 (hexanes:$CHCl_3$, 2:3): ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.92 (d, 1H, J = 1.7 Hz, Ar-H_3), 6.78 (d, J = 1.7 Hz, 2H, Ar-H_5, Ar-H_6), 6.00 (dd, 1H, J = 17.1, 11.0 Hz), 5.51 (s, OH), 5.09 (m, 1H), 5.07 (dd, 1H, J = 11.0, 1.0 Hz), 5.07 (m, 1H), 5.03 (dd, 1H, J = 17.1, 1.0 Hz), 3.87 (s, 3H, OMe), 2.04 (m, 2H), 1.95 (m, 2H), 1.86 (m, 2H), 1.77 (m, 2H), 1.68 (s, 3H), 1.59 (s, 3H), 1.52 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 147.3, 145.3, 144.7, 141.3, 131.5, 135.1, 124.8, 124.6, 118.2, 113.5, 111.7, 110.4, 56.2 (OMe), 44.0, 41.4, 39.9, 26.9, 25.9, 25.2, 23.4, 17.9, 16.1. m/z 329.2498 $[M + H]^+$ ($C_{22}H_{33}O_2^+$, m_{calc} = 329.2475).

5.1.4. Preparation of O,O-diacetyl 6,10-diepoxy derivatives *8*

Compound **1** (150 mg, 0.5 mmol) was treated with Ac_2O (1 mL) and C_5H_5N (1 mL) under N_2 and magnetic stirring at room temperature for 24 h. The *O,O*-diacetyl derivative (171.4 mg; 90.2%) obtained was dissolved in CH_2Cl_2 and the reaction flask was placed in a NaCl–H_2O(s) bath (−5 °C) and then treated with *m*-CPBA (220.5 mg, 1.3 mmol, 1.2 equiv) in CH_2Cl_2 (10 mL) with stirring under N_2. After 2 h, the reaction mixture was allowed to warm to room temperature with stirring for 11 days. After this period, the reaction mixture was transferred to a separation funnel and washed with aqueous $Na_2S_2O_3$, followed by aqueous $NaHCO_3$ and water. The organic phases were dried with anhydrous Na_2SO_4 (method adapted from Ref. [20]). The crude product was purified by chromatography (hexanes:AcOEt, 3:2 to 1:1) yielding a diastereomeric mixture of diepoxy derivatives **8** (viscous, yellowish-clear oil, 77.2 mg, 56.3%), R_f 0.36 (hexanes:AcOEt, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (m, 1H, Ar-H_3), 7.44 (m, 1H, Ar-H_6), 7.10 (m, 1H, Ar-H_5), 5.97 (m, 1H), 5.10 (m, 2H), 3.31 (m, 2H), 2.27 (2, 3H, COOMe), 2.26 (s, 3H, COOMe), 2.05 (m, 2H), 1.91 (m, 2H), 1.81 (m, 2H), 1.75 (m, 2H), 1.57 (s, 3H), 1.51 (s, 3H), 1.35 (s, 3H), 1.23 (s, 3H). m/z 431.2437 $[M + H]^+$ ($C_{25}H_{35}O_6^+$, m_{calc} = 431.2428).

5.2. In vitro anti-plasmodial activity

P. falciparum strain K1 (MRA-159, ATCC) was used in our study and was acquired from MR4 (Malaria Research and Reference Reagent Resource Center, Manassas, Virginia, US). *P. falciparum* was cultivated by modification of the Trager and Jensen method [21]. The procedures used for assaying antimalarial activity are described in Ref. [8]. Briefly, in a preliminary screen, active compounds inhibited the growth of parasites by 80–100%, partially active (PA) compounds by 50–79% and inactive (I) compounds by < 50% at concentrations of 50 and 2.5 μg/mL. Active compounds were further evaluated at different dilutions for probit analysis.

5.3. In vitro antitumour activity

The human tumor cell lines used were HCT-8 (human colon carcinoma), SF-295 (human nervous system), MDA/MB-435 (melanoma) and HL-60 (human myeloblastic leukemia) which were donated by the Mercy Children's Hospital (United States). They were cultivated in RPMI 1640 medium which was supplemented with 10% bovine fetal serum and 1% antibiotics and maintained in an incubator at 37 °C and an atmosphere containing 5% CO_2. Samples were diluted in DMSO at a stock concentration of 5 mg/mL. The cytotoxicity of the samples was evaluated using the MTT method [22]. The cells were plated in 96-well test plates in the following densities: 0.7×10^5 (HCT-8), 0.6×10^5 (SF-295) and 0.3×10^6 (HL-60). The samples were incubated for 72 h at a single concentration (100 μg/mL). Absorbance was measured with the aid of a plate spectrophotometer operating at 550 nm.

The experiments were analyzed using averages and the corresponding confidence intervals based on the non-linear regression generated using *GraphPad Prism*. Each sample was tested in triplicate in two independent experiments. An intensity scale was used to evaluate the cytotoxic potential of the tested samples.

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