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Synthesis, biological evaluation and mechanistic studies of totarol amino alcohol derivatives as potential antimalarial agents

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ABSTRACT

Herein we report on the semisynthesis and biological evaluation of β-amino alcohol derivatives of the natural product totarol and other simple aromatic systems. All β-amino alcohol derivatives of totarol exhibited higher antiplasmodial activity than totarol [IC_{50}: 11.69 μM (K1, chloroquine and multi-drug resistant strain), and 11.78 μM (D10, chloroquine sensitive strain)]—**12e** was the most active [IC_{50}: 0.63 μM (K1), and 0.61 μM (D10)]. The phenyl and naphthyl β-amino alcohol derivatives were much less active than their corresponding totarol equivalents. The majority of the β-amino alcohol derivatives of totarol were more active against K1 than the D10 strains of *Plasmodium falciparum*, a trend similar to the inverse relationship observed with the established aryl-amino alcohol antimalarial mefloquine. Selected compounds were shown to affect erythrocyte morphology, inhibit erythrocyte invasion and trigger CQ accumulation.

1. Introduction

Malaria remains a dominant health issue in large parts of the developing world. According to the World Health Organization (WHO) 2010 World Malaria Report, malaria is responsible for 225 million clinical cases and 781,000 deaths annually, especially among children and pregnant women.[1] *Plasmodium falciparum* is the most virulent species of the malaria parasite and the cause of the bulk of the mortality associated with malaria. The major challenge to the effective chemotherapy of this disease has been the emergence over time of drug resistance of *P. falciparum* to virtually all known classes of antimalarial drugs.[2–4] Of particular concern are reports from Southeast Asia of the emergence of resistance to artemisinin-based antimalarials, a situation that could lead to the loss of clinical efficacy of the currently first-line artemisinin-combination therapy (ACT) strategies.[5,6] This situation has emphasized the need for the development of new drugs to supplement available antimalarial drugs, and has triggered extensive drug discovery efforts aimed at identifying and developing novel compounds that exhibit excellent experimental and clinical antimalarial efficacy without showing any evidence of resistance.[2]

An impressive range of structurally diverse natural products have shown notable antiplasmodial activity,[7–9] and one approach has been to identify such promising antimalarial compounds and then modify them through medicinal chemistry in an effort to increase their potency, study their structure–activity relationships (SARs) or optimize their physicochemical and ADME (absorption, distribution, metabolism and excretion) characteristics.[10,11]

In the course of our research on the antiplasmodial activity of South African medicinal plants, two diterpenes, 8,11,13-totaratriene-12,13-diol (**1**) and 8,11,13-abietatriene-12-ol (ferruginol, **2**) (Fig. 1), were isolated and found to show notable in vitro antiplasmodial activity and little cytotoxicity against a mammalian cell line.[12] Considering their selective activity and different structural features

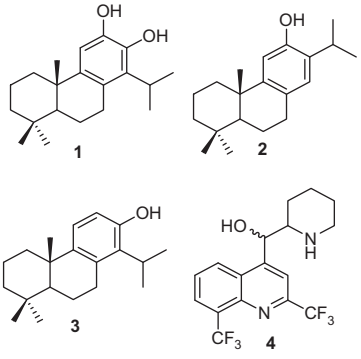

Figure 1. Chemical structures of diterpenes (**1**) and (**2**), totarol (**3**) and mefloquine (**4**).

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to existing antimalarial agents, **1** and **2** offered themselves as suitable hits for use as the basis for the design and semisynthesis of a series of potential superior antiplasmodial compounds. However, the low yields obtained from the extraction of the two isolated compounds necessitated the use of the commercially available structural analog totarol (**3**) (Fig. 1) as the template for chemical modification. Totarol itself is a naturally occurring phenolic diterpene isolated from numerous plants of the *Podocarpaceae* and *Cupressaceae* families, and has been shown to exhibit various biological activities including antibacterial,[13,14] antifungal[15] and antimalarial[16] activities.

We have previously reported on the design, semisynthesis and biological evaluation of an exploratory library of novel β-amino alcohol derivatives of totarol.[16] The β-amino alcohol moiety is present as a pharmacophore in a number of potent antimalarial drugs exemplified by mefloquine (**4**) (Fig. 1), and on this basis the combination of an antimalarial amino alcohol pharmacophore with a natural product-derived template totarol was explored. All of the synthesized compounds showed an improved antiplasmodial activity,[16] and these encouraging in vitro results warranted further exploration of the SAR and possible mechanisms of action of this type of compounds.

We hypothesized that, for these compounds, the totarol backbone may simply be acting as a hydrophobic group that may be replaceable by simpler aromatic ring systems such as phenyl and naphthyl. We further reasoned that the nature of the β-amino group vis-à-vis electronic and steric properties could influence the antiplasmodial activity and selectivity, and that this could be investigated by synthesizing and evaluating a variety of secondary and tertiary β-amino alcohol derivatives. Specifically, electronic properties of the β-amino group have a bearing on basicity and therefore uptake and accumulation into low pH compartments within *P. falciparum*, such as the digestive food vacuole. Also, steric properties of the β-amino group can, amongst other things, influence binding to potential targets.

Herein we report on the synthesis and biological evaluation of β-amino alcohol compounds derived from totarol (**12**, Fig. 2), as well as simple aromatic ring systems (**13–14**). These target compounds along with their starting materials and intermediates were tested in vitro against a chloroquine (CQ)-sensitive (D10) and CQ-resistant (K1) strain of *P. falciparum*. Promising compounds were also tested for cytotoxicity against the Chinese hamster ovarian (CHO) mammalian cell line. In order to gain insight into the possible mechanism of action of totarol and selected derivatives, selected compounds were studied for their effect on erythrocytic morphology as well as for their ability to inhibit erythrocytic invasion by merozoites. Selected compounds were also studied for their effect on chloroquine uptake.

2. Synthesis

The target compounds **12–15** were synthesized in two steps from their respective starting materials via an epoxide intermediate. The starting phenols (totarol (**3**), phenol (**5**), 1-naphthol (**6**) and 2-naphthol (**7**)) were dissolved in DMF and alkylated with epichlorohydrin in the presence of sodium hydride, resulting in the formation of the epoxide intermediates **8–11**. These intermediates were then dissolved in methanol, except in the reactions involving phthalimide where a catalytic amount of potassium phthalimide and DMF (solvent) were used. In the epoxide ring opening reactions, an excess of amines was used, resulting in the formation of the respective β-amino alcohol derivatives **12–15** with percentage yields in the range of 29–93% (Scheme 1).[16,17]

All β-amino alcohol target compounds were chiral compounds and were isolated either as single diastereomers (in case of totarol derivatives) or as racemic mixtures (in case of phenyl and naphthyl derivatives). Compounds were characterized by ^{1}H NMR, ^{13}C NMR, Low Resolution Mass Spectrometry, IR spectroscopy, elemental analysis and melting point determination.

3. Biological evaluation

3.1. In vitro antiplasmodial activity and cytotoxicity of β-amino alcohol derivatives of totarol

The antiplasmodial activities of totarol, intermediate **8** and the β-amino alcohol derivatives of totarol are shown in Table 1. Compounds showing activity greater than that of totarol on the

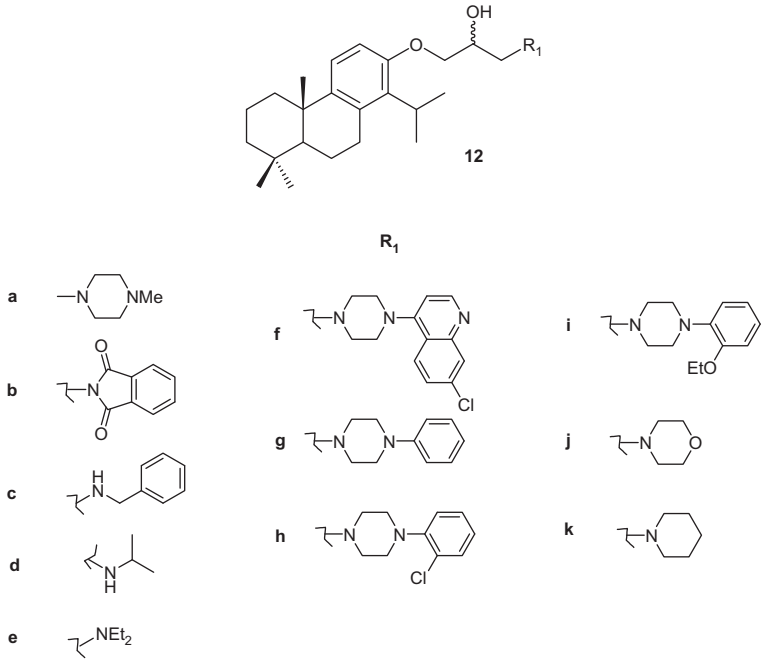
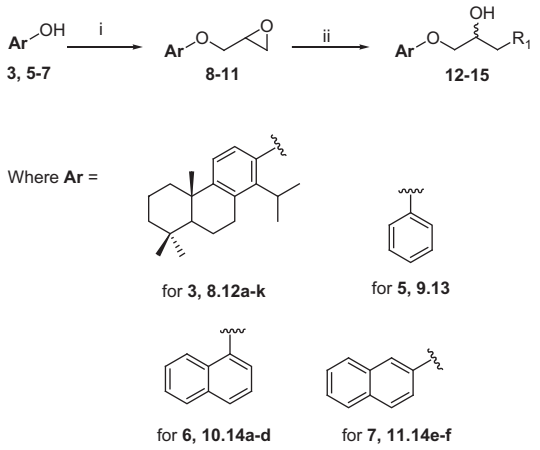


Figure 2. Structures of the target β-amino alcohol derivatives of totarol, compounds **12a–k**.



Scheme 1. General synthesis of the target β-amino alcohol derivatives **12**–**14**. *Reagents and conditions:* (i) epichlorohydrin, NaH, DMF, 0–25 °C, N_2, 12 h; (ii) amine, MeOH/DMF, 50 °C, 12–24 h.

K1 strain (compounds **12a, 12c–k**) were tested for cytotoxicity against the CHO mammalian cell line, and their selectivity indices calculated. The in vitro antiplasmodial activities of compounds **12e–k** have been previously reported,[16] and are included in Table 1 for ease of comparison and discussion.

From Table 1 it can be seen that the all β-amino alcohol derivatives exhibited higher antiplasmodial activity than totarol (except **12b** on the K1 strain). Moreover, compounds **12c–f**, **12h**, **12j** and **12k** all exhibited sub-micromolar IC_{50} values against one or both of the *P. falciparum* strains. Compound **12e** appears to be the most active compound across both strains, with IC_{50} values of 0.61 and 0.63 μM against the D10 and K1 strains of *P. falciparum*, respectively. Compound **12d** is the most active against the CQ-resistant K1 strain (IC_{50} 0.17 μM).

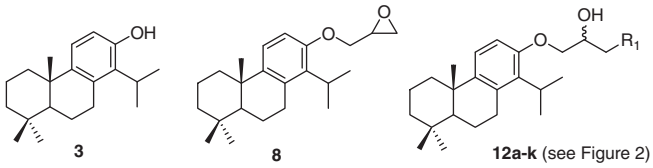
Interestingly, the majority of the β-amino alcohol derivatives are more active against the CQ-resistant K1 strain than the CQ-sensitive D10 strain, with the exception of compounds **12a**, **12b** and **12e**. This inverse relationship in sensitivity between CQ and these compounds in the selected *P. falciparum* strains is similar to that observed with established aryl-amino alcohol containing antimalarials, such as mefloquine (Table 1), quinine and halofantrine.[18] This similarity may arise due to structural similarities arising from the presence of the β-amino alcohol moiety in these compounds, and may indicate that mechanisms by which chloroquine (CQ) resistance is conferred may influence the activity of these β-amino alcohol derivatives of totarol.

Compounds **12a** and **12f–i** have related structures in that they all possess a piperazinyl moiety forming the amino portion of the β-amino alcohol. Their antiplasmodial activities are also comparable and, with the exception of **12h**, do not vary greatly between CQ-sensitive and resistant strains.

Compounds **12a–e** were relatively cytotoxic against the CHO mammalian cell line, with all exhibiting lower CHO IC_{50} values and poorer selectivity indices relative to totarol. Only compounds **12f–i** exhibited selectivity indices superior to totarol. Notably, these compounds all possessed an aryl-piperazinyl group as the amino component of the β-amino alcohol moiety, implying that this group may play a part in reducing cytotoxicity towards the CHO mammalian cell line, thereby improving the selectivity of these compounds.

In general, the addition of a β-amino alcohol side chain notably improved the antiplasmodial activity of these compounds relative to totarol, though this improvement was not universal. This leads to the conclusion that the addition of the β-amino alcohol moiety, and by extension the introduction of protonatable nitrogen(s), is a useful strategy for the enhancement of the antiplasmodial activity of totarol and related compounds. The poor selectivity indices of some of these compounds is an alert to possible in vivo toxicity, though the incorporation of aryl-piperazinyl groups as the amino

Table 1
In vitro antiplasmodial activities and cytotoxicity of totarol (**3**) and derivatives **8** and **12**



3 8 **12a-k** (see Figure 2)

Compound	*P. falciparum* IC_{50} (μM)[a]		R_I	CHO IC_{50} (μM)[a]	S_I	
	D10	K1			D10	K1
3 (Totarol)	11.78	11.69	0.99	170.46	14.5	14.6
8	10.33	14.04	1.36	ND	–	–
12a	1.36	1.39	1.03	3.73	2.7	2.7
12b	9.55	48.33	5.06	ND	–	–
12c	6.47	0.65	0.10	14.41	2.2	22.3
12d	1.05	0.17	0.14	1.28	1.2	8.7
12e[b]	0.61	0.63	1.04	7.73	12.7	12.2
12f[b]	1.42	0.93	0.64	94.19	67.3	>100
12g[b]	2.56	1.81	0.71	105.44	41.2	58.1
12h[b]	3.25	0.94	0.23	186.25	57.3	206.9
12i[b]	1.62	1.01	0.62	182.84	112.9	181.0
12j[b]	2.07	0.56	0.27	5.61	2.7	10.0
12k[b]	1.08	0.75	0.70	2.99	2.8	4.0
Mefloquine	0.018	0.008	0.44	–	–	–
Emetine	–	–	–	0.14	–	–

ND = not determined;
R_I = IC_{50} (K1)/IC_{50} (D10);
S_I = IC_{50} (CHO)/IC_{50} (D10 or K1).
[a] Each compound was assayed in duplicate, on two separate occasions.
[b] The in vitro antiplasmodial activities of compounds **12e–k** have been previously reported,[16] and are included here for ease of comparison.

component of the β-amino alcohol moiety was associated with attenuated cytotoxicity.

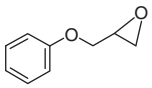
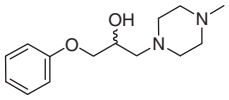
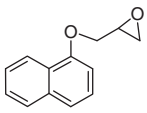
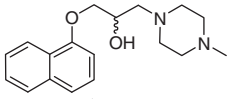
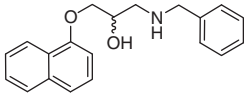
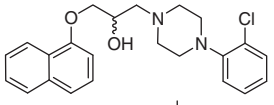
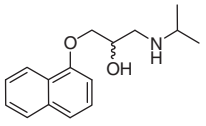
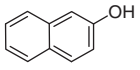
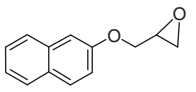
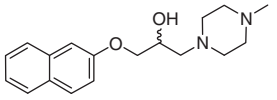
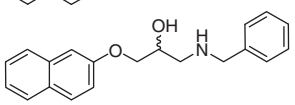
3.2. In vitro antiplasmodial activity and cytotoxicity of simplified β-amino alcohols

The role of the totarol moiety in imparting antiplasmodial activity was investigated by synthesizing and evaluating compounds **13–14**. These compounds were designed by simply replacing the totarol diterpenyl moiety of the totarol β-amino alcohol derivatives **12a**, **12c**, **12d** and **12h** with phenyl, 1-naphthyl or 2-naphthyl groups. The results from the in vitro antiplasmodial and cytotoxicity testing of these compounds and their intermediates are shown in Table 2.

The phenolic starting materials **5–7**, the epoxide intermediates **9–11** and some of the β-amino alcohol targets (**13** and **14c**) were found to possess no antiplasmodial activity against the D10 *P. falciparum*, at the maximum concentration of 100 μM. For this reason further antiplasmodial and cytotoxicity testing on these compounds was not carried out.

In general, the phenyl, 1-naphthyl or 2-naphthyl derivatives **13–14** were much less active against *P. falciparum* than their corresponding totarol analogs. This is unsurprising as totarol exhibits antiplasmodial activity (Table 1) that is significantly higher than the activity exhibited by phenol, 1-naphthol or 2-naphthol (Table 2). Notably, the 1-naphthyl analog with a benzylamine side chain (**14b**) displayed similar activity to its totarol counterpart **12c** against both strains of *P. falciparum*; the 2-naphthyl analog with a

Table 2
In vitro antiplasmodial activities and cytotoxicity of phenyl and naphthyl amino alcohols

Compound	Structure	*P. falciparum* IC_{50} (μM)[a]		R_I	CHO IC50 (μM)[a]	S_I	
		D10	K1			D10	K1
5		>100	ND	–	ND	–	–
9		77.04	ND	–	ND	–	–
13		>100	ND	–	ND	–	–
6		56.51	ND	–	ND	–	–
10		>100	ND	–	ND	–	–
14a		41.81	7.23	0.17	261.35	6.2	36.1
14b		5.18	0.73	0.14	8.27	1.6	11.2
14c		>100	ND	–	ND	–	–
14d		37.68	10.19	0.27	>300	NC	NC
7		>100	ND	–	ND	–	–
11		>100	ND	–	ND	–	–
14e		42.94	8.21	0.19	148.70	3.5	18.1
14f		6.77	13.53	2.00	123.40	18.2	9.1

ND = Not determined;
$R_I = IC_{50}$ (K1)/IC_{50} (D10);
$S_I = IC_{50}$ (CHO)/ IC_{50} (D10 or K1).
[a] Each compound was assayed in duplicate, on two separate occasions.

benzylamine side chain (**14f**) displayed similar activity to **12c** only against the D10 strain of *P. falciparum*, but was much less active against the K1 strain.

Incidentally, compound **14b**, the most active of this set of analogs against K1, also appears to be the most cytotoxic (CHO IC_{50} 8.27 μM), slightly more so than its totarol counterpart **12c** (CHO IC_{50} 14.41 μM). The other β-amino alcohol derivatives of this series exhibited negligible cytotoxicity, all having IC_{50} values >100 μM against the CHO mammalian cell line. Despite this low cytotoxicity, the poor antiplasmodial activity exhibited by these compounds resulted in overall poor selectivity indices.

One may therefore tentatively conclude that the presence of the totarol moiety in the β-amino alcohols **12** contributes to their superior antiplasmodial activity relative to **13–14**. Disappointingly, this superior antiplasmodial activity is accompanied by higher cytotoxicity and poor selectivity indices except for compounds **12f–i**, possibly due to the presence of the aryl-piperazinyl side chains. A possible explanation for this is that totarol may be facilitating the promiscuous binding of the totarol-based derivatives to multiple receptor sites, contributing to both the antiplasmodial activity and the cytotoxicity observed with these compounds.

3.3. Effects of selected totarol derivatives and analogs on erythrocytic morphology and lysis

The growth of *P. falciparum* is dependent on erythrocytic transport processes and hence drug effects on erythrocytes, and particularly on erythrocyte membranes, may indirectly influence parasite viability and growth.[19]

Natural products related to totarol have been shown to incorporate into the erythrocyte membrane due to the apolar and amphiphilic nature of these compounds; totarol itself has also been shown to incorporate into erythrocytic membranes. This has led to suggestions that any observed antiplasmodial effects may be an indirect effect on the host cell and hence this type of compounds may not be genuine antiplasmodial agents.[20–23]

It is therefore important to investigate their possible membrane effects on host cells in order to identify genuine antiplasmodial effects at sublytic concentrations. Such investigations are based on the fact that, in general, amphiphilic and lipophilic compounds and other compounds which incorporate into membranes cause membrane modifications that manifest as morphological changes.[20,21]

Three β-amino alcohol totarol derivatives were therefore selected and studied for their effect on erythrocyte morphology. Erythrocytic morphology was examined by separately incubating un-parasitized erythrocytes for 48 h in the presence of a 6.25 μg/ml concentration of totarol, **12a**, **12c** or **12d**. This 6.25 μg/ml concentration was selected as a compromise (moderate) test concentration that could be easily prepared by serial dilution from the 100 μg/ml stock solutions of the compounds. At the end of the incubation period, the shape of the erythrocytes was assessed using phase contrast light microscopy. The results are shown in Figure 3.

From the phase contrast micrographs, it can be observed that totarol and compounds **12c** and **12d** did not cause morphological changes to the erythrocytes. The erythrocytes exposed to these compounds retained morphologies similar to the control (unexposed) erythrocytes. As the concentrations of 6.25 μg/ml used for this experiment were higher than the antiplasmodial IC_{50} values of these three compounds, it may be concluded that effects on erythrocyte membranes are unlikely to contribute to the observed antiplasmodial activities of these compounds.

However, compound **12a** was observed to cause modification of erythrocytic morphology as evidenced by the presence of a large proportion of erythrocytes that appeared to be contracted and deformed. This implied possible deleterious effects of **12a** on the integrity of the erythrocyte membrane, and suggested that adverse effects on erythrocytes could possibly contribute to the overall observed antiplasmodial activity of this compound.

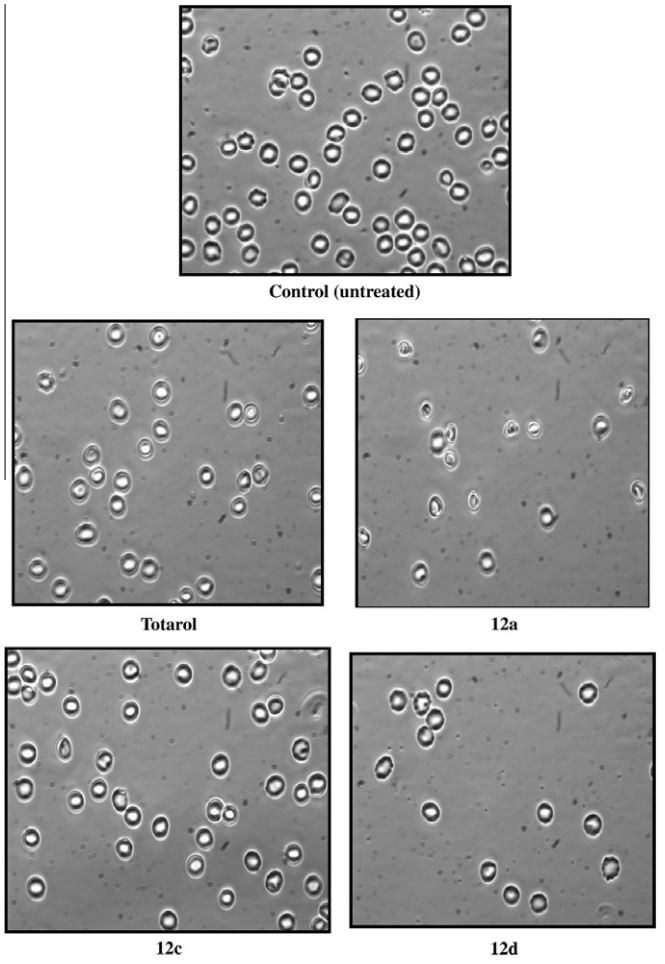


Figure 3. Phase contrast micrographs showing the effect of totarol and selected totarol derivatives on erythrocyte morphology.

3.4. Effect of selected totarol derivatives on chloroquine accumulation

Basic requirements for a general CQ resistance (CQR) reversal pharmacophore have been proposed as consisting of at least one hydrophobic aromatic function linked to a secondary or tertiary nitrogen (acting as a hydrogen-bond acceptor); various forms and connectivities of these components of the proposed CQR reversal pharmacophore are permissible with retention of CQR reversal activity.[24]

The β-amino alcohol derivatives of totarol display structural elements comparable to those of CQR reversal agents: as an example, compound **12a** is composed of a diterpene-like totarol portion, which is hydrophobic and contains a planar aromatic ring. In addition, the *N*-methylpiperazine portion of **12a** has two protonatable tertiary nitrogens. It is therefore possible that this compound could act as a CQR reversal agent.

A decrease in CQ accumulation appears central to the resistant phenotype, with CQ-resistant parasites being observed to accumulate less CQ than their susceptible counterparts.[25] Compounds which increase CQ accumulation may therefore play a role in potentiating CQ action, and CQ accumulation experiments are useful in identifying such compounds

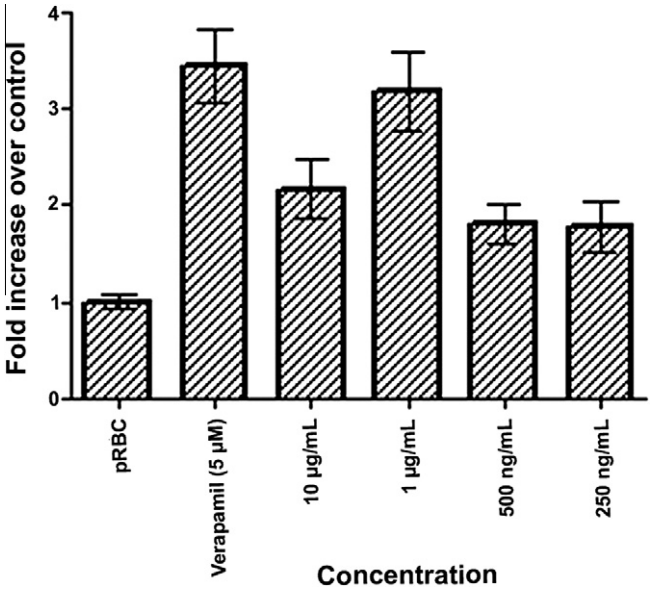


Figure 4. Accumulation of ^{3}H-CQ in K1 *P. falciparum* in the presence of 5 μM verapamil or various concentrations of compound 1**2a** and expressed relative to CQ accumulation in untreated control.

As a preliminary test of the hypothesis that the β-amino alcohol derivatives of totarol may act as CQR sensitizing agents, the effect of various concentrations of compound **12a** on ^{3}H-CQ accumulation in parasitized erythrocytes was studied. Figure 4 shows the results from this study.

The clinically available Ca^{2+} channel blocker verapamil enhances CQ susceptibility of CQ resistant *P. falciparum* at non-toxic concentrations, and has been observed to enhance CQ accumulation in CQ resistant strains.[26] The results presented in Figure 4 are in agreement with these observations whereby verapamil, which was used as a positive control, was found to enhance CQ accumulation more than three-fold at a concentration of 5 μM.

Interestingly, a significant increase in ^{3}H-CQ accumulation (comparable to that of 5 μM verapamil) was noted for compound **12a** at a concentration of 1 μg/ml. This is quite remarkable considering that the IC_{50} value of **12a** is 1.39 μM (0.67 μg/ml) for the resistant (K1) strain.

Though these results were of a preliminary nature and were for only one compound, they nevertheless indicate that compounds of this type appear to hold potential as possible CQ chemosensitizers, and a more extensive and in-depth investigation may be warranted.

3.5. Effect of selected compounds on erythrocytic invasion

Compound **14d** is the racemic form of the well-known β-adrenergic receptor antagonist propranolol, which has been shown to inhibit the invasion of erythrocytes by *P. falciparum* as well as reduce the parasitaemia of *Plasmodium berghei* infections in vivo.[27] These observations and the broad structural similarities between β-adrenergic receptor antagonists and the β-amino alcohol analogs synthesized in this study prompted a preliminary investigation and comparison of the effect of compounds **14b** and **14d**, and their respective totarol derivatives **12c** and **12d**, on erythrocytic invasion.

Briefly, parasitized erythrocytes in the schizont stage of development were exposed to the selected compounds at concentrations equivalent to their respective IC_{50} values until all obvious schizonts had burst and invasion was assumed to have occurred (as evidenced by a new ring stage in the control culture). Parasitemia was then determined from Giemsa stained smears, counting a minimum number of 1000 erythrocytes per slide. Table 3 shows the %parasitemia achieved by the cultures incubated with the various compounds.

Table 3
%Parasitemia achieved by cultures treated with compounds **12d**, **14d**, **12c** and **14b**

Compound	%Parasitemia[a]	Change in %parasitemia relative to control
Control (untreated)	8.83	—
12d	5.43	−3.40 (−38.5%)
14d	5.35	−3.48 (−39.4%)
12c	8.31	−0.52 (−0.06%)
14b	10.24	1.41 (0.16%)

[a] The experiment was performed on three separate occasions.

Compounds **12d** and **14d**, tested at their respective IC_{50} values, lowered the parasitemia relative to the control culture by 38.5% and 39.4%, respectively, while compounds **12c** and **14b** had no significant effect on parasitemia.

The inhibition of erythrocyte invasion by compound **14d** is in agreement with the experimental findings alluded to above.[27] The fact that compound **12d** also shows comparable inhibition implies that the replacement of the 1-naphthyl moiety of **14d** with totarol is associated with retention of this activity. Furthermore, the fact that these two compounds show close to 40% inhibition of erythrocyte invasions at concentrations equivalent to their antiplasmodial IC_{50} values strongly suggests that inhibition of erythrocyte invasion may contribute markedly to their overall antiplasmodial activity.

The same cannot be said for compounds **12c** and **14b** as these show negligible inhibition of erythrocyte invasion. This suggests that inhibition of erythrocyte invasion does not contribute significantly to their overall antiplasmodial activity, and that different mechanisms of antiplasmodial action may be at play. The fact that these compounds only differ from **12d** and **14d** in their respective side chains (isopropyl- vs benzyl-) clearly demonstrates the importance of the side chain in determining this activity.

4. Conclusion

The addition of a β-amino alcohol side chain increases the activity of totarol in most cases, and the side chain containing a protonatable nitrogen appears to be necessary for activity. Furthermore, the diterpene-like backbone of totarol appears to be important for antiplasmodial activity, as substitution with simpler aromatic groups generally led to decreased activity; however, this was usually also accompanied by a decrease in cytotoxicity.

Inhibition of erythrocyte invasion may be a possible mechanism by which some of these compounds elicit their antiplasmodial activity, though this is unlikely to be universal. Also, the antiplasmodial activity displayed by totarol derivatives does not appear to be the result of the effects of these compounds on the erythrocyte membrane. Compounds of this type also appear to hold potential as possible CQ potentiating agents.

5. Experimental data

Chemicals and reagents were purchased from either Sigma–Aldrich or Merck, South Africa. Chromatography solvents were purchased from Kimix Chemicals or Protea Chemicals, South Africa, as Chemically Pure (CP grade) solvents and distilled before use.

5.1. General procedure for the synthesis of epoxide intermediates 8–11

Sodium hydride (1.5 equiv) was added to a solution of the respective starting phenol (1 equiv) in DMF at 0 °C under nitrogen. The

reaction mixture was raised to 25 °C and epichlorohydrin (5 equiv) was added dropwise over 2 min. The reactions were complete after 12 h as confirmed via TLC. The mixture was diluted with water (100 ml), extracted with EtOAc (3 × 100 ml) and dried over $MgSO_4$. Excess epichlorohydrin and EtOAc was removed in vacuo to yield an oil which was purified by silica gel column chromatography.

5.1.1. Compound 8

11.1 g (3.88 mmol) of totarol (**3**), 0.14 g (5.82 mmol) NaH and 1.52 (19.41 mmol) epichlorohydrin used, resulting oil purified via silica gel chromatography, eluting with Et_2O/Hex (1:12), R_f = 0.56, giving an isolated yield of 84% (1.12 g). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 7.07 (d, *J* = 8.8, H-11), 6.67 (d, *J* = 8.8, H-12), 4.16 (t, *J* = 10.9, 3.0, H-21a), 4.14 (t, *J* = 10.9, 3.0, H-21b), 3.98 (ddd, *J* = 10.8, 5.2, 2.4, H-23α), 3.95 (ddd, *J* = 10.8, 5.2, 2.4, H-23β), 3.36 (m, H-22), 3.3 (sept, *J* = 7.2, H-15), 2.95 (dd, *J* = 16.9, 6.0, H-7β), 2.75 (m, H-7α), 2.24 (br d, *J* = 11.6, H-1β), 1.91 (dd, *J* = 7.9, 1.9, H-6α), 1.73 (dt, *J* = 13.4, 3.9, H-2β), 1.55–1.72 (m, H-6β), 1.49 (dt, *J* = 13.2, 3.2, H-2α), 1.47 (br d, *J* = 13.2, H-3β), 1.36 (d, *J* = 7.1, H-17), 1.34 (d, *J* = 7.1, H-16), 1.18 (s, H-20), 1.16–1.30 (m, H-1α, H-3α, H-5), 0.95 (s, H-18), 0.92 (s, H-19). Compound previously reported.[16]

5.1.2. Compound 9

Reaction was performed at 50 °C. 1.07 g (11.39 mmol) of phenol (**5**), 0.82 (34.17 mmol) NaH and 4.45 ml (56.95 mmol) epichlorohydrin used, resulting oil purified via silica gel chromatography, eluting with EtOAc/Hex (1:12), R_f = 0.48, giving an isolated yield of 88% (1.50 g). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 4.21 (dd, *J* = 14.8, 4.4, H-7a), 3.98 (dd, *J* = 11.1, 5.4, H-7b), 3.33–3.38 (m, H-8), 2.90 (m, H-9α), 2.75 (dd, *J* = 5.1, 3.3, H-9β). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 69.0 (C-7), 50.2 (C-8), 44.7 (C-9).

5.1.3. Compound 10

1.02 g (7.08 mmol) of 1-naphthol (**6**), 0.25 g (10.5 mmol) NaH and 2.74 ml (35 mmol) of epichlorohydrin used, resulting oil purified via silica gel chromatography, eluting with Et_2O/Hex (3:7), R_f = 0.40, giving an isolated yield of 76% (1.08 g). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 4.40 (dd, *J* = 11.2, 3.2, H-9a), 4.17 (dd, *J* = 11.0, 5.4, H-9b), 3.48–3.51 (m, H-10), 2.97 (dd, *J* = 5.0, 4.2, H-11α), 2.85 (dd, *J* = 5.0, 2.6, H-11β). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 69.0 (C-9), 50.2 (C-10), 44.7 (C-11).

5.1.4. Compound 11

0.5 g (3.47 mmol) of 2-naphthol (**7**), 0.125 g (5.2 mmol) NaH and 1.35 ml (17.3 mmol) of epichlorohydrin used, resulting oil purified via silica gel chromatography, eluting with Et_2O/Hex (3:7), R_f = 0.51, giving an isolated yield of 92% (632.6 mg). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 4.34 (dd, *J* = 11.0, 3.4, H-9a), 4.09 (dd, *J* = 10.8, 5.6, H-9b), 3.42 (m, H-10), 2.94 (dd, *J* = 4.8, 4.0, H-11α), 2.81 (dd, *J* = 5.2, 2.8, H-11β). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 68.8 (C-9), 50.1 (C-10), 44.7 (C-11).

5.2. General procedure for synthesis of β-amino alcohols 12–14

The respective amine (1.1 equiv) was added to 1 equiv of the respective epoxide in MeOH (1 ml per 0.06 mmol starting material). The mixture was stirred at 50 °C, for 12–24 h, until the reaction was complete (verified via TLC). The excess solvent was removed under reduced pressure and the resulting oil purified using either preparative TLC or column chromatography.

5.2.1. Compound 12a

101.3 mg (0.30 mmol) of totarol epoxide (**8**) used, 0.036 ml (0.33 mmol) *N*-methylpiperazine used, resulting oil purified using preparative TLC, DCM/MeOH (9:1), R_f = 0.56, to yield 29% (38 mg). IR ν_{max} (KBr)/cm^{-1} 3134 (OH), 2805–2938 (CH aliphatic), 1590 (C=C aromatic); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 7.00 (d, *J* = 8.8, H-11), 6.51 (d, *J* = 8.8, H-12), 4.11 (m, H-22), 3.98 (dd, *J* = 12.4, 6.4, H-21a), 3.91 (dd, *J* = 12.4, 6.8, H-21b), 3.30 (sept, *J* = 7.2, H-15), 2.94 (dd, *J* = 17.0, 6.2, H-7β), 2.75 (m, H-7α), 2.57–2.60 (m, H-23), 2.34–2.56 (m, H-24, H-25, H-26, H-27), 2.30 (s, H-28), 2.23 (br d, *J* = 11.6, H-1β), 1.92 (dd, *J* = 7.7, 1.6, H-6α), 1.73 (dt, *J* = 13.7, 3.3, H-2β), 1.55–1.70 (m, H-6β), 1.49 (dt, *J* = 13.2, 3.2, H-2α), 1.47 (br d, *J* = 13.2, H-3β), 1.36 (d, *J* = 7.1, H-17), 1.34 (d, *J* = 7.1, H-16), 1.18 (s, H-20), 1.17–1.24 (m, H-1α, H-3α, H-5), 0.95 (s, H-18), 0.92 (s, H-19). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 155.1 (C-13), 143.3 (C-9), 133.7 (C-8), 133.2 (C-14), 122.7 (C-11), 109.8 (C-12), 80.0 (C-21), 70.1 (C-22), 65.9 (C-23), 65.7 (C-24), 61.0 (C-28), 55.1 (C-25), 53.2 (C-26), 49.5 (C-5), 45.9 (C-3), 41.5 (C-1), 39.6 (C-10), 37.6 (C-4), 33.1 (C-18), 28.7 (C-7), 27.4 (C-15), 22.9 (C-20), 21.5 (C-19), 20.6 (C-16, C-17), 19.4 (C-2), 19.3 (C-6), 17.0 (C-28); APCI *m/z* 443.5 $(M^{+1})^+$. Found: C, 76.0; H, 10.0; N, 6.2. $C_{28}H_{46}N_2O_2$ requires C, 76.0; H, 10.5; N, 6.3. Mp 123 °C.

5.2.2. Compound 12c

76.8 mg (0.22 mmol) of totarol epoxide (**8**) used, 0.027 ml (0.25 mmol) benzylamine used, resulting oil purified via silica gel chromatography, eluting with DCM/MeOH (9:1), R_f = 0.55, giving an isolated yield of 30.5% (30.5 mg). IR ν_{max} (KBr)/cm^{-1} 3687 (OH), 2953 (CH aliphatic), 1603, 1453 (C=C), 707 (C–N); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 7.26–7.37 (m, H-26, H-27, H-28, H-29, H-30), 7.00 (d, *J* = 8.8, H-11), 6.51 (d, *J* = 8.8, H-12), 4.16 (m, H-22), 4.00 (dd, *J* = 9.5, 5.4, H-21a), 3.92 (dd, *J* = 9.5, 5.4, H-21b), 3.50 (s, H-24), 3.28 (br s, H-15), 2.94 (m, H-7β), 2.75 (m, H-7α), 2.96–3.00 (m, H-23), 2.23 (br d, *J* = 11.6, H-1β), 1.92 (dd, *J* = 13.2, 7.6, H-6α), 1.73 (dt, *J* = 13.7, 3.3, H-2β), 1.55–1.70 (m, H-6β), 1.47 (dt, *J* = 13.2, 3.2, H-2α), 1.47 (br d, *J* = 13.2, H-3β), 1.36 (d, *J* = 3.6, H-17), 1.34 (d, *J* = 1.34, H-16), 1.19 (s, H-20), 1.20–1.39 (m, H-1α, H-3α, H-5), 0.96 (s, H-18), 0.93 (s, H-19). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 155.0 (C-13), 143.5 (C-9), 138.5 (C-25), 133.8 (C-8), 133.1 (C-14), 128.4 (C26, C-27, C-28, C-29, C-30), 122.8 (C-11), 115.5 (C-12), 80.0 (C-21), 70.1 (C-22), 53.4 (C-23), 51.3 (C-24), 49.5 (C-5), 41.5 (C-3), 39.6 (C-1), 37.6 (C-10), 33.2 (C-4), 33.1 (C-18), 28.7 (C-7), 27.3 (C-15), 25.1 (C-16, C-17), 21.5 (C-20), 20.7 (C-19), 19.4 (C-2), 19.3 (C-6); EIMS *m/z* 449 (M^+). Mp 69–74 °C.

5.2.3. Compound 12d

Reaction was performed at 31 °C. 229.9 mg (0.67 mmol) of totarol epoxide (**8**) used, 3 equiv (0.3 ml, 3.52 mmol) isopropylamine used, resulting oil purified via silica gel chromatography, eluting with DCM/MeOH (9:1), R_f = 0.37, giving an isolated yield of 70% (189.3 mg). IR ν_{max} (KBr)/cm^{-1} 3313 (OH, H bonded), 2960, 2867 (C–H aliphatic), 1590 (C=C aromatic); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 7.09 (d, *J* = 8.8, H-11), 6.72 (d, *J* = 8.8, H-12), 4.11 (m, H-22), 3.96 (2 X ddd, *J* = 9.4, 5.1, 1.8, H-21a and b), 3.30 (br s, H-15), 2.84–3.00 (m, H-7β), 2.76–3.00 (m, H-7α, H-23), 2.74 (br s, H-24), 2.25 (d, *J* = 12.4, H-1β), 1.92 (dd, *J* = 13.3, 8.1, H-6α), 1.74 (dt, *J* = 13.6, 3.3, H-2β), 1.63–1.72 (m, H-6β), 1.59 (dt, *J* = 13.6, 3.4, H-2α), 1.47 (br d, *J* = 14.0, H-3β), 1.20–1.39 (m, H-1α, H-3α, H-5, H-16, H-17), 1.19 (s, H-20), 1.13 (d, *J* = 6.4, H-25, H-26), 0.95 (s, H-18), 0.93 (s, H-19). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 155.1 (C-13), 143.5 (C-9), 133.8 (C-8), 133.2 (C-14), 122.8 (C-11), 110.0 (C-12), 70.2 (C-21), 69.6 (C-22), 49.61 (C-23), 49.59 (C-24), 49.5 (C-5), 49.1 (C-24), 41.6 (C-3), 39.6 (C-1), 37.7 (C-10), 33.2 (C-4), 33.2 (C-18), 28.7 (C-7), 27.4 (C-15), 25.1 (C-16), 22.7 (C-17), 21.6 (C-25), 20.7 (C-26), 20.7 (C-20, C-19), 19.5 (C-2), 19.4 (C-6); APCI *m/z* 402.5 $(M^{+1})^+$. Found: C, 77.3; H, 10.2; N, 3.2. $C_{26}H_{43}NO_2$ requires C, 77.8; H, 10.8; N, 3.5. Mp 39–42 °C.

5.2.4. Compound 13

500 mg (3.61 mmol) of phenyl epoxide (**9**) used, 0.44 ml (3.97 mmol) *N*-methylpiperazine used, resulting oil purified via

silica gel chromatography, eluting with DCM/MeOH (9:1), R_f = 0.43, giving an isolated yield of 67% (608.3 mg). IR ν_{max} (KBr)/cm^{-1} 3348 (OH H-bonded), 2938, 2806 (C–H aliphatic), 1596 (C=C aromatic). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 4.05–4.13 (m, H-8), 3.98 (d, J = 5.1, H-7), 2.73 (br d, J = 5.4, H-9), 2.59–2.51 (m, H-10, H-11, H-12, H-13), 2.31 (s, H-14). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 70.2 (C-8), 65.6 (C-7), 60.5 (C-9), 55.1 (C-10, C-13), 53.1 (C-11, C-12), 45.9 (C-14); APCI m/z 251.2 $(M^{+1})^+$. Found: C, 66.2; H, 8.6; N, 11.0. $C_{14}H_{22}N_2O_2{\cdot}0.2H_2O$ requires C, 66.2; H, 8.7; N, 11.0. Mp 51–60 °C.

5.2.5. Compound 14a

190 mg (0.95 mmol) of 1-naphthyl epoxide (**10**) used, 0.12 ml (1.1 mmol) *N*-methylpiperazine used, resulting oil purified via silica gel chromatography, eluting with MeOH, R_f = 0.26. The compound was further purified via recrystallization with hexane and dried under high vacuum giving an isolated yield of 68% (192.3 mg). IR ν_{max} (KBr)/cm^{-1} 3162 (OH), 3058 (C–H aromatic), 2964–2923, 2878–2782 (C–H aliphatic), 1578 (C=C aromatic). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 4.19–4.25 (m, H-9a and b), 4.14–4.16 (m, H-10), 2.67–2.69 (m, H-11a and b), 2.76 (br s, H-12, H-15), 2.50 (br s, H-13, H-14), 2.31 (s, H-16). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 70.6 (C-9), 65.7 (C-10), 60.8 (C-11), 55.2 (C-12, C-15), 53.3 (C-13, C-14), 46.0 (C-16). APCI m/z 301.1 $(M^{+1})^+$. Found: C, 70.2; H, 7.7; N, 9.0. $C_{18}H_{24}N_2O_2{\cdot}0.5H_2O$ requires C, 69.9; H, 7.8; N, 9.1. Mp 66–77 °C.

5.2.6. Compound 14b

0.89 mmol of 1-naphthyl epoxide (**10**) used, 1.1 mmol benzylamine used, resulting oil purified via silica gel chromatography, eluting with EtOAc/Hex (1:1), R_f = 0.63, giving an isolated yield of 34% (93.4 mg). IR ν_{max} (KBr)/cm^{-1} 3279 (OH H-bonded), 3050 (C-H aromatic), 2942 (C-H aliphatic), 1580 (C=C aromatic). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 7.26–7.51 (m, H-3, H-4, H-6, H-7, H-14 to 18), 4.29 (m, H-10), 4.10–4.21 (m, H-9a and b), 3.93 (d, J = 4, H-12), 2.92–3.06 (m, H-11a and b), 2.88 (NH). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 138.4 (C-13), 128.6 (C-15, C-17), 128.5 (C-14, C-18), 127.5 (C-16), 70.5 (C-10), 68.1 (C-9), 53.5 (C-12), 51.2 (C-11). APCI m/z 308.4 $(M^{+1})^+$. Found: C, 78.1; H, 6.6; N, 4.6. $C_{20}H_{21}NO_2$ requires C, 78.2; H, 6.9; N, 4.6. Mp 112–113 °C.

5.2.7. Compound 14c

Neutralization of 1-(2-chlorophenyl)-piperazine monohydrochloride (271 mg, 2.40 mmol) with MP-carbonate (3.65 mg, 9.6 mmol) in MeOH (7 ml) at 20 °C for 7 h yielded amine. 134.4 mg (0.67 mmol) of 1-naphthyl epoxide (**10**) used. Resulting oil purified via silica gel chromatography, eluting with EtOAc/Hex (1:1), R_f = 0.72, giving an isolated yield of 55% (147 mg). IR ν_{max} (KBr)/cm^{-1} 3345 (OH H-bonded), 3054 (C–H aromatic), 2807–2933 (C–H aliphatic), 1579 (C=C aromatic). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 7.26 (d, J = 0.9, H-18), 7.24 (m, H-20), 7.21 (dd, J = 8.1, 1.8, H-21), 7.02 (td, J = 7.6, 1.6, H-19), 4.35 (quintet, J = 8.6, H-10), 4.15–4.28 (m, H-9a and b), 3.16 (m, H-12, H-15), 2.752–2.819 (m, H-13, H-14), 2.94–3.00 (m, H-11a and b). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 149.0 (C-16), 130.7 (C-18), 128.8 (C-17), 125.6 (C-20), 123.9 (C-21), 121.9 (C-19), 70.5 (C-10), 65.6 (C-9), 61.1 (C-12, C-15), 53.6 (C-13, C-14), 51.1 (C-11). APCI m/z 397.3 $(M^{+1})^+$. HPLC indicated that the compound was pure. Mp 107–117 °C.

5.2.8. Compound 14d

Reaction was performed at 31 °C. 167 mg (0.83 mmol) of 1-naphthyl epoxide (**10**) used, 3 equiv (0.30 ml, 3.52 mmol) isopropylamine used, resulting oil purified via silica gel chromatography, eluting with EtOAc, R_f = 0.11, giving an isolated yield of 93% (200.6 mg). IR ν_{max} (KBr)/cm^{-1} 3271 (OH H-bonded), 3054 (C–H aromatic), 2963, 2923, 2834 (C–H aliphatic), 1583 (C=C aromatic). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 4.13–4.21 (m, H-10, OH), 3.90 (m, H-9a), 3.62 (dd, J = 11.2, 3.2, H-9b), 3.39 (dd, J = 11.0, 7.8, H-11a), 3.01 (dd, J = 16, 4.8, H-11b), 2.88 (m, H-12), 2.20 (NH), 1.11 (d, J = 3.1, H-13, H-14). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 70.7 (C-10), 68.5 (C-9), 68.0 (C-11), 49.0 (C-12), 23.0 (C-13), 22.9 (C-14). APCI m/z 260.3 $(m^{+1})^+$. Found: C, 71.7; H, 7.9; N, 4.9. $C_{16}H_{21}NO_2{\cdot}0.5H_2O$ requires C, 71.6; H, 7.9; N, 5.2. Mp 35–79 °C.

5.2.9. Compound 14e

313.7 mg (1.57 mmol) of 2-naphthol epoxide (**11**) used, 0.19 ml (1.72 mmol) *N*-methylpiperazine used, resulting oil purified via silica gel chromatography, eluting with MeOH, R_f = 0.18. The compound was further purified via recrystallization with hexane and EtOAc and dried under high vacuum giving an isolated yield of 48% (226.2 mg). IR ν_{max} (KBr)/cm^{-1} 3365 (OH), 3025 (C–H aromatic), 2942, 2807 (C–H aliphatic), 1631–1600 (C=C aromatic). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 4.16 (m, H-10), 4.10 (d, J = 3, H-9a and b), 2.73 (br d, J = 5.7, H-12, H-15), 2.63–2.59 (m, H-11a and b), 2.50 (s, H-13, H-14), 2.31 (s, H-16). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 70.4 (C-10), 65.6 (C-9), 60.5 (C-12, C-15) 55.1 (C-11, C-15), 53.1 (C-13, C-14), 45.9 (C-16). APCI m/z 301.3 $(M^{+1})^+$. Found: C, 68.6; H, 7.2; N, 9.1. $C_{18}H_{24}N_2O_2{\cdot}0.8H_2O$ requires C, 68.9; H, 7.7; N, 9.0. Mp 132 °C.

5.2.10. Compound 14f

225.7 mg (1.13 mmol) of 2-naphthol epoxide (**11**) used, 0.135 ml (1.24 mmol) benzylamine used, resulting oil purified via silica gel chromatography, eluting with MeOH, R_f = 0.47, giving an isolated yield of 67% (230.7 mg). IR ν_{max} (KBr)/cm^{-1} 3266 (OH H-bonded), 3053 (C–H aromatic), 2716–2899 (C–H aliphatic), 1601–1629 (C=C aromatic). ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 7.44 (dt, J = 10.2, 1.6, H-14, H-18) 7.13–7.36 (m, H-2, H-5, H-6, H-8, H-15, H-16, H-17), 4.19 (m, H-10), 4.10 (d, J = 6.4, H-9a and b), 3.89 (br s, H-12a and b), 2.84–3.00 (m, H-11a, b and NH). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 128.5 (C-14, C-18), 128.3 (C-15, C-17), 127.3 (C-16), 70.4 (C-10), 68.2 (C-9), 53.6 (C-12) 51.1 (C-11). APCI m/z 308.4 $(M^{+1})^+$. Found: C, 77.3; H, 6.6; N, 4.5. $C_{20}H_{21}NO_2{\cdot}0.1H_2O$ requires C, 77.7; H, 6.8; N, 4.5. Mp 95–102 °C.

5.3. Compound 12b

Compound **8** (294.3 mg, 0.86 mmol), potassium phthalimide (7.7 mg, 0.04 mmol) and phthalimide (142,2 mg, 0.946 mmol) were dissolved in DMF (13 ml). The reaction mixture was stirred for 12 h at 90 °C. Completion of reaction was confirmed by TLC, Et_2O/Hex (3:10), R_f = 0.15. The reaction mixture was diluted with water (50 ml) and extracted with $CHCl_3$ (3 × 50 ml). The extracts were washed with water (2 × 50 ml) and brine (2 × 50 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a yellowish oil. The oil was dissolved in EtOAc (100 ml), washed with water (3 × 100 ml), dried and loaded onto a silica gel column. The column was eluted with Et_2O/Hex (3:10) and once the spot of interest had been eluted, baseline material was washed out with Et_2O/Hex (1:1). Fractions were concentrated under reduced pressure and dried on high vacuum, resulting in 0.25 g (63%) of compound **6**. IR ν_{max} (KBr)/cm^{-1} 3417 (OH), 2930 (CH aliphatic), 1702 (C=O), 1443 (C=C aromatic), 1263 (C–O), 1073 (C–N); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 7.88 (dd, J = 5.6, 3.0, H-26, H-29), 7.73 (dd, J = 5.4, 3.2, H-27, H-28) 7.00 (d, J = 8.9, H-11), 6.51 (d, J = 8.9, H-12), 4.32 (m, H-22), 4.03 (m, H-21, H-23), 3.34 (sept, J = 7.2, H-15), 2.94 (dd, J = 17.2, 6.2, H-7β), 2.75 (m, H-7α), 2.25 (br d, J = 11.2, H-1β), 1.92 (dd, J = 7.7, 1.6, H-6α), 1.73 (dt, J = 17.0, 3.4, H-2β), 1.55–1.70 (m, H-6β), 1.47 (dt, J = 13.2, 3.2, H-2α), 1.47 (br d, J = 13.2, H-3β), 1.35 (dd, J = 10.1, 3.6, H-16, H-17), 1.18 (s, H-20), 1.18–1.26 (m, H-1α,, H-3α, H-5), 0.95 (s, H-18), 0.92 (s, H-19). ^{13}C NMR δ_C (100.6 MHz; $CDCl_3$) 168.7 (C-24,

C-31), 154.9 (C-25, C-30), 143.8 (C-13), 134.1 (C-26, C-29), 133.4 (C-27, C-28), 132.0 (C-14), 123.5 (C-11), 122.9 (C-12), 109.9 (C-22), 69.6 (C-21), 69.3 (C-5), 49.5 (C-3), 41.6 (C-23), 41.5 (C-1), 39.6 (C-10), 37.7 (C-4), 33.3 (C-18), 33.2 (C-7), 29.7 (C-15), 28.8 (C-16), 25.2 (C-17), 21.6 (C-20), 20.9 (C-19), 19.5 (C-2), 19.4 (C-6); *m/z* 512 $(M^{+Na})^+$. HPLC indicated that the compound was pure. Mp 148 °C.

NB: Compounds **12e–k** were synthesized as described previously.[16]

5.4. In vitro antiplasmodial activity

D10 and K1 strains of *P. falciparum* were maintained in continuous in vitro culture by standard methods.[28] The cultured parasites were, whenever necessary, synchronized by treatment with 5% D-sorbitol (Sigma) at the ring stage.[29]

The in vitro antiplasmodial assays were carried out using the parasite lactate dehydrogenase assay as previously reported by Guantai et al. 2010.[30]

5.5. In vitro cytotoxicity

Cytotoxicity assays were carried out on a Chinese hamster ovarian (CHO) cell line using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Briefly, cell cultures were routinely maintained in Dulbecco's Modified Eagles' Medium (DMEM): Hams (1:1) supplemented with heat inactivated fetal calf serum (FCS) (10%) and gentamicin (0.04 μg/ml). The cells were maintained in 75 cm^3 flasks in a 5% CO_2 humidified atmosphere at 37 °C.

For the cytotoxicity evaluations, 1×10^4 cells in 180 μl medium were seeded in each well of a 96-well plate and incubated at 37 °C under a 5% CO_2 atmosphere for 24 h. Aliquots of 20 μl of serial dilutions of the test compounds (stocks in DMSO) were added to the wells (concentration range of 100 μg–0.01 μg/ml). Untreated control wells received 20 μl of culture medium, while additional solvent controls were prepared with medium containing DMSO to account for any possible effects of DMSO on cell viability. The plates were then incubated at 37 °C under an atmosphere of 5% CO_2 for 48 h.

After the incubation period, 20 μl MTT (5 mg/ml) was added to each well. The plates were further incubated for an additional 4 h at 37 °C under an atmosphere of 5% CO_2, and then centrifuged for 10 min at 800*g*. The supernatant was carefully aspirated from each well without disturbing the pellet, and the cells were washed by addition of 150 μl of phosphate-buffered saline (PBS) followed by centrifugation for 10 min at 800*g*. The supernatant was again carefully aspirated, and the plates were left to dry off at 37 °C for an hour. Ethanol (100 μl) was added to each well to solubilize the resultant formazan crystals, aided by gentle mechanical shaking for 1–2 h. Absorbances were measured on a Universal Microplate Reader (ELx800 UV, Bio-tek Instruments) at a wavelength of 570 nm and used to calculate percentage cell growth in drug-treated wells; these in turn were plotted versus log drug concentration and used to determine the corresponding IC_{50} values by non-linear regression analysis.

5.6. Tritiated chloroquine accumulation

Synchronized parasitized erythrocytes at the trophozoite stage (hematocrit 1%, parasitemia 5%) in the presence of the appropriate concentration of the test compounds were incubated at 37 °C for 15 min before being exposed to 4 nM ^{3}H-CQ in a microcentrifuge tube. The tubes were then incubated at 37 °C for an hour, after which 100 μl of dibutylphthalate (DBP) was added and the tubes centrifuged and the supernatant aspirated. The microcentrifuge tip containing the parasites was then cut off and placed in a scintillation vial. 100 μl of Solvable, 25 μl of EDTA (0.1 M) and 100 μl of peroxide (30%) were added, followed by 2 ml of scintillation fluid (Pakard Bioscience). The radioactivity within the vials was determined using a Packard Tri-Carb 2100TR liquid scintillation spectrophotometer and used to quantify the extent of accumulation of ^{3}H-CQ relative to the untreated controls.

5.7. Erythrocytic membrane modifying effect

Unparasitized erythrocytes (100 μl, hematocrit 2%) were incubated for 48 h in the presence of 6.25 μg/ml of the totarol derivatives and analogs, after which 100 μl of 1% gluteraldehyde (Sigma) in paraformaldehyde was added. The erythrocytic shape of 20 μl samples was assessed using phase contrast light microscopy (×1000).

5.8. Effect of selected compounds on erythrocytic invasion

Parasitized erythrocytes (hematocrit 1%, parasitemia 2%) in the schizont stage of development were exposed to the selected compounds at their respective IC_{50} values, until all obvious schizonts had burst and invasion was assumed to have occurred (as evidenced by a new ring stage in the control culture). Parasitemia was then determined from Giemsa stained smears, counting a minimum number of 1000 erythrocytes per slide. The experiment was performed on three separate occasions.

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