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Synthesis of Totarol Amino Alcohol Derivatives and Their Antiplasmodial Activity and Cytotoxicity

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Abstract—The previously unknown antiplasmodial activity of the plant derived natural product totarol is reported. Novel β-amino alcohol derivatives based on this natural product were designed, synthesised and evaluated for in vitro antiplasmodial activity and cytotoxicity. These derivatives showed antiplasmodial IC_{50} values in the range of 0.6–3.0 μM and were equally active against a chloroquine-sensitive and resistant strain of *Plasmodium falciparum*, while showing little cytotoxicity against a mammalian cell line (CHO). In terms of lead development, two of the compounds based on substituted phenylpiperazine warrant further investigation as potential antiplasmodial leads. In addition to their selective antiplasmodial activity and lack of chloroquine cross-resistance, these compounds are structurally different to any of the available antimalarial drugs.

Introduction

Malaria continues to be one of the leading health problems in sub-Saharan Africa and is responsible for over 1 million deaths per year.[1] The rapid spread of *Plasmodium falciparum* resistance to available antimalarial drugs has highlighted the need to identify alternative antimalarial compounds. Natural products are biologically validated starting points for drug discovery. Recent examples in the literature attest to the fact that screening libraries of natural product derivatives is even more effective than screening natural products alone.[2–5]

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 (**2**) (Fig. 1), were isolated and found to show significant in vitro antiplasmodial activity and little cytotoxicity.[6] Considering their selective activity and different structural features to current antimalarial agents, **1** and **2** were used as hits to design a series of potential antiplasmodial lead compounds. Due to the low yields of the isolated compounds, a commercial analogue (totarol) was identified and used for chemical modification purposes.

Totarol (**3**) has been isolated from a large variety of plants,[7,8] and several studies have shown that it exhibits potent in vitro antibacterial activity.[9–13] In this study totarol was used as a scaffold to synthesise a series of β-amino alcohol derivatives (Scheme 1). The rationale for choosing this class of derivatives is based on the fact that the β-amino alcohol moiety is present in a number of potent antimalarial drugs exemplified by quinine (**4**) and mefloquine (**5**) (Fig. 2).[14] Moreover, structural requirements for antiplasmodial activity of amino alcohol antiplasmodial agents include the presence of an aromatic portion and amino alcohol portion in such a way that the amino and alcohol groups are separated by two to three carbon atoms.[15] Thus, the combination of a potentially active moiety with a natural product hit to create a hybrid compound was explored.

This paper describes the synthesis of the totarol derivatives and reports on their in vitro antiplasmodial activity against a drug sensitive and resistant strain of *P. falciparum*. In order to determine the selectivity of the antiplasmodial activity, the in vitro cytotoxicity against a mammalian cell line is also reported.

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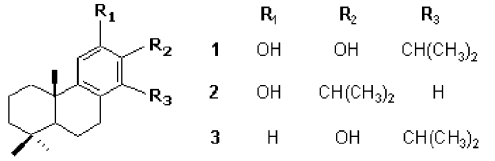

Figure 1. Structures of the isolated compounds (**1** and **2**) and totarol (**3**).

Results and Discussion

Chemistry

The target exploratory compounds were synthesised in two steps from totarol via the intermediate epoxide **6**. Treatment of totarol with epichlorohydrin in the presence of sodium hydride gave **6**, which was in turn reacted with selected secondary amines to produce β-amino alcohols (**7**–**13**) (Scheme 1).

In vitro antiplasmodial activity and cytotoxicity

The 50% inhibitory concentrations (IC_{50}) of the synthesised β-amino alcohols (**7**–**13**) as well as the corresponding amines are listed in Table 1. For comparative purposes, the activity of totarol (**3**) and compounds **1** and **2** are also listed in Table 1.

Prior to any modifications, totarol (**3**) was tested for in vitro activity and showed marked antiplasmodial activity against the CQ-resistant and sensitive strain of *P. falciparum*. While the activity of **3** was comparable to the isolated compounds (**1** and **2**), it was slightly less active. The cytotoxicity assays revealed that the antiplasmodial activity of **3** was 40-fold greater than the cytotoxic effect, demonstrating some selectivity. Although the in vitro cytotoxicity of totarol has previously been reported,[11] this is the first time, to the best of our knowledge, that totarol has been shown to possess in vitro antiplasmodial activity.

Not surprisingly, the aminoquinoline-based piperazine was the most active amine, and showed strong antiplasmodial activity against D10 and K1. This compound and chloroquine both possess a 4-amino-7-chloroquine structure that has been shown to exhibit moderate in vitro antiplasmodial activity.[16] The fact that the activity is not as strong as CQ, and that CQ cross resistance is not observed is probably due to the absence of the aminoalkyl side chain. Among the amines tested, the most interesting results were those of the three phenylpiperazines. These three amines showed little activity against the CQ-sensitive strain (D10), but were highly active against the CQ-resistant strain (K1). The resistance indexes (RI) indicate that the amines were approximately 20 times more potent to K1 than D10. Furthermore, the presence of a Cl or OEt group in the *ortho* position of phenylpiperazine provided a 2-fold increase in potency against both D10 and K1. At present, neither the mode of action of the amines nor the precise mechanism of CQ resistance is known. Thus, the exact reason for the increased sensitivity of K1 to these amines in unclear.

Out of the β-amino alcohols, **7** showed the highest antiplasmodial activity against D10 and K1. As indicated by the selectivity index (SI), the antiplasmodial activity was found to be only 12 times greater than the cytotoxic effect. Similar results were observed for compounds **12** and **13**. The low selective indexes of **7**, **12**, and **13** suggests a slender safety margin, which would limit their potential as lead compounds. The next most active β-amino alcohol was **8**, although it was not as active as its starting amine. This suggests that the synthesised β-amino alcohol prevents the 4-amino-7-chloroquine structure from either (i) being delivered to the requisite site of action within the parasite, or (ii) prevents it from interacting with its target. Compounds **9**, **10**, and **11** showed a remarkable improvement in activity compared to their respective amines. The antiplasmodial activity of these compounds followed the same trend as their starting amines, with **10** and **11** showing the greatest activity followed by **9**. It is interesting to note that **10** and **11** were the least cytotoxic out of the tested compounds. The cytotoxicity IC_{50} values for these compounds could not be determined since 100% cell viability was observed even at the highest concentrations (100 μg/mL) tested.

Although the series of β-amino alcohols synthesised is relatively small, a number of interesting preliminary generalisations can be made on the basis of the data presented. The addition of a phenylpiperazine nucleus to totarol causes an increase in selective antiplasmodial activity, which is enhanced by the attachment of a Cl or OEt substituent to the *ortho* position of the phenylpiperazine aromatic ring. Attachment of a 4-amino-7-chloroquinoline group to totarol reduces the activity of the former, while the attachment of diethylamine, piperidine or morpholine to totarol greatly increases its

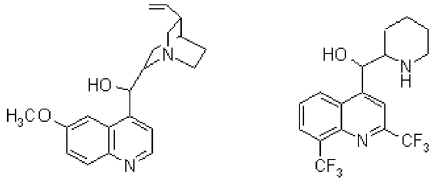

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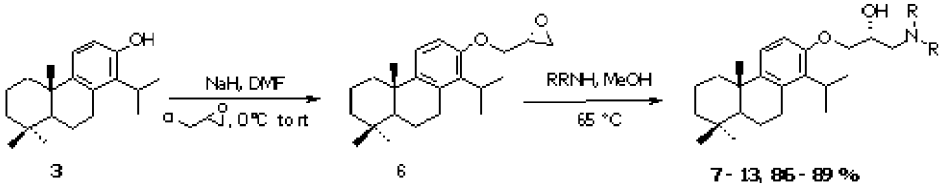
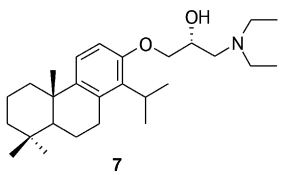
Figure 2. Structures of quinine (**4**) and mefloquine (**5**).



Scheme 1. Synthesis of totarol derived β-amino alcohols.

Table 1. In vitro antiplasmodial activity and cytotoxicity[a]



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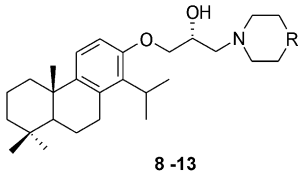

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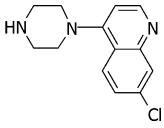
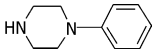
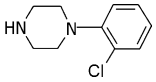
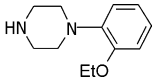
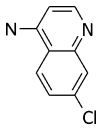
Compd	R	IC_{50} D10	IC_{50} K1	IC_{50} CHO	SI[b]	RI[c]
1	—	2.51	2.75	170.24	61.99	1.09
2	—	3.32	2.20	180.59	82.05	0.67
3	—	7.51	4.29	170.46	39.66	0.57
	—	>1367	1172	ND[d]	—	—
	—	1.18	0.97	ND	—	0.82
	—	367.05	20.46	ND	—	0.06
	—	169.46	8.39	ND	—	0.05
	—	188.81	9.36	ND	—	0.05
	—	290.35	69.29	ND	—	0.24
	—	166.29	32.53	ND	—	0.20
7	—	0.61	0.63	7.73	12.21	1.04
8		1.40	0.90	94.19	104.89	0.64
9		2.56	1.81	105.44	58.13	0.71
10		3.25	0.90	>186	>207	0.23
11		1.62	1.01	>182	>180	0.62
12	O	2.07	0.56	5.61	10.04	0.27
13	CH_2	1.08	0.75	2.99	4.00	0.70
CQ	—	0.023	0.352	ND	—	15.30
DN	—	ND	ND	2.50	—	—

[a]Results are expressed as mean IC_{50} (μM) of three independent experiments each performed in duplicate.
[b]Selectivity index (SI) = cytotoxicity IC_{50}/antiplasmodial IC_{50}.
[c]Resistance index (RI) = K1 IC_{50}/D10 IC_{50}.
[d]ND, not determined.

antiplasmodial activity as well as its cytotoxicity. While definite conclusions regarding structure–activity relationships cannot be made at this point due to the limited number of compounds studied, these results indicate favorable modifications for antiplasmodial lead development.

Conclusions

In comparison to totarol, all of the new compounds showed improved antiplasmodial activity and no cross-resistance with CQ. The most promising lead compounds in terms of selectivity and antiplasmodial

activity are **10** and **11**. Both of these compounds exhibited significant antiplasmodial activity, high selective indexes and low resistance indexes. Although the antiplasmodial activity of **11** was comparable to that of the isolated compounds, **11** was less cytotoxic and would have a larger safety margin. In light of the above, compounds **10** and **11** showed the greatest potential for further lead development. However, before this work is pursued, it will be necessary to determine the antiplasmodial activity of both the opposite enantiomer and racemic forms of totarol in order to establish the most active form. Nevertheless, the wide range of amines commercially and readily available presents opportunities for potential generation of diverse libraries of totarol derived β-amino alcohols. This work along with structure–activity studies initially focusing on compounds **10** and **11** is underway in our laboratories and will be reported in due course.

Experimental

General

The 1-phenylpiperazine and 1-(2-chlorophenyl)-piperazine monohydrochlorides were obtained from Lancaster, B&M Scientific, South Africa and the totarol was purchased from Sequoia Chemicals, UK. The remaining reagents were obtained from Sigma-Aldrich, South Africa and the common solvents were purchased from KIMIX, South Africa. Column chromatography was carried out on Merck Kieselgel silica gel 60 and preparative TLC was performed on silica 60 F_{254} coated plates (20 × 20 cm × 0.55 mm) (Merck). All reactions were monitored by TLC, which was carried out on silica 60 F_{254} coated aluminium sheets (Merck). Melting points were measured on a Reichert-Jung Thermovar hot stage microscope and are uncorrected. IR spectra were recorded using NaCl discs on a Thermo Mattson FTIR spectrometer in the range of 4000–500 cm^{-1}. Optical rotations were measured in $CHCl_3$ on an ADP220 Polarimeter. High-resolution mass spectra (HREIMS) were obtained on a VG-70-SEQ mass spectrometer operating at 70 eV. Low-resolution mass spectra of **12** and **13** were recorded on an AMD 604 spectrometer operating at 70 eV. ^1H, ^{13}C, HSQC, HMBC and COSY NMR spectra were recorded on Varian 300 MHz (VXR 300) or Varian 400 MHz (VXR 400) instruments with TMS as an internal reference. Chemical shifts are reported in parts per million (ppm) and coupling constants are given in hertz (Hz).

In vitro antiplasmodial activity

A chloroquine-sensitive strain (D10) of *P. falciparum* and a chloroquine resistant strain (K1) were continuously cultured according to the methods described by Trager and Jensen.[17] Parasite lactate dehydrogense (pLDH) activity was used to measure parasite viability and assays were performed as previously described.[6] No attempt was made to determine 50% inhibitory concentration (IC_{50}) values in excess of 100 μg/mL. Chloroquine diphosphate (CQ) was used as a positive standard for the pLDH assays.

In vitro cytotoxicity

Cytotoxicty assays were performed on Chinese Hamster Ovarian (CHO) cells using the MTT colorimetric assay[18] as reported previously.[6] Daunorubicin hydrochloride (DN) served as the positive control.

Preparation of the amines

Diethylamine, 1-phenylpiperazine, piperidine and morpholine were obtained from Sigma-Aldrich and were used without further purification. The amines 1-(2-ethoxyphenyl)-piperazine and 1-(2-chlorophenyl)-piperazine were obtained as amine hydrochloride salts and were neutralised using the polymer-supported tetraalkylammonium carbonate macroporous triethylammonium methylpolystyrene carbonate (MP-carbonate) (Argonaut Technologies). Neutralisation of 1-(2-chlorophenyl)-piperazine monohydrochloride (237.12 mg, 1.02 mmol) with MP-carbonate (1.55 mg, 4.08 mmol) in MeOH (3 mL) at 20 °C for 7 h yielded the free base. Reaction of 1-(2-ethoxyphenyl)-piperazine monohydrochloride (235.39 mg, 0.97 mmol) with MP-carbonate (1.47 mg, 3.88 mmol) in MeOH (3 mL) at 20 °C for 7 h gave the free base. The resins were removed by filtration, washed twice with MeOH and the resulting filtrate was concentrated under reduced pressure to yield the neutralised amines. The aminoquinoline-based piperazine was not available commercially and was synthesised according to the following procedure:[19] A mixture of piperazine (10.875 mg, 126.45 mmol), potassium carbonate (1.047 g, 7.58 mmol), triethylamine (5.28 mL, 37.88 mmol) and 4,7-dichloroquinoline (5.00 g, 25.25 mmol) was stirred in *N*-methyl-2-pyrrolidinone (17.7 mL) under nitrogen at 135 °C for 2 h and after cooling to room temperature was diluted with CH_2Cl_2 (200 mL). The reaction mixture was washed with brine (2 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The resulting oil was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (4:1) as the eluent and gave the amine (5.433 g, 87.5%). Mp 113–115 °C; ^1H NMR ($CDCl_3$) δ 8.72 (d, J = 5.0, H-2), δ 8.04 (d, J = 2.1, H-8), δ 7.96 (d, J = 9.0, H-5), δ 7.42 (dd, J = 9.0 and 2.1, H-6), δ 6.84 (d, J = 5.0, H-3), δ 3.18 (m, H-2′, H-3′, H-5′ and H-6′), δ 1.65 (s, H-4′); ^{13}C NMR ($CDCl_3$) δ 151.9 (C-2), 150.2 (C-4), 134.9 (C-7), 128.9 (C-8), 126.1 (C-6), 125.2 (C-5), 121.9 (C-4a), 108.9 (C-3), 53.6 (C-2′ and C-6′), 46.1 (C-3′ and C-5′).

6. Epichlorohydrin (1.88 μL, 2.4 mmol) was stirred in a solution of sodium hydride (60%, 77 mg, 1.92 mmol) and totarol (0.274 g, 0.96 mmol) in DMF (3 mL) at 0 °C under nitrogen. The suspension was allowed to warm to room temperature (20 °C), heated to 50 °C and stirred for 16 h. Excess epichlorohydrin was removed in vacuo and the resulting product was extracted into ethyl acetate, washed with water, dried ($MgSO_4$) and concentrated under reduced pressure. Purification of 18 mg of the product (254.3 mg, 77.6%) on preparative TLC using hex/EtO_2 (9.5:0.5), R_f (0.35) gave the epoxide. ^1H NMR ($CHCl_3$) δ 7.08 (d, J = 8.7, H-11), 6.68 (d, J = 8.7, H-12), 4.17 (dd, J = 3.5, 2.1, H-21a), 4.13 (dd, J = 3.5,

2.1, H-21b), 3.36 (m, H-22), 3.29 (s, $J=7.0$, H-15), 2.89, (dd, $J=5.0$, 4.4, H-23a), 2.75 (dd, $J=5.0$, 2.7, H-23b), 2.95 (dd, $J=16.7$, 5.9, H-7β), 2.82–2.70 (m, H-7α), 2.24 (d, $J=12.5$, H-1β), 1.92 (dd, $J=13.2$, 7.9, H-6α), 1.73 (dt, $J=13.5$, 3.4, H-2β), 1.72–1.59 (m, H-6β), 1.47 (dt, $J=13.5$, 3.2, H-2α), 1.47 (d, $J=13.1$, H-3β), 1.29–1.19 (m, H-1α, H-3α, H-5), 1.32 (d, $J=7.0$, H-17), 1.31 (d, $J=7.0$, H-16), 1.18 (s, H-20), 0.95 (H-18), 0.92 (H-19); ^{13}C NMR ($CDCl_3$) δ 155.1 (C-13), 143.7 (C-9), 133.9 (C-8), 133.6 (C-14), 122.8 (C-11), 110.2 (C-12), 68.7 (C-21), 50.4 (C-22), 49.5 (C-5), 44.8 (C-23), 41.6 (C-3), 39.6 (C-1), 37.7 (C10), 33.3 (C-4), 33.2 (C-18), 28.7 (C-7), 27.5 (C-15), 25.2 (C-20), 21.6 (C-19), 20.5 (C-17), 20.4 (C-16), 19.5 (C-2), 19.4 (C-6).

General procedures for the synthesis of compounds 7–13

The amine (1.05 equiv) was added to a stirred solution of the epoxide (1.0 equiv) in MeOH (5 mL for 0.10 mmol scale of epoxide). The resulting mixture was stirred for 16 h at 65 °C, concentrated under reduced pressure and purified using preparative TLC.

Compound 7. TLC (Et_2O, R_f 0.17). Mp 71–72 °C; +24.4° (*c* 4.50, $CHCl_3$); IR (NaCl) ν_{max} 3412 (OH), 2925, 1455, 1260, 1114, 754; ^{1}H NMR ($CHCl_3$) δ 7.07 (d, $J=8.8$, H-11), 6.71 (d, $J=8.8$, H-12), 4.05 (m, H-22), 4.01 (dd, $J=9.2$, 4.6, H-21a), 3.90 (dd, $J=9.2$, 5.8, H-21b), 3.39 (br m, H-15), 2.95 (dd, $J=16.8$, 6.6, H-7β), 2.78–2.69 (m, H-7α), 2.74–2.69 (m, H-25), 2.71–2.64 (m, H-23), 2.67–2.6 (m, H-27), 2.25 (br d, $J=12.8$, H-1β), 1.91 (dd, $J=13.5$, 8.0, H-6α), 1.78 (dt, $J=13.6$, 3.2, H-2β), 1.72–1.56 (m, H-6β), 1.46 (br d $J=12.9$, H-3β), 1.29–1.21 (m, H-5, H-3α, H-1α), 1.32 (d, $J=7.0$, H-17), 1.31 (d, $J=7.0$, H-16), 1.18 (s, H-20), 1.08 (t, $J=7.1$, H-28, H-26), 0.94 (s, H-18), 0.92 (s, H-19); ^{13}C NMR ($CHCl_3$) δ 155.2 (C-13), 143.3 (C-9), 133.7 (C-8), 133.1 (C-14), 122.8 (C-11), 109.8 (C-12), 70.1 (C-21), 65.9 (C-22), 56.8 (C-23), 49.5 (C-5), 47.4 (C-25, C-27), 41.6 (C-3), 39.6 (C-1), 37.7 (C-10), 33.3 (C-4), 33.2 (C-18), 28.7 (C-7), 27.5 (C-15), 25.2 (C-20), 21.9 (C-19), 20.6 (C-16, C-17), 19.5 (C-2), 19.4 (C-6), 11.6 (C-26, C-28); EIMS *m/z* (rel. int.) 415 [M^+] (9), 130 (8), 116 (4), 87 (23), 86 (100), 72 (4), 69 (6), 58 (7); HREIMS *m/z* 415.34612 (calcd for $C_{27}H_{45}NO_2$ 415.34503).

Compound 8. TLC [Et_2O/EtOAc (1:1), R_f 0.60]. Mp 86–87 °C; +20.7° (*c* 10.15, $CHCl_3$); IR (NaCl) ν_{max} 3400 (OH), 2924, 1575, 1456, 1260, 930, 872, 823, 736; ^{1}H NMR ($CHCl_3$) δ 8.72 (d, $J=5.0$, H-2′), 8.05 (d, $J=2.1$, H-8′), 7.95 (d, $J=8.9$, H-5′), 7.42 (dd, $J=8.9$, 2.1, H-6′) 7.10 (d, $J=8.9$, H-11), 6.85 (d, $J=5.0$, H-3′), 6.73 (d, $J=8.9$, H-12), 4.21 (m, H-22), 4.04 (dd, $J=9.5$, 5.1, H-21a), 3.97 (dd, $J=9.5$, 5.3, H-21b), 3.28 (m, H-26, H-28), 3.33–3.23 (m, H-15), 3.01–2.92 (m, H-7β, H-25), 2.82–2.71 (m, H-7α, H-23, H-27), 2.25 (d, $J=12.5$, H-1β), 1.92 (dd, $J=13.1$, 8.0, H-6α), 1.74 (dt $J=13.6$, 3.3, H-2β), 1.73–1.57 (m, H-6β), 1.43 (br d, $J=13.6$, H-3β), 1.35 (d, $J=6.9$, H-17), 1.34 (d, $J=6.9$, H-16), 1.29–1.21 (m, H-1α, H-3α, H-5), 1.19 (s, H-20), 0.95 (s, H-18), 0.92 (s, H-19); ^{13}C NMR ($CHCl_3$) δ 156.8 (C-8a′), 155.0 (C-13), 151.9 (C-2′), 150.2 (C-4′), 143.6 (C-9), 134.9 (C-7′), 134.0 (C-8), 133.2 (C-14), 128.9 (C-8′), 126.2 (C-6′), 125.1 (C-5′), 122.9 (C-11), 121.9 (C-4′a), 109.9 9C-12), 109.0 (C-3′), 70.1 (C-21), 66.1 (C-22), 61.3 (C-23), 53.4 (C-25, C-27), 52.3 (C-26, C-28), 49.6 (C-5), 41.6 (C-3), 39.7 (C-1), 37.7 (C-10), 33.4 (C-4), 33.3 (C-18), 28.8 (C-7), 27.5 (C-15), 25.2 (C-20), 21.6 (C-19), 20.8 (C-16, C-17), 19.6 (C-2), 19.5 (C-6); EIMS *m/z* (rel. int.) 589 [M^+] (9), 262 (32), 261 (17), 260 (100), 86 (5), 70 (9); HREIMS *m/z* 589.34432 (calcd for $C_{36}H_{48}N_3O_2Cl$ 589.34351).

Compound 9. TLC [Et_2O/Hex (1:1) R_f 0.29); mp 50–52 °C; +24.3° (*c* 10.29, $CHCl_3$); IR (NaCl) ν_{max} 3435 (OH), 2925, 1599, 1455, 1263, 928, 802, 738; ^{1}H NMR ($CHCl_3$) δ 7.28–7.24 (m, H-3′, H-5′), 7.01 (d, $J=8.8$, H-11), 6.92 (d, $J=8.6$, H2′, H-6′), 6.85 (2d, $J=7.3$, 0.7, H-4′), 6.71 (d, $J=8.8$, H-12), 4.16 (m, H-22) 4.01 (dd, $J=7.0$, 5.1, H-21a), 3.94 (dd, $J=7.0$, 5.2, H-21b), 3.23 (t, $J=3.3$, H-28), 3.29 (m, H-15), 3.21 (t, $J=3.3$, H-26), 2.94 (dd, $J=12.8$, 6.7, H-7β), 2.86–2.80 (m, H-25), 2.79–2.71 (m, H-7α), 2.66–2.64 (m, H-23, H-27), 2.24 (d, $J=12.6$, H-1β), 1.91 (dd, $J=9.9$, 7.9, H-6α), 1.76 (dt, $J=13.8$, 3.5, H-2β), 1.67–1.63 (m, H-6β), 1.58 (dt, $J=13.8$, 3.3, H-2α), 1.46 (d, $J=13.2$, H-3β), 1.32 (d, $J=7.0$, H-17), 1.31 (d, $J=7.0$, H-16), 1.28–1.19 (m, H-1α, H-3α, H-5), 1.17 (s, H-20), 0.94 (s, H-18), 0.91 (s, H-19); ^{13}C NMR ($CHCl_3$) δ 155.0 (C-13), 151.3 (C-1′), 143.6 (C-9), 134.0 (C-8), 133.3 (C-14), 129.2 (C-3′, C-5′), 122.9 (C-11), 116.3 (C-2′), 119.9 (C-4′), 116.3 (C-6′), 110.0 (C-12), 70.3 (C-21), 66.1 (C-22), 61.4 (C-23), 53.6 (C-25, C-27),49.7 (C-5), 49.4 (C-26, C-28), 41.7 (C-3), 39.8 (C-1), 37.8 (C-10), 33.4 (C-4), 33.3 (C-18), 28.9 (C-7), 27.6 (C-15), 25.3 (C-20), 21.7 (C-19), 20.8 (C-16, C-17), 19.6 (C-2), 19.5 (C-6); EIMS *m/z* (rel. int.) 504 [M^+] (8), 211 (5), 209 (15), 176 (17), 175 (100), 132 (5), 70 (9); HREIMS *m/z* 504.37256 (calcd for $C_{33}H_{48}N_2O_2$ 504.37158).

Compound 10. TLC [Et_2O/Hex (1:1), R_f 0.25]. Mp 55–56 °C; +21.8° (*c* 6.42, $CHCl_3$); IR (NaCl) ν_{max} 3434 (OH) 2941, 1588, 1479, 1263, 935, 802, 739; ^{1}H NMR ($CHCl_3$) δ 7.36 (dd, $J=8.0$, 1.5, H-3′), 7.21 (m, H-5′), 7.05 (dd, $J=8.1$, 1.5, H-6′), 7.09 (d, $J=8.9$, H-11), 6.98 (2dd, $J=7.7$, 1.5, H-4′), 6.73 (d, $J=8.9$, H-12), 4.18 (m, H-22), 4.03 (dd, $J=9.5$, 5.0, H-21a), 3.95 (dd, $J=9.5$, 5.3, H-21b), 3.30 (m, H-15), 3.11 (m, H-26, H-28), 2.96 (m, H-7β), 2.92–2.79 (m, H-25), 2.76–2.65 (m, H-7α, H-23, H-27), 2.25 (d, $J=12.4$, H-1β), 1.92 (dd, $J=13.2$, 8.0, H-6α), 1.78 (dt, $J=13.8$, 3.3, H-2β), 1.72–1.57 (m, H-6β), 1.47 (m, H-2α), 1.47 (d, $J=13.3$, H-3β), 1.34 (d, $J=6.8$, H-17) 1.32 (d, $J=6.8$, H-16), 1.30–1.21 (m, H-1α, H-3α, H-5), 1.19 (s, H-20), 0.95 (s, H-18), 0.92 (s, H-19); ^{13}C NMR ($CHCl_3$) δ 155 (C-13), 149.2 (C-1′), 143.4 (C-9), 133.9 (C-8), 133.2 (C-14), 130.7 (C-3′), 127.6 (C-5′), 128.8 (C-2′), 123.8 (C-4′), 122.8 (C-11), 120.4 (C-6′), 109.8 (C-12), 70.2 (C-21), 65.9 (C-22), 61.2 (C-23), 53.6 (C-25, C-27), 51.3 (C-26, C-28),49.6 (C-5), 41.6 (C-3), 39.7 (C-1), 33.3 (C-4), 33.2 (C-18), 28.8 (C-7), 27.5 (C-15), 25.2 (C-20), 21.6 (C-19), 20.7 (C-16, C-17), 19.5 (C-2), 19.4 (C-6); EIMS *m/z* (rel. int.) 538 [M^+] (4), 212 (6), 211 (41), 210 (18), 209 (100), 166 (5), 70 (13); HREIMS *m/z* 538.33254 (calcd for $C_{33}H_{47}N_2O_2Cl$ 538.33261).

Compound 11. TLC [Et_2O/Hex (1:1), R_f 0.39]; mp 50–51 °C; +21.1° (*c* 10.92, $CHCl_3$); IR (NaCl) ν_{max} 3434

(OH), 2941, 1588, 1479, 1263, 935, 802, 739; ^{1}H NMR ($CHCl_3$) δ 7.09 (d, J=8.8, H-11), 7.00–6.92 (m, H-3′), 6.97–6.92 (m, H-5′), 694–6.90 (m, H-4′), 6.85 (d, J=7.2, H-6′), 6.73 (d, J=8.8, H-12), 4.17 (m, H-22), 4.10 (q, 7.0, H-1″), 4.02 (dd, J=9.5, 5.0, H-21a), 3.94 (dd, J=9.5, 5.2, H-21b), 3.14 (m, H-26, H-28), 3.30 (m, H-15), 2.95 (dd, J=16.9, 6.1, H-7β), 2.91–2.78 (m, H-25), 2.85–2.73 (m, H-7α), 2.70–2.64 (H-23, H-27), 2.25 (d, J=13.0, H-1β), 1.91 (dd, J=13.5, 8.0, H-6α), 1.77–1.56 (m, H-2α, H-2β, H-3β, H-6β), 1.45 (t, J=7.0, H-2″), 1.34 (d, J=1.34, H-17), 1.32 (d, J=7.0, H-16), 1.29–1.21 (m, H-1α, H-3α, H-5), 1.18 (s, H-20), 0.95 (s, H-18), 0.92 (s, H-19); ^{13}C NMR ($CHCl_3$) δ 155.2 (C-13), 151.6 (C-2′), 141.3 (C-1′), 143.4 (C-9), 133.2 (C-8, C-14), 122.8 (C-3′, C-11), 121.0 (C-5′), 118.2 (C-4′), 112.6 (C-6′), 109.8 (C-12), 70.2 (C-21), 65.9 (C-22), 63.6 (c-1″), 61.4 (C-23), 53.7 (C-25, C-27), 50.6 (C-26, C-28), 49.5 (C-5), 41.6 (C-3), 39.7 (C-1), 37.7 (C-10), 33.3 (C-4), 33.2 (C-18), 28.8 (C-7), 27.5 (C-15), 25.2 (C-20), 21.6 (C-19), 20.7 (C-16, C-17), 19.5 (C-2), 19.4 (C-6), 14.9 (C-2″); EIMS m/z (rel. int.) 548 [M^+] (7), 220 (20), 219 (100), 204 (5), 70 (6); HREIMS m/z 548.39818 (calcd for $C_{35}H_{52}N_2O_3$ 548.39779).

Compound 12. Obtained as a white paste. TLC [Et_2O/Hex (1:9) R_f 0.10]; IR (NaCl) ν_{max} 3400 (OH), 2924, 1423, 1201, 872, 737; ^{1}H NMR ($CHCl_3$) δ 7.07 (d, J=9.0, H-11), 6.71 (d, J=9.0, H-12), 4. 10 (m, H-22), 4.01 (dd, J=9.3, 4.8, H-21a) 3.90 (dd, J=9.6, 5.7, H-21b), 3.27 (br m, H-15), 2.95 (dd, J=16.8, 6.6, H-7β), 2.82–2.68 (m, H-7α), 2.66–2.57 (m, H-25), 2.56–2.47 (m, H-23), 2.40 (m, H-29), 2.25 (br d, J=12.3, H-1β), 1.91 (dd, J=8. 1, 13.2, H-6α), 1.74 (dt, H-2β), 1.71–1.64 (m, H-6β), 1.63–1.56 (m, H-26, H-27, H-28), 1.45 (br d, J=12.9, 3α) 1.29–121 (m, H-5, H-3α, H-1α), 1.32 (d, J 6.9, H-17), 1.31 (d, J 6.9, H-16), 1.18 (s, H-20), 0.94 (s, H-18), 0.91 (s, H-19). ^{13}C NMR ($CHCl_3$) δ 155.1 (C-13), 143.2 (C-9), 133.6 (C-8), 133.2 (C-14), 122.8 (C-11), 109.8 (C-12), 70.3 (C-21), 65.7 (C-22), 65.6 (C-23), 54.8 (C-22, 29), 49.5 (C-5), 41.6 (C-3), 41.0 (C-26, C-28), 39.6 (C-1), 37.7 (C-10), 33.3 (C-4), 33.2 (C-18), 28.7 (C-7), 26.1 (C-27), 25.2 (C-20), 24.2 (C-27), 21.6 (C-19), 20.6 (C-16, C-17), 19.5 (C-2), 19.4 (C-6); EIMS m/z (rel. int.) 427 [M^+] (5) 175 (5), 149 (7), 98 (100), 86 (27), 55 (12), 43 (13).

Compound 13. Obtained as a cream paste. TLC [Et_2O/Hex (9:1), R_f 0.10]. IR (NaCl) ν_{max} 3434 (OH), 2942, 1588, 1480, 935, 801, 738; ^{1}H NMR ($CHCl_3$) δ 7.07 (d, J=9.0, H-11), 6.71 (d, J=9.0, H-12), 4.10 (m, H-22), 4.01 (dd, J=9.3, 4.8, H-21a) 3.90 (dd, J=9.6, 5.7, H-21b), 3.29 (br m, H-15), 2.95 (dd, J=16.8, 6.6, H-7β), 2.82–2.68 (m, H-7α), 2.66–2.67 (m, H-25), 2.56–2.47 (m, H-23), 2.52–2.42 (m, H-27), 2.25 (br d, J=12.3, H-1β), 1.91 (dd, J=8.1, 13.2, H-6α), 1.74 (dt, H-2β), 1.71–1.56 (m, H6β), 1.46 (br d, J=12.9, H-3β), 1.32 (d, J=6.9, H-17), 1.31 (d, J=6.9, H-16) 1.29–21 (m, H-5, H-3α, H-1α), 1.18 (s, H-20), 0.94 (s, H-18), 0.91 (s, H-19). ^{13}C NMR ($CHCl_3$) δ 155.1 (C-13), 143.5 (C-9), 133.8 (C-8), 133.2 (C-14), 122.6 (C-11), 109.8 (C-12), 70.1 (C-21), 67.0 (C-26, C-28), 65.8 (C-22), 61.76 (C-25, C-27), 53.9 (C-23), 49.5 (C-5), 41.6 (C-3), 39.6 (C-1), 37.7 (C-10), 33.3 (C-4, C-18), 28.7 (C-7), 27.4 (C- 5), 25.2 (C-20), 2 1.6 (C-19), 20.7 (C-16, C-17), 19.5 (C-2, 6); EIMS m/z (rel. int.) 429 [M^+] (5) 416 (3), 271 (5), 100 (100), 86 (84), 55 (8), 43 (10).

Totarol (3). Mp 126–127 °C; $[\alpha]_D^{23}$ (c 10.15, $CHCl_3$); IR (NaCl) ν_{max} 3435 (OH), 2943, 2867, 1636, 1456, 1271; ^{1}H and ^{13}C NMR spectral chemical shifts were similar to published data.[20]

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