Simplified tricyclic model of quassinoids with *in vitro* antiparasitic activity

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Dedicated to Dr. Manuel Gonzalez Sierra on the occasion of his 65th birthday

Abstract
This is a report of the synthesis of BCD ring partial analogs of quassinoids, and the evaluation of their biological activity so as to elucidate minimal functional requirements as potential antimalarial and leishmanicidal agents.

Keywords: Quassinoids, benzochromenes, acetals, antiparasitic activity

Introduction

Many infections are caused by protozoan parasites. Among them are trypanosomiasis, leishmaniasis and malaria.[1-4] Malaria is the world´s second major killer after tuberculosis. The most deadly of the four *Plasmodium* species that causes human malaria is the protozoan parasite *Plasmodium falciparum*.[5]

The development of new compounds for the treatment of these diseases is based on the available drugs which are few, inadequate in terms of efficiency, and often toxic. The synthesis of new chemotherapeutic agents requires a suitable selection of the molecular target that will be used in the compounds design.

Natural products have been and still are the main source of structurally diverse compounds as well as the main source of small molecule drugs.[6]

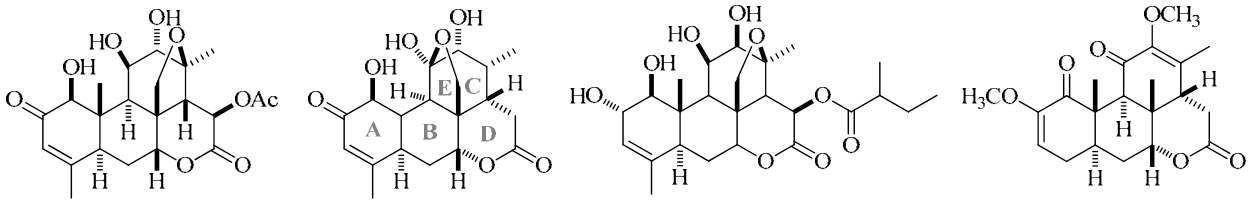
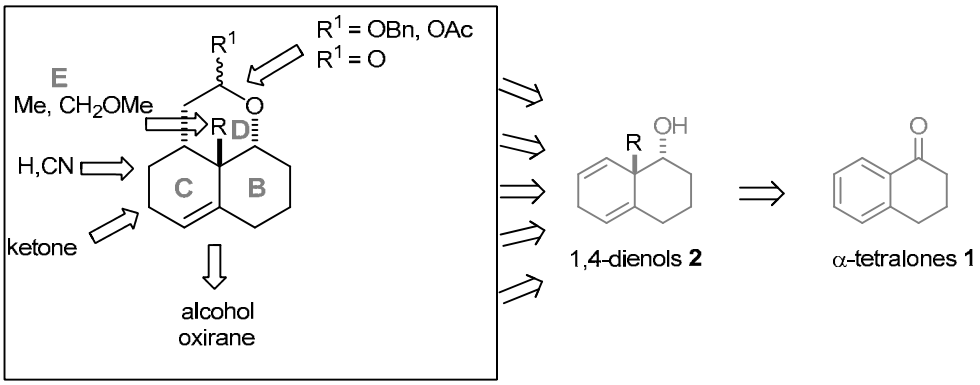


Figure 1

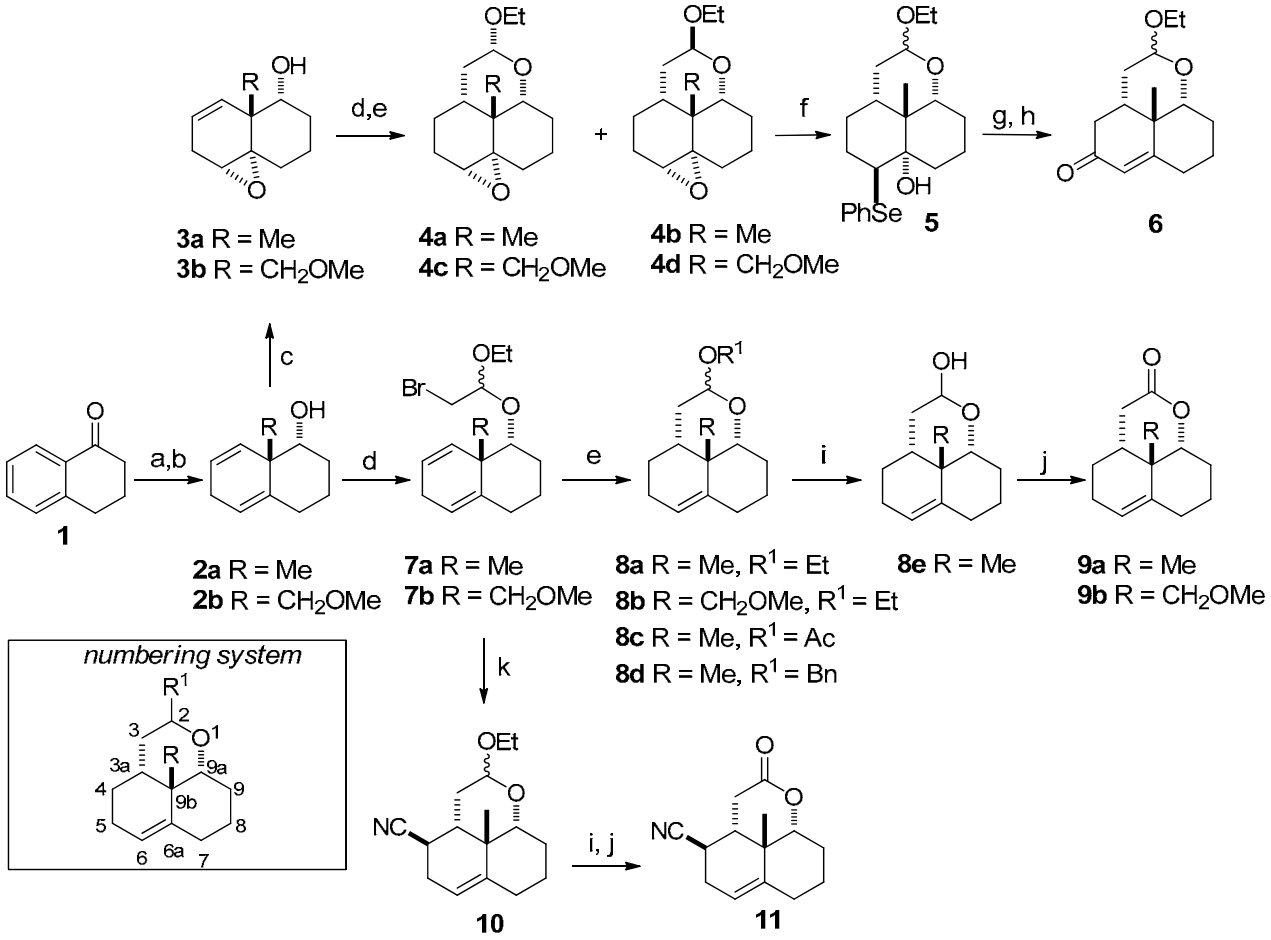
Quassinoids are complex natural triterpenes with a high degree of oxygenation. Their diverse biological activities, such as antitumoral, antifeedant, insecticidal, antimalarial, among others, motivated investigation related to their organic chemistry and pharmacology.[7] Many of these natural products, isolated from plants, such as chaparrinone,[8] dihydroxyeuricomanol,[9] samaderines X and Z, orinocinolide, excelsite, and quassin,[10-13] (Figure 1), have shown antimalarial activity. Owing to the wide spectrum of biological activities shown by this family of natural products, we postulate that their structure can be used as a privileged scaffold to prepare new bioactive compounds. Recently, we have been working on the search of new synthetic compounds with antiparasitic activity. Using 1,4-diene **2** as key intermediates in synthetic sequences,[14] we have been able to prepare different saudin model compounds and A/B rings of ouabain. Following the same strategy, we envisioned that the same intermediates could also be useful to prepare simplified quassinoid analogs. In particular, in this opportunity, we were able to apply our experience on 1,4-diene to obtain tricyclic acetals and lactones as mimetics of the B/C/D cyclic system of the natural product and study if that could fill the minimum structural features to produce activities in the antiparasitic assay. The proposed structures hold different positions where it should be possible to introduce diversity over rings C and D and also on the axial substituent of the ring fusion, Scheme 1. The products can be prepared following our reported procedure[14d] that has the 1,4-dienol **2** as key intermediate and that is the product of the reductive alkylation of α-tetralone, followed by the selective reduction of the ketone.



Scheme 1. Retrosynthetic analysis for the target compounds prepared.

Results and Discussion

As shown in Scheme 2, from α-tetralone, by means of our well-known methodology of alcohol-diene preparation by Birch alkylation reaction (NH_3, K, alkylating reagents methyl iodide, methoxymethyl iodide), we prepared two series of compounds, R = methyl and R = methoxymethyl.



Scheme 2. a: 1) Et_2O, ^{t}BuOH, $NH_{3(l)}$/K, -78 °C, 2) RX, -40 °C to r.t. b: 1M L-Selectride®, THF, -78 °C then 30% H_2O_2, 1M NaOH. c: *m*-CPBA, 0.5M $NaHCO_3$, CH_2Cl_2, 4 °C, overnight. d: $BrCH_2CH(OEt)Br$, $C_6H_5N(CH_3)_2$, CH_2Cl_2, r.t., overnight. e: $NaBH_3CN$, AIBN, Bu_3SnCl, ^{t}BuOH, reflux. f: $NaBH_4$, Ph_2Se_2, EtOH, reflux. g: 30% H_2O_2, $NaHCO_3$, THF, r.t. h: PCC, Al_2O_3, CH_2Cl_2, r.t. i: 6M HCl, THF/H_2O, 40 °C. j: Jones reagent, acetone; k: e + *t*BuNC.

The selective ketone reduction of **1** leads to the dienols **2a-b**. α-alcohols were obtained when L-Selectride® was used; while β-alcohols were generated with $NaBH_4$, MeOH, at -78 °C. These dienols were converted to the corresponding bromoacetals **7** which have been the starting materials for obtaining the tricyclic compounds **4** and **8** by radical cyclizations with $NaBH_3CN$,

AIBN, and Bu_3SnCl in *t*BuOH. The presence of the products **8** has demonstrated that the reactivity of the C_{3a}-C_4 double bond towards radical attack is directed by the stereochemistry of the alcohol. Therefore the reaction gives exclusively six membered rings *via* a regioselective 6-exo-trig closure.[14,15] This outcome was rationalized assuming that the conformer that leads to a chair-axial-chair transition state is responsible for the before mentioned stereocontrol.

We have been able to extend the cyclization reaction toward a cyanation of the incipient radical using *t*Bu-isocyanide as intermolecular radical trap. Thus, the reaction performed in a ratio of *t*BuNC : substrate **7a** of 10:1, led stereoselectively to the β-cyano **10**. In this case the cyclization followed the same ring closure stereochemical behavior, and the radical intermediate reacted with the isocyanide by the opposite face of the new formed bond.

Rearrangement of the epoxides **4**, obtained from **2** in three steps, was thought as method for introducing oxygen functionalities on C_5, C_6 and C_{6a}. The cleavage of **4** with $NaBH_4$, Ph_2Se_2, EtOH, reflux, then oxidation of the resultant selenide (30% H_2O_2, $NaHCO_3$, THF, r.t.) and further allyl rearrangement with PCC, Al_2O_3, CH_2Cl_2 at r.t., generated the unsaturated ketone **6**.

The conversions from acetals to lactones **10** → **11** and **8** → **9** have been accomplished employing the hydrolysis reaction with 6M HCl, THF/H_2O, 40 ^oC and further oxidation of corresponding acetals with Jones reagent in acetone.

Thus, functional groups transformations of 1,4-dienes **2** (B/C rings) gave products with B/C/D rings of quassinoids possessing substitutions on C_4 (R = H, CN), C_5 (C=O), C_6 (oxirane, double bond), C_{6a} (OH), C_{9b} (R = Me, CH_2OMe), acetals **4**, **5**, **6**, **8**, **10** (C_2 R = OEt, OAc, OBn) and lactones **9** and **11**, on the D-ring.

These prepared substructures of quassinoids were screened *in vitro* for their antimalarial and antileishmanial activities (Table 1).

As a result of this screening only the compound **8a** showed activity on the chloroquine sensitive *Plasmodium falciparum* strain (D6 clone) with an IC_{50} value of 550 ng/mL and 870 ng/mL on the chloroquine resistant (W2 clone). Under the same experimental conditions chloroquine exhibited an IC_{50} value lower than 9.7 and 148.5 ng/mL against D6 and W2 clones respectively.

The presence of an acetal group at C_2 and a methyl group at C_{9b} on D ring of **8a** (R = Me, R^1 = OEt) seems of pivotal importance, whereas a small structural variation of the acetal function **8c** (R^1 = Ac) or **8d** (R^1 = Bn) induced the total loss of antimalarial activity. Similarly, the relative lactone **9a** obtained from the oxidation of **8e** was equally ineffective against *Plasmodium falciparum* strains. Conversely **8d** (R^1 = Bn) that has proven to be ineffective against malaria, displayed activity against *L. donovani* with an IC_{50} value of 13 μg/mL. The incorporation of larger acetal substituents **8d** (R^1 = Bn) at C_2 had a better result than the acetyl group in compound **8c**. The lack of antileishmanial activity of product **8c** could be a consequence of the ester hydrolysis by an esterase producing the hemiketal **8e** that is inactive, but that hypothesis should be studied further.

Table 1. Antiparasitic activities of synthetic substructures of quassinoids

Compounds	P. falciparum (D6)		P. falciparum (W2)		L. donovani	Cytotoxicity (Vero cells)
	IC_{50} (ng/mL)	SI	IC_{50} (ng/mL)	SI	IC_{50} (μg/mL)	IC_{50} (ng/mL)
4a	NA		NA		NA	NC
4d	NA		NA		NA	NC
6	NA		NA		NA	NC
8a	550	≥ 8.6	870	> 5.5	NA	NC
8b	NA		NA		NA	NC
8c	NA		NA		NA	NC
8d	NA		NA		13.7+3.1*	NC
8e	NA		NA		NA	NC
9a	NA		NA		19.1+2.9*	NC
9b	NA		NA		NA	NC
11	NA		NA		NA	NC
Cloroquine	9.7		148.5		NT	NT
Artemisinin	5.3		4.9		NT	NT
Pentamidine	NA		NA		2.1+0.5*	NT
Amphoteric in B	NA		NA		0.34+0.04*	NT

NA: inactive, NC: no cytotoxicity, NT: not tested; SI- Selectivity index= IC_{50} against vero cells/IC_{50} against parasite; The highest concentration tested for antimalarial activity and vero cell cytotoxicity is 4760 ng/mL. The highest concentration tested against *L. donovani* promastigotes is 40 μg/mL. *Values are mean ± S.D. of three observations.

The comparison of the antiparasitic activity developed by **8a** with the inactive compound **8b** against *Plasmodium falciparum* strains, shows an interesting input on the requirement of the oxygen function on the E ring, Figure 1. The introduction of the methoxy methyl group at C_{9b} which mimics a tetrahydrofuran, suggested that E ring might not be needed for bioactivity of this core of quassinoids.

In general, the antimalarial activity cannot be ascribed to the C_5-carbonyl group of the α,β-unsaturated ketone **6** (C_5-C=O, R = Me) of the C-ring because it was an activity inhibitor structural feature. The structural modifications introduced at C_{6a}, C_6 (**4a**, **4d**), and at C_4 (**10**)

along with its corresponding lactone **11** showed no contribution for the activity against *Plasmodium falciparum* and *Leishmania donovani*. The transformation of **8a** into **9** gave a lactone group on D-ring, present in bruceantin[16] and its related compounds already described as antimalarials. **9a** was active for *L. donovani* with an IC_{50} of 19 μg/mL, but it was inactive for antimalarial activity on *Plasmodium falciparum.* All these synthetic compounds were non-cytotoxic to the Vero cells at the maximum concentration tested (4.75 μg/mL). This result is not negligible due to one of the main drawbacks of quassinoids is their cytotoxicity although they have high potency as antimalarial compounds.

Conclusions

We synthesized two series of compounds (R = Me, R = CH_2OMe) as substructures of quassinoids for the understanding of structure-antiparasitic activity relationships. This exploratory study allowed us to validate the method in order to discover the minimal tricyclic structure having bioactivity. In this way the above-mentioned compounds are the starting point to obtain new analogs for improving presented activities.

Experimental Section

General. All chemicals and solvents are commercially available and were used after distillation or treatment with drying agents. Melting points are uncorrected. ^{1}H (300 MHz) and ^{13}C NMR (75.13 MHz) spectra were recorded in $CDCl_3$ (with TMS for ^{1}H and chloroform-*d* for ^{13}C as the internal standard). Numbering of compounds is according to tricyclic structure as we have shown in Scheme 2.

Synthesis of 6-ethoxy-7b-methoxymethyl-decahydro-1,5-dioxacyclopropa[*c*]phenalene

The compounds **4 a-d** were obtained following the synthetic sequence of reactions previously reported as communication.[17]

(4c (α-acetal)). colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.43 (dd, *J* = 2.5 and 9.8 Hz, 1H, H-acetalic), 3.75 (br s, 1H, CH-O), 3.51 (q, *J* = 8.3 Hz, 1H, OCH_2CH_3), 3.48 (q, *J* = 8.7 Hz, 1H, OCH_2CH_3), 3.42 (m, 2H, CH_2OCH_3), 3.34 (s, 3H, OCH_3), 2.78 (d, *J* = 3.5 Hz, 1H, CH-epoxide), 2.58 (dt, *J* = 4.2, 13.0 Hz, 1H, H-9), 2.00-1.68 (m, 10H), 1.21 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3). ^{13}C NMR (75.13 MHz, $CDCl_3$) *δ* 97.4 (C2), 69.9 (C9a), 63.3 (C6a), 62.4 (9b-CH_2OCH_3), 56.5 (C6), 33.9 (C9b), 32.4 (C3), 30.9 (C9), 27.0 (C4), 22.6 (C7), 20.2 (C3a), 19.6 (C7), 18.8 (C8), 15.1 (OCH_2CH_3). IR (film, cm^{-1}) 2937, 1457, 1442, 1369, 1339, 1286, 1247, 1217, 1139, 1125, 1071, 1027, 993, 959, 876, 768. EM: (relative intensity) *m/z* 252 (14), 234 (M^+-H_2O, 5), 206 (16), 181 (50), 145 (34), 118 (48), 108 (100), 79 (83), 41 (79). EIRMS $C_{16}H_{26}O_4$ Calcd for 282.1831, Experimental for (M^+) *m/z* 282.1829.

4d. (β-acetal, less polar than **4c)**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.92 (dd, *J* = 4.2, and 1.1 Hz, 1H, H-acetalic), 3.74 (br s, 1H, CH-O), 3.65 (q, *J* = 8.3 Hz, 1H, OC*H*$_2$CH$_3$), 3.48 (dd, *J* = 6.5, and 3.8 Hz, 2H, C*H*$_2$OCH$_3$), 3.46 (q, *J* = 8.7 Hz, 1H, OC*H*$_2$CH$_3$), 3.34 (s, 3H, OCH$_3$), 2.79 (d, *J* = 3.9 Hz, 1H, CH-epoxide), 2.60 (dt, *J* = 4.1, 12.9 Hz, 1H, H-9), 2.02-1.63 (m, 10H), 1.21 (t, *J* = 7.1 Hz, 3H, OCH$_2$C*H*$_3$). ^{13}C NMR (75.13 MHz, $CDCl_3$) *δ* 97.1 (C2), 74.8 (C9a), 65.2 (C6a), 61.9 (9b-*C*H$_2$OCH$_3$), 58.8 (C6), 38.2 (C9b), 32.1 (C3), 30.8 (C9), 27.7 (C4), 27.0 (C7), 20.4 (C3a), 19.7 (C7), 18.2 (C8), 14.9 (OCH$_2$*C*H$_3$). EIRMS $C_{16}H_{26}O_4$ calc. for (M^+) *m/z* 282.1831, Experimental for (M^+) *m/z* 282.1833.

General procedure for bromoketal generation

1,2-dibromoethyl ether (1.1 eq) was added to a solution of the alcohol **2b** (1.1 eq) with *N*,*N*-dimethylaniline (1.5 eq) in dichloromethane (10 mL) at 0 °C. The solution was stirred at room temperature over 3 h and then 1,2-dibromoethyl ether (1.1 eq) and *N*,*N*-dimethylaniline (1.5 eq) were added. The mixture was stirred overnight and cold sat. $NaHCO_3$ (20 mL) was added. The reaction mixture was extracted three times with dichloromethane (3 × 15 mL), water (1 × 10 mL) and dried (Na_2SO_4).

A stirred solution of the dienone (1 mmol) in THF (5 mL/mmol) at -78 °C was treated with 2 mmol of a 1.0 M solution of L-selectride® in THF. After 1.5 h 2 mL/mmol of 1.0 M aqueous solution NaOH was added, followed by of 0.6 mL/mmol 30% H_2O_2. The heterogeneous solution was stirred for 2 h at 45 °C. The combined organic extract was washed with water (30 mL), dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. The crude residue was purified by column chromatography.

2-Ethoxy-9b-methyl-6-phenylselanyl-decahydro-benzo[de]chromen-6a-ol (5 α-acetal**).** Colorless crystals, 87%. M.p. = 155–156 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (m, 2H, Ph-Se), 7.23 (m, 3H, Ph-Se), 5.12 (s, 1H, OH), 4.97 (t, *J* = 4.4 Hz, 1H, H-2), 3.75 (br s, *J* = 2.2 Hz, 1H, H-9a), 3.60 (dq, *J* = 7.1 and 9.8 Hz, 1H, OC*H*$_2$CH$_3$), 3.45 (dq, *J* = 7.1 and 9.8 Hz, 1H, OC*H*$_2$CH$_3$), 3.20 (br s, 1H, H-6), 2.7-1.50 (complex signal, 13 H, H-9, H-8, H-7, H-5, H-4, H-3a, H-3), 1.22 (t, *J* = 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.15 (s, 3H, 9b-C*H*$_3$). ^{13}C NMR (75.13 MHz, $CDCl_3$) *δ* 133.3, 131.3, 128.8 and 126.5 (Ph-Se), 98.0 (C2), 77.8 (C6a), 73.2 (C9a), 63.6 (O*C*H$_2$CH$_3$), 52.7 (C6), 36.3 (C9b), 34.7 (C3), 33.5 (C3a), 32.7 (C9), 25.1(C8), 24.5 (C7), 22.7 (C9b-C*H*$_3$), 21.7 (C5), 17.2 (C4), 14.9 (OCH$_2$*C*H$_3$). IR (KBr, cm^{-1}) 3464, 3443, 2962, 1579, 1476, 1432, 1369, 1232, 1125, 1115, 1071, 1056, 1027, 978, 929, 856, 710, 690, 671. EM: (relative intensity) *m/z* 410 (M^+, 22), 207 (92), 189 (69), 147 (78), 145 (100), 118 (60), 105 (62), 92 (73), 91 (82). EIHRMS $C_{21}H_{30}O_3Se$ calc. for (M^+) *m/z* 410.1360. Experimental for (M^+) *m/z* 410.1354.

(5 β-acetal**)**: Oil, 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (m, 2H, Ph-Se), 7.24 (m, 3H, Ph-Se), 5.2 (s, 1H, OH), 4.55 (dd, *J* = 10.5 and 3.0 Hz, 1H, H-2), 3.90 (m, 1H, OC*H*$_2$CH$_3$), 3.55 (m, 1H, OC*H*$_2$CH$_3$), 3.30 (br s, 1H, H-9a), 3.20 (br s, 1H, H-6), 2.6-1.40 (complex signal, 13 H, H-9, H-8, H-7, H-5,H-4, H-3a, H-3), 1.23 (t, *J* = 7.1 Hz, 3H, OCH$_2$C*H*$_3$), 1.11 (s, 3H, 9b-C*H*$_3$). ^{13}C NMR (75.13 MHz, $CDCl_3$) *δ* 132.2, 128.8 and 126.7 (Ph-Se), 102.7 (C2), 76.6 (C6a), 82.1 (C9a), 64.1 (O*C*H$_2$CH$_3$), 52.8 (C6), 36.1 (C9b), 34.6 (C3), 38.9 (C3a), 25.4 (C8), 24.7 (C7), 24.5 (C9), 22.5

(C9b-C*H*$_3$), 22.1 (C5), 17.3 (C4), 15.0 (OCH$_2$*C*H$_3$). IR (KBr, cm^{-1}) 3442, 2986, 2938, 2882, 1576, 1450, 1388, 1185, 1140, 1078, 1016, 952, 890, 752, 702. EM: (relative intensity) *m/z* 410 (M$^+$, 8), 207 (76), 189 (44), 174 (28), 145 (100), 105 (16), 91 (14).

2-Ethoxy-9b-methyl-2,3,3a,7,8,9,9a,9b-octahydro-4H-benzo[de]chromen-5-one (6) (α-acetal). Colorless crystals, 83%. M.p. = 138–139 °C. ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.81 (br s, 1H, H-6), 4.50 (dd, *J* = 10.6 and 2.9 Hz, 1H, H-2), 3.90 (m, 1H, OC*H*$_2$CH$_3$), 3.47 (d, *J* = 3 Hz, 1H, H-9a), 3.45 (m, 1H, OC*H*$_2$CH$_3$), 2.6-1.40 (complex signal, 11 H, H-9, H-8, H-7, H-4, H-3a, H-3), 1.28 (s, 3H, 9b-C*H*$_3$), 1.20 (t, *J* = 7 Hz, 1H, OCH$_2$C*H*$_3$). ^{13}C NMR (75.13 MHz, CDCl$_3$) *δ* 197.7 (C5), 165.8 (C6a), 125.7 (C6), 101.0 (C2), 78.9 (C9a), 63.8 (O*C*H$_2$CH$_3$), 40.0 (C3a), 39.9 (C4), 38.3 (C9b), 33.8 (C7), 31.7 (C9), 26.9 (C8), 22.1 (s, 3H, 9b-C*H*$_3$), 19.8 (C-3), 15.0 (OCH$_2$*C*H$_3$). IR (KBr, cm^{-1}) 2915, 2840, 1720, 1610, 1450, 1430, 1370, 1270, 1290, 1250, 1230, 1170, 1030, 980, 905, 780. EM: (relative intensity) *m/z* 252 (M$^+$, 2), 205 (19), 158 (23), 145 (90), 134 (100), 122 (45), 107 (24), 91 (71), 79 (42), 61 (19), 55 (18). EIRMS C$_{15}$H$_{22}$O$_3$ calc. for (M$^+$) *m/z* 250.1569, Experimental for (M$^+$) *m/z* 250.1560.

6 (β-acetal). Colorless crystals, 78%. Mp = 145–146 °C. ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.83 (br s, 1H, H-6), 4.80 (d, *J* = 3.3 Hz, 1H, H-2), 3.81 (t, *J* = 3.3 Hz, 1H, H-9a), 3.67 (dq, *J* = 14.2 and 7.1 Hz, 1H, OC*H*$_2$CH$_3$), 3.44 (dq, *J* = 14.2 and 7.1 Hz, 1H, OC*H*$_2$CH$_3$), 2.6-1.40 (complex signal, 11 H, H-9, H-8, H-7, H-4, H-3a, H-3), 1.31 (s, 3H, 9b-C*H*$_3$), 1.22 (t, 3H, OCH$_2$C*H*$_3$). ^{13}C NMR (75.13 MHz, CDCl$_3$) *δ* 198.1 (C5), 165.9 (C6a), 125.6 (C6), 96.3 (C2), 34.7 (C3a), 70.7 (C9a), 62.4 (O*C*H$_2$CH$_3$), 26.9 (C3), 39.9 (C4), 38.4 (C9b), 34.7 (C9), 32.1 (C8), 31.8 (C7), 22.7 (s, 3H, 9b-C*H*$_3$), 15.0 (OCH$_2$*C*H$_3$). IR (KBr, cm^{-1}) 2940, 1670, 1425, 1370, 1340, 1240, 1135, 1060, 1030, 980, 950, 875. Anal. Calcd. for C$_{15}$H$_{22}$O$_3$: C,71.95; H, 8.86. Found: C, 71.50; H, 8.82.

4-(2-Bromo-1-ethoxyethyloxy)-4a–methoxymethyl-1,2,3,4,4a,7-hexahydronaphthalene (7b). Colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.76 (m, 2H, H-8, H-9), 5.60 (m, 1H, H-6), 4.69 (m, 1H, H-acetalic), 3.85 (m, 1H, H-1), 3.79 (m, 2H, OC*H*$_2$CH$_3$), 3.69 (m, 2H, C*H*$_2$OCH$_3$), 3.31 (s, 3H, OCH$_3$), 2.65 (br s, 2H, H-allylic), 2.3-1.7 (m, 6H), 1.19 (t, 3H, OCH$_2$C*H*$_3$). ^{13}C NMR (75.13 MHz, CDCl$_3$) *δ* 150.3 (C5), 133.0 (C8), 103.7 (C-acetal), 114.9 (C6), 72.3 (C1), 72.2 (*C*H$_2$OCH$_3$), 72.1 (CH$_2$Br), 54.8 (O*C*H$_2$CH$_3$), 54.7 (CH$_2$O*C*H$_3$), 44.5 (C10), 24.8 (C3), 35.6 (C4), 34.1 (C2), 30.6 (C7), 15.8 (OCH$_2$*C*H$_3$). IR (film, cm^{-1}) 2980, 1630, 1465, 1430, 1370, 1345, 1270, 1140, 1090, 1053, 995, 960, 880, 765. EIHRMS C$_{16}$H$_{25}$BrO$_3$ calc. for (M$^+$) *m/z* 344.0987. Experimental for (M$^+$) *m/z* 344.0989.

2-Ethoxy-9b-methyl-2,3,3a,4,5,7,8,9,9a,9b-decahydro-benzo[de]chromene (8a) (β-acetal). 67%. ^{1}H NMR (300 MHz, CDCl$_3$) *δ* 5.66 (dt, *J* = 11.0 and 3.1 Hz, 1H, H-5), 5.35 (br s, 1H, H-6), 4.86 (d, *J* = 3.4 Hz, 1H, H-2), 4.61 (s, 1H, OH), 3.70 (m, 1H, OC*H*$_2$CH$_3$), 3.68 (s, 1H, H-9a), 3.45 (m, 1H, OC*H*$_2$CH$_3$), 2.10-1.40 (complex signal, 11H, H-3, H-3a, H-4, H-7, H-8, H-9), 1.26 (t, *J* = 7.0 Hz, 1H, OCH$_2$C*H*$_3$), 1.14 (s, 3H, C9b-Me). ^{13}C NMR (75.13 MHz, CDCl$_3$) *δ* 96.9 (C2), 20.9 (C3),* 32.5 (C3a), 21.1 (C4),* 27.5 (C5),* 120.4 (C6), 136.6 (C6a), 30.5 (C7),* 31.7 (C8),* 22.5 (C9),* 70.8 (C9a), 36.5 (C9b), 15.1 (C9b-Me), 62.1 (OC*H*$_2$CH$_3$), 15.1 (OCH$_2$C*H*$_3$). *interchangeable carbon assignments. IR (film, cm^{-1}) 3045, 2895, 1455, 1435, 1370, 1325, 1215, 1170, 1115, 1045, 1010, 995, 870, 760. EM: (relative intensity) *m/z* 236 (M$^+$, 10), 191 (15), 164

(100), 146 (40), 131 (80), 118 (60), 105 (62), 91 (82), 77 (38), 41 (44). EIRMS $C_{15}H_{24}O_2$ calc. for (M^+) *m/z* 236.1776. Experimental for (M^+) *m/z* 236.1777.

2-Ethoxy-9b-methoxymethyl-2,3,3a,4,5,7,8,9,9a,9b-decahydro-benzo[de]chromene (8b). Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.36 (br s, 1H, H-6), 4.88 (d, J = 3.9 Hz, 1H, H-2), 4.00 br s, 1H, H-9a), 3.67 (m, 1H, OCH_2CH$_3$), 3.49 (s, 2H, CH_2OCH$_3$), 3.45 (m, 1H, OCH_2CH$_3$), 3.34 (s, 3H, CH$_2$OCH_3), 1.95-1.30 (m, 13H), 1.21 (t, J = 10.0 Hz, 3H, OCH$_2$CH_3). ^{13}C NMR (75.13 MHz, $CDCl_3$) δ 148.6 (C6a), 122.4 (C6), 97.1 (C2), 72.6 (C9a), 69.5 (*C*H$_2$OCH$_3$), 59.7 (O*C*H$_2$CH$_3$), 69.3 (OCH$_3$), 45.3 (C9b), 33.3 (C9), 33.2 (C3), 32.9 (C7), 27.8 (C4), 27.1 (C5), 26.1 (C3a), 24.5 (C8), 19.8 (OCH$_2$CH_3). IR (film, cm^{-1}) 3045, 2895, 1647, 1455, 1435, 1370, 1325, 1215, 1170, 1115, 1045, 1010, 995, 870, 760. EIRMS $C_{16}H_{26}O_3$ calc. for (M+) *m/z* 266.1882. Experimental for (M^+) *m/z* 266.1912.

Acetic acid 9b-methyl-2,3,3a,4,5,7,8,9,9a,9b-decahydrobenzo[*de*]chromen-2-yl Ester (8c). A solution of **8a** (1.8 mmol) in pyridine (5 mL) was cooled at 0 °C. Ac_2O (2.6 mmol) was added and dimethylaminopyridine (catalytic amount). After the reaction mixture was left for 24 h, it was then poured on 10% de HCl (25 mL/5mL Py). The mixture was extracted with Et_2O (5 × 15 mL), washed with water, and dried (Na_2SO_4), filtered and the solvent was evaporated. Colorless oil, 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.68 (dm, 1H, H-2), 5.36 (m, 1H, H-6), 3.47 (t, J = 2.7 Hz, 1H, H-9a), 2.09 (s, 3H, OC(O)CH_3), 2.05 (m, J = 3.3 Hz, 1H, H-7), 1.98 (m, J = 2.9 Hz, 1H, H-7), 1.93 (m, 2H, H-9), 1.82 (m, 2H, H-5), 1.71 (m, 1H, H-3a), 1.70 (m, 2H, H-3), 1.54 (m, 2H, H-4), 1.48 (m, 2H, H-8), 1.11 (9b-CH$_3$). ^{13}C NMR (75.13 MHz, $CDCl_3$) δ 169.6 (C=O), 138.2 (C6a), 120.9 (C6), 95.2 (C2), 80.4 (C9a), 38.2 (C-3a), 36.4 (C-9b), 31.7 (C-7), 31.3 (C-3), 27.6 (C-5), 24.4 (CH$_3$), 22.8 (C-4), 21.4 (C-9), 21.2 (C-8), 21.1 (OC(O)*C*H$_3$). IR (film, cm^{-1}) 3015, 2935, 2866, 2360, 1747, 1729, 1441, 1366, 1229, 1136, 1051, 1033, 911, 723. EIRMS $C_{15}H_{22}O_3$ calc. for (M^+) *m/z* 250.1569. Experimental for (M^+) *m/z* 250.1549.

2-Benzyloxy-9b-methyl-2,3,3a,4,5,7,8,9,9a,9b-decahydrobenzo[*de*]chromene (8d). A solution of the compound **8a** (6.6 mmol) in THF (11 mL) was added into a vessel with NaH (6.7 mmol) in THF (2mL). To this mixture was added BnBr (6.8 mmol). The mixture was stirred at room temperature for 48 h. Florisil® (46 mg) was incorporated to the mixture. The solvent was evaporated and the residue filtered through a sintered glass funnel with hexane. The organic layer was dried (Na_2SO_4), filtered and the solvent was evaporated to give a crude which was purified by column chromatography. Colorless oil, 96%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (dm, 2H, Ar-H), 7.33 (m, 1H, Ar-H), 7.27 (dm, 2H, Ar-H), 5.34 (m, 1H, H-6), 4.87 (d, J = 12.2 Hz, 1H, CH_2-Ar), 4.60 (d, J = 12.2 Hz, 1H, CH_2-Ar), 4.50 (dd, J = 9.3 and 2.1 Hz, 1H, H-2), 3.30 (br s, 1H, H-9a), 2.09-1.90 (m, 2H, H-9), 1.86-1.51 (m, 2H, H-8), 2.23-2.08 (m, 2H, H-5), 1.90 (m, 2H, H-4), 1.88 (m, 2H, H-7), 1.72-1.51 (m, 2H, H-3), 1.58 (m, 1H, H-3a), 1.09 (9b-CH$_3$). ^{13}C NMR (75.13 MHz, $CDCl_3$) δ 138.5 (C1-Ar), 129.0 (C3-Ar), 128.4 (C-2-Ar), 127.5 (C4-Ar), 138.3 (C-6a), 120.8 (C6), 100.7 (C2), 79.4 (C9a), 69.5 (CH$_2$Ar), 38.5 (C3a), 31.8 (C7), 32.6 (C3), 27.8 (C5), 24.5 CH$_3$), 23.2 (C4), 23.1 (C9), 21.3 (C8). IR (film, cm^{-1}) 3015, 2927, 2866, 2360, 1640, 1454, 1363, 1196, 1108, 1070, 980, 734, 697. EIRMS $C_{20}H_{26}O_2Na$ calc. for (M^+) *m/z* 321.1831. Experimental for (M^+) *m/z* 321.1844.

General procedure for the syntheses of compounds (9)

To a solution of the compound **8a** (50 mg) in THF/H_2O (3 mL, 2:1) 6M HCl (0.5 mL) was added. The mixture was maintained at 40 ^oC for 48 h. After this time, a sat. soln. of $NaHCO_3$ (10 mL) was added and the aqueous phase was extracted with Et_2O (3 × 5 mL), and the organic extract was dried (Na_2SO_4). The solvent was evaporated and the crude product was purified by column chromatography yielding the hemiketal **8e** (93%). Compound **9a** was directly obtained from the crude product by oxidation with Jones reagent in acetone or from the pure **8e**.

9b-Methyl-2,3,3a,4,5,7,8,9,9a,9b-decahydrobenzo[*de*]chromen-2-ol (8e). oil (93%). ^1H NMR (300 MHz, $CDCl_3$) δ 5.36 (br s, 1H, H-6), 5.33 (d, *J* = 2.7 Hz, 1H, H-2), 3.59 (m, 1H, H-9a), 1.98 (bs, 1H, HO), 1.97 (m, 4H, H-5, H-7), 1.70-1.64 (m, 5H), 1.46-1.34 (m, 4H), 1.26 (s, 3H, 9b-CH_3). ^{13}C NMR (75.13 MHz, $CDCl_3$) *δ* 147.0 (C6a), 122.7 (C6), 92.6 (CH-OH), 76.4 (C9a), 44.3 (C9b), 35.7 (C3), 35.5 (C7), 33.6 (C9), 32.8 (C4), 31.4 (C3a), 7.2 (C5), 24.0 (C8), 13.8 (CH_3).

9b-Methyl-3a,4,5,7,8,9,9a,9b-octahydro-3*H*-benzo[*de*]chromen-2-one (9a). Colorless crystals, 86%. M.p. 81.7-83 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 5.54 (br s, 1H, H-6), 4.26 (d, *J* = 2.7 Hz, 1H, H-9a), 2.62 (m, 2H, H-5), 2.58 (dd, *J* = 17.2 and 9.5 Hz, 1H, H-4), 2.33 (dd, *J* = 17.2 and 5.6 Hz, 1H, H-4), 2.12-1.89 (m, 9H), 1.24 (s, 3H, 9b-CH_3). ^{13}C NMR (75.13 MHz, $CDCl_3$) *δ* 173.2 (C=O), 136.5 (C6a), 123.3 (C6), 82.5 (C9a), 38.4 (C9b), 36.5 (C9), 36.3 (C3a), 32.5 (C3), 30.8 (C7), 26.5 (C5), 24.5 (CH_3), 20.4 (C4), 20.1 (C8). IR (KBr, cm^{-1}) 2910, 2830, 1715, 1425, 1350, 1310, 1240, 1160, 1120, 1095, 1075, 1040, 1010, 995, 975, 940, 850, 735, 700. EM: (relative intensity) *m/z* 206 (M^+, 12), 164 (58), 146 (88), 131 (100), 118 (69), 105 (73), 91 (96), 77 (59), 65 (27), 41 (54). EIRMS $C_{13}H_{18}O_2$ calc. for (M^+) *m/z* 206.1307. Experimental for (M^+) *m/z* 206.1306.

9b-Methoxymethyl-3a,4,5,7,8,9,9a,9b-octahydro-3*H*-benzo[*de*]chromen-2-one (9b). Oil, 67%. ^1H NMR (300 MHz, $CDCl_3$) δ 5.69 (br s, 1H, H-6), 4.61 (dd, *J* = 5.3 and 3.0 Hz, 1H, H-9a), 3.69 and 3.27 (d, *J* = 3.3 Hz, 2H, C*H*$_2$OCH$_3$), 3.32 (s, 3H, OCH_3), 2.70 (t, 2H, H-5), 2.63-1.40 (m, 11H). ^{13}C NMR (75.13 MHz, $CDCl_3$) *δ* 173.8 (C=O), 133.8 (C6a), 126.6 (C6), 78.0 (C9a), 75.0 (*C*H$_2$OCH$_3$), 59.2 (OCH_3), 41.7 (C9b), 33.5 (C3), 31.4 (C7), 31.1 (C3a), 30.1 (C9), 26.3 (C4), 20.7 (C5), 20.1 (C8). EIRMS $C_{14}H_{20}O_3$ calc. for (M^+) *m/z* 236.1412. Experimental for (M^+) *m/z* 236.1412.

Synthesis of 2-Ethoxy-9b-methyl-2,3,3a,4,5,7,8,9,9a,9b-decahydro-benzo[de]chromene-4-carbonitrile (10). To a degassed solution of the bromoketal **7a** (0.63 mmol) in *t*BuOH (17 mL), was added $NaCNBH_3$ (1.27 mmol), AIBN (0.06 mmol), and finally Bu_3SnCl (0.06 mmol). The mixture was refluxed over 10 h over argon and the *t*BuOH was evaporated. The residue was dissolved in dichloromethane, washed with brine (2 × 5 mL), dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography. Oil 87%. ^1H NMR (300 MHz, $CDCl_3$) δ 5.32 (br s, 1H, H-6), 4.88 (t, *J* = 2.7 Hz, 1H, H-2), 3.70 (m, 1H, H-9a), 3.41 (m, 1H, C*H*$_2$CH$_3$), 3.10 (m, 1H, C*H*$_2$CH$_3$), 2.65 (m, 1H, H-4), 2.40-2.13 (m, 6H), 1.90-1.61 (m, 5H), 1.22 (t, 3H, CH$_2$C*H*$_3$), 1.14 (s, 3H, 9b-CH_3). ^{13}C NMR (75.13 MHz, $CDCl_3$) *δ* 139.4 (C6a), 121.5

(C6), 118.9 (nitrile), 98.1 (C2), 70.0 (C9a), 62.3 (*C*H_2CH_3), 36.6 (C9b), 35.4 (C7), 31.3 (C9), 29.5 (C3), 27.1 (C3a), 25.3 (C4), 24.8 (C5), 24.6 (C8), 20.7 (CH_2*C*H_3), 14.9 (9b-CH_3).

Synthesis of 9b-Methyl-2-oxo-2,3,3a,4,5,7,8,9,9a,9b-decahydro-benzo[de]chromene-4-carbonitrile (11). Compound **10** was treated with the same reaction conditions for the conversion **8** →**9**, ketal hydrolysis with 6M HCl, THF/H_2O, 40 ^oC and subsequent Jones reagent in acetone. Oil 86%. ^{1}HNMR (300 MHz, $CDCl_3$) δ 5.52 (br s, 1H, H-6), 4.35 (br s, 1H, H-9a), 3.01 (dt, *J* = 7.8 and 3.3 Hz, 1H, H-4), 2.83 (dd, *J* = 17.0 and 10.4 Hz, 1H, H-3), 2.59 (dd, *J* = 17.0 and 4.2 Hz, 1H, H-3), 2.40-1.60 (m, 9H), 1.28 (s, 3H, 9b-CH_3). ^{13}CNMR (75.13 MHz, $CDCl_3$) *δ* 171.0 (C=O), 137.2 (C6a); 120.6 (C6), 120.4 (nitrile), 81.4 (C9a), 38.6 (C3a), 37.5 (C9b), 30.3 (C3), 29.0 (C5), 27.0 (C4), 25.9 (C8), 24.1 (C9), 23.9 (CH_3), 19.8 (C7). IR (film, cm^{-1}) 2950, 2840, 2256, 1735, 1635, 1420, 1350, 1240, 1160, 1100, 1040, 970, 735. EM: (relative intensity) *m/z* 232 (M+1, 100), 219 (21). EIRMS $C_{14}H_{18}O_2N$ calc. for $(M+H)^+$ *m/z* 232.1331. Experimental for $(M+H)^+$ *m/z* 232.1338.

In vitro antimalarial and antileishmanial assays

Antimalarial activity of the compounds was determined *in vitro* on chloroquine sensitive (D6, Sierra Leone) and resistant (W2, IndoChina) strains of *Plasmodium falciparum* . The 96 well microplate assay is based on evaluation of the effect of the compounds on growth of asynchronous cultures of *P. falciparum*, determined by the assay of parasite lactate dehydrogenase (pLDH) activity.[18] The appropriate dilutions of the compounds were prepared in DMSO or RPMI-1640 medium and added to the cultures of *P. falciparum* (2% hematocrit, 2% parasitemia) set up in clear flat bottomed 96 well plates. The plates were placed into the humidified chamber and flushed with a gas mixture of 90% N_2, 5% CO_2 & 5% O_2. The cultures were incubated at 37 °C for 48 h. Growth of the parasite in each well was determined by pLDH assay using Malstat® reagent. The medium and RBC controls were also set-up in each plates. The standard antimalarial agents, chloroquine and artemisinin, were used as the positive controls while DMSO was tested as the negative control. Antileishmanial activity of the compounds was tested in vitro on a culture of *Leishmania donovani* promastigotes (Strain S1). In a 96 well microplate assay the compounds with appropriate dilution were added to the leishmania promastigotes culture (2×10^6 cell/mL) to get the final concentrations of 40, 8 and 1.6 μg/mL. The plates were incubated at 26 °C for 72 h and growth of leishmania promastigotes was determined by Alamar blue assay.[19] Pentamidine and Amphotericin B were used as the standard antileishmanial agents. All the analogs were simultaneously tested for cytotoxicty on VERO (monkey kidney fibroblast) cells by Neutral Red assay.[20] IC_{50} value for each compound was computed from the growth inhibition curve.

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