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Bioorganic & Medicinal Chemistry 14 (2006) 1750–1760
Bioorganic & Medicinal Chemistry

# Biomimetic synthesis, antimicrobial, antileishmanial and antimalarial activities of euglobals and their analogues

Sandip B. Bharate,[a] Kamlesh K. Bhutani,[a] Shabana I. Khan,[b] Babu L. Tekwani,[b] Melissa R. Jacob,[b] Ikhlas A. Khan[b] and Inder Pal Singh[a,*]

[a]*Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER),*[†] *Sector-67, S.A.S. Nagar, Punjab 160062, India*

[b]*National Centre for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA*

Received 16 September 2005; revised 14 October 2005; accepted 15 October 2005
Available online 7 November 2005

**Abstract**—In the present communication, naturally occurring phloroglucinol–monoterpene adducts, euglobals G1–G4 (**3b/a** and **4a/b**) and 16 new analogues (**13a/b**–**18a/b** and **19**–**22**) were synthesized by biomimetic approach. These synthetic compounds differ from natural euglobals in the nature of monoterpene and acyl functionality. All of these compounds were evaluated for their antibacterial, antifungal, antileishmanial and antimalarial activities. Analogue **17b** possessed good antibacterial activity against methicillin-resistant *Staphylococcus aureus*, while analogues **19**–**22** possessed potent antifungal activity against *Candida glabrata* with $IC_{50}$s ranging from 1.5 to 2.5 μg/mL. Euglobals along with all synthesized analogues exhibited antileishmanial activity. Amongst these, euglobal G2 (**3a**), G3 (**4a**) and analogues **13a** and **14a** showed potent antileishmanial activity with $IC_{50}$s ranging from 2.8 to 3.9 μg/mL. Analogue **16a** possessed antimalarial activity against chloroquine sensitive D6 clone of *Plasmodium falciparum*. None of the compounds showed toxicity against mammalian kidney fibroblasts (vero cells) upto the concentration of 4.76 μg/ml.


## 1. Introduction

Parasitic diseases such as leishmaniasis, malaria and trypanosomiasis have a significant impact in developing countries and affect millions of people and are cause of high mortality rate. Leishmaniasis is a group of tropical diseases caused by a number of species of protozoan parasites belonging to the genus *Leishmania*. This ailment affects around 12 million people in 80 countries and it is estimated that there are about two to three million new cases each year. It is also considered that presently there exists a population of 350 million people under risk of infection.[1] Moreover, a person with HIV infection whose immune system is suppressed is at high risk of leishmaniasis. Visceral leishmaniasis is the most severe clinical form of the disease caused by parasite *Leishmania donovani* and it can be fatal when not treated; it is characterized by its effect on the internal organs, particularly the liver, the spleen and the bone marrow.[1]

Leishmaniasis remains a significant health issue in large part of the world due to the lack of effective and affordable drugs and increasing resistance against existing drugs. The available chemotherapeutic agents for treatment of leishmaniasis include antimony compounds (e.g., sodium stibogluconate and meglumine antimonite) and amphotericin B. However, the antimonials cause serious side effects that include pain at the site of injection, stiff joints, gastrointestinal problems, cardiotoxicity and, in some cases, hepatic and renal insufficiency. These drugs also require lengthy treatments and their cost is rather high. Amphotericin B is also associated with a number of side effects, including the alteration of the renal function in approximately 80% of treated individuals. Amongst the natural products, different classes of secondary metabolites have been reported to possess antileishmanial activity.[2] These include quinones, alkaloids (quinoline, isoquinoline, indole, and steroidal), terpenes and phenolics (chalcones, flavonoids and lignans). Although several phloroglucinols have been reported to possess antiparasitic activities such as

*Keywords*: Euglobals; Biomimetic synthesis; Antimicrobial; Antileshmanial; antimalarial.
* Corresponding author. Tel.: +91 172 2214682; fax: +91 172 2214692; e-mail: ipsingh@niper.ac.in
† NIPER communication number 363.

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doi:10.1016/j.bmc.2005.10.027

antimalarial robustadials,[3] to our knowledge none of the phloroglucinol derivatives have been evaluated for antileishmanial activity.

There are a number of antileishmanial drugs available but to date none of them has been demonstrated to be fully effective against *Leishmania* parasites. Therefore, there is an urgent need to search for new classes of antileishmanial agents that are fully effective against *Leishmania* parasites.[2]

Malaria is another most important parasitic disease in tropical areas. Four parasitic species of the genus *Plasmodium* infect human beings, but two cause majority of the infections. Nearly all malaria deaths and a large proportion of morbidity are caused by *Plasmodium falciparum.* More than a third of the world's population (about two billion people) lives in malaria-endemic areas. Majority of deaths caused by falciparum malaria occur in sub-Saharan Africa, primarily among children younger than 5 years and pregnant women living in remote rural areas with limited access to health services.[4] The estimates for annual malaria mortality range from 0.5 to 3.0 million people. This increase in mortality can be attributed to spread of *Plasmodium falciparum* that is resistant to antimalarial drugs. In last few decades, resistance to several antimalarials became widely disseminated, while the cost of effective treatment is prohibitive for the large majority of the populations in these areas. Thus, rapid development of resistance by *Plasmodium falciparum* to the conventional drugs like chloroquine necessitates search for new antimalarial drugs.[4] Similarly, increasing number of multidrug-resistant microbial pathogens have become a serious problem particularly during the last decade and provide impetus for the search and discovery of novel antibacterial and antifungal agents active against these pathogens.[5]

Naturally occurring phloroglucinol compounds possess a number of biological activities such as antibacterial, antimalarial, antiviral and antifouling, etc.[6] This class of compounds is of wide occurrence in myrtaceae family, particularly in *Eucalyptus* spp. and also in *Hypericum* spp. Phloroglucinol compounds of *Eucalyptus* spp. include monomeric compounds such as grandinol (**1**), jensenone (**2**), phloroglucinol-monoterpene adducts such as euglobals and phloroglucinol–sesquiterpene adducts such as macrocarpals. Grandinol and jensenone are proposed to be the key precursors in the biogenesis of many bioactive phloroglucinol molecules found in *Eucalyptus*.[6] These include Epstein–Barr virus inhibitory euglobals[7] (**3a**, **3b**, **4a**, **4b**), antimalarial robustadials[3] (**5a**, **5b**), antibacterial and anti-HIV macrocarpals[8,9] (**6**) and antifouling sideroxylonals (**7**) and grandinal[10] (**8**). Apart from antimalarial robustadials, a number of other acyl phloroglucinols (robustaol[3] (**9**), sarothralen[11] (**10**), japonicine[12] (**11**) and *O*-prenylated phloroglucinol derivative[13] (**12**)) have also been reported to possess antimalarial activity (Fig. 1).

Euglobals constitute an important group of bioactive phloroglucinol compounds. Structurally euglobals differ from each other in the nature of terpenoid moiety, with nine different terpenes implicated in the formation of about thirty naturally known euglobals.[14]

The euglobals G1 (**3b**), G2 (**3a**), G3 (**4a**) and G4 (**4b**) have a pinane skeleton as the terpenic component. Euglobals G1 and G2 show α-pinene fused to dihydropyran ring, while Euglobal G3 and G4 have β-pinene joined to pyran ring to form spiro compounds. These have been isolated from leaves of *Eucalyptus grandis* and have structural similarity with antimalarial robustadials. Euglobals G3 (**4a**) and G4 (**4b**) differ from robustadials (**5a** and **5b**) in the location of isobutyl chain, present either on aromatic ring or the pyran ring.

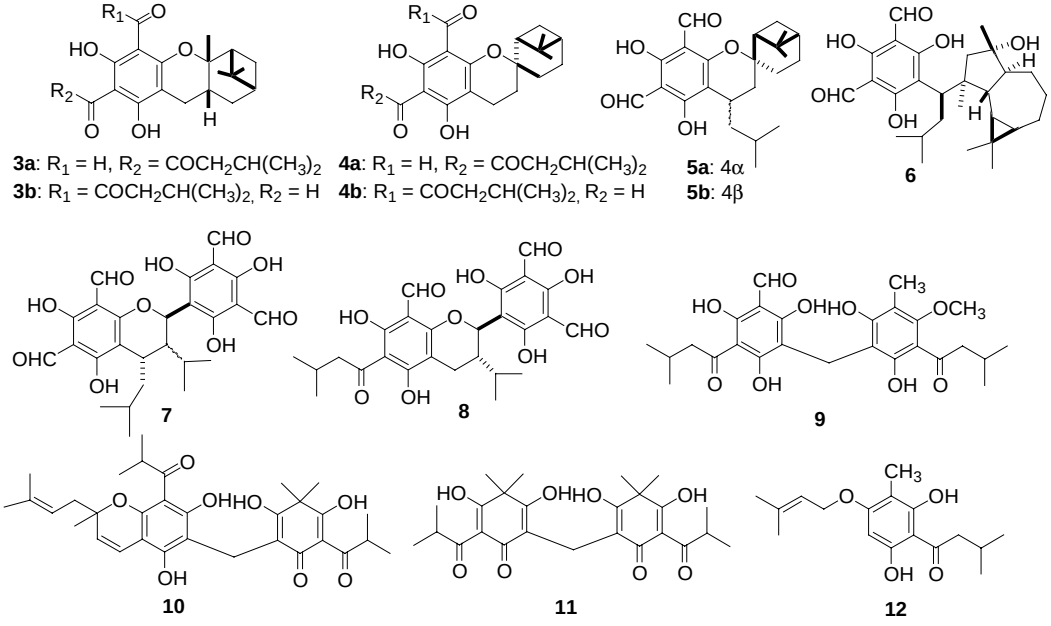



**Figure 1.** Bioactive phloroglucinol compounds.

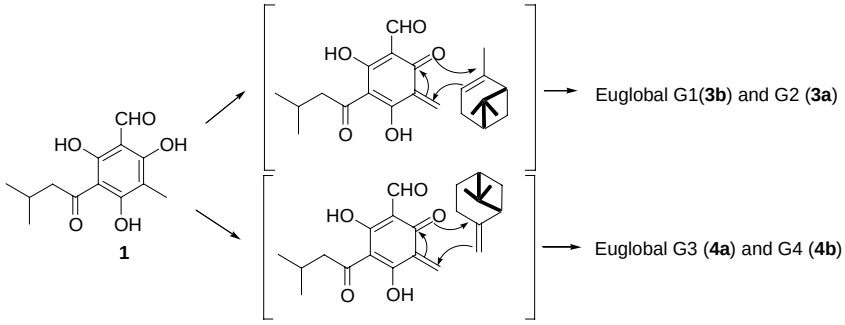



**Figure 2.** Proposed biogenesis of euglobals G1–G2 (**3b/a**) and G3–G4 (**4a/b**).

Biogenetically, the robustadials and euglobals have been proposed to be formed by Diels–Alder type cycloaddition of *O*-quinone methides derived from oxidation of phloroglucinol derivatives jensenone (**2**) or grandinol (**1**) with various mono- or sesqui-terpenes as depicted in Figure 2.[14]

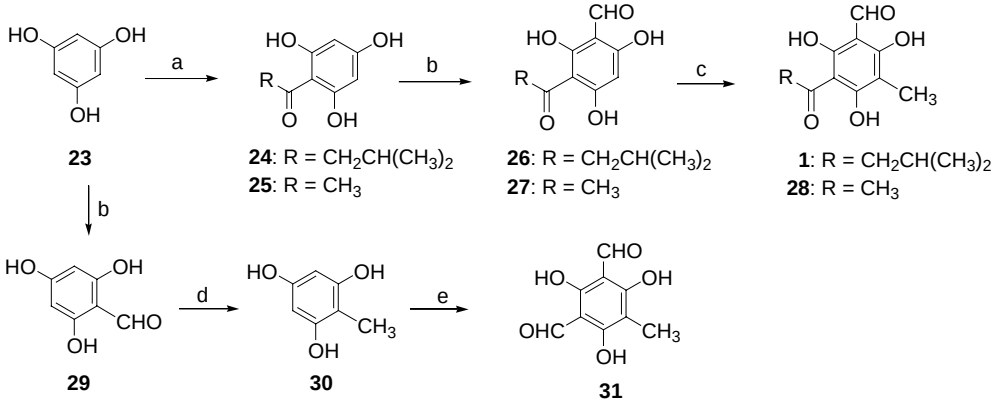
Based on the interesting biological activity profile of these phloroglucinol–terpene adducts, we planned to synthesize natural and unnatural euglobals by biomimetic approach. Some of the structural analogues of euglobals were designed and synthesized for evaluation as antimicrobial, antileishmanial and antimalarial agents. These included adducts with monoterpenes not encountered in natural euglobals.

## 2. Results and discussion

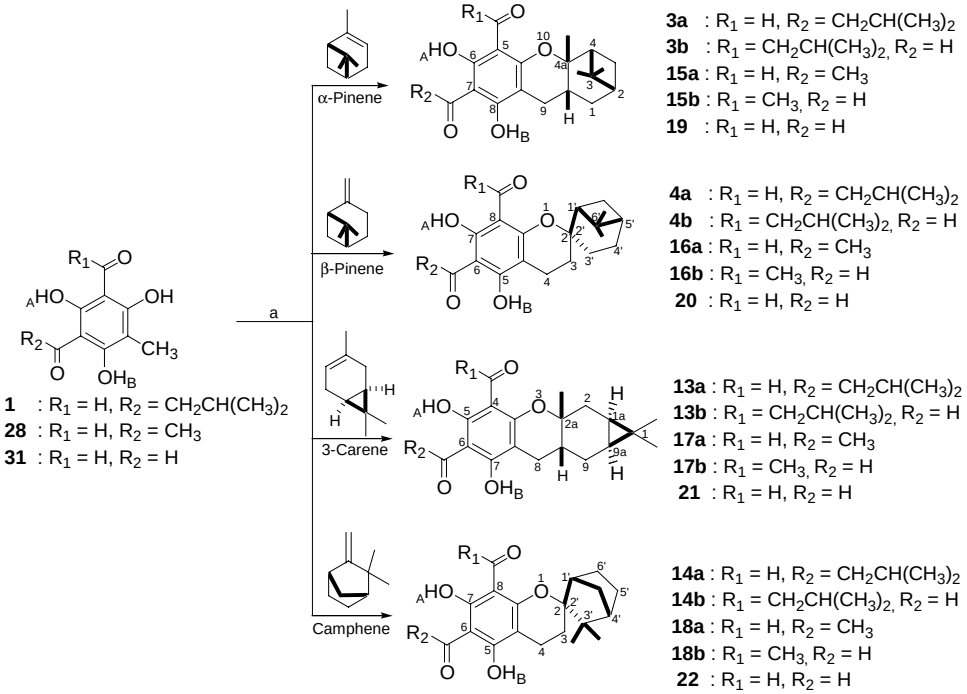
### 2.1. Synthesis of euglobals and their analogues

We have synthesized naturally occurring phloroglucinol–terpene adducts, euglobals G1–G4 (**3b/a** and **4a/b**) and analogues (**13a/b**–**18a/b** and **19**–**22**) by biomimetic approach in four steps starting from commercially available phloroglucinol (**23**). Our synthetic strategy involves initial synthesis of the key precursors required for synthesis of target phloroglucinol–terpene adducts. Synthesis of key precursors, **1**, **28**, and **31** is shown in Scheme 1. The Friedel–Crafts acylation of **23** in the presence of aluminium chloride resulted in formation of acyl phloroglucinols (**24** and **25**) in 70% yield. Acyl phloroglucinols (**24** and **25**) upon Vilsmeier–Haack formylation using phosphorus oxychloride and dimethylformamide resulted in the formation of formylated acyl phloroglucinols (**26** and **27**), which upon methylation with iodomethane in the presence of potassium hydroxide resulted in formation of C-methylated products, **1** and **28** (overall yield from **23**, 25%).[15,16]

In order to synthesize diformylated phloroglucinol–terpene adducts (**19**–**22**), the key precursor **31** was essential and has been synthesized from **23** in three steps. Formylation of **23** with Vilsmeier–Haack reagent resulted in the formation of **29**, which upon deoxygenation with sodium cyanoborohydride led to formation of 2,4,6-trihydroxytoluene, **30**. The conversion of **30** to **31** was found to be challenging since the introduction of one formyl group onto an aromatic ring deactivates the phloroglucinol nucleus for second formylation. A number of reactions and different experimental conditions ($POCl_3$/DMF, $CH(OC_2H_5)_3$/DCM) were tried to achieve diformylation of **30** to obtain **31** but in all cases monoformylated product was predominating. The most exciting and striking results were obtained with modified Duff reaction. Refluxing of **30** with two equivalents of hexamethylene tetramine in trifluoroacetic acid for 20 h at 70 °C resulted in exclusive formation of diformylated product **31** in 40% yield.[16]




**Scheme 1.** Reagents and conditions: (a) RCOCl, $AlCl_3$, 50 °C, 70%; (b) $POCl_3$, DMF, rt, 70%; (c) $CH_3I$, KOH, MeOH, 70 °C, 50%; (d) $NaBH_3CN$, THF, pH 4, rt, 15 h, 60%; (e) $(CH_2)_6N_4$, TFA, 70 °C, 43%.




**Scheme 2.** Reagents and conditions: (a) $PhNO_2$, DDQ, 50 °C, 1 h.

After synthesis of the precursors, the key step involved in the synthesis of phloroglucinol–terpene adducts is the oxidative activation of compounds, **1**, **28**, and **31** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in nitromethane leading to the generation of corresponding *O*-quinone methides which further undergo intermolecular cycloaddition with monoterpenes such as α-pinene, β-pinene, (+)-3-carene and (−)-camphene (Scheme 2). Several strategies have been employed to generate *O*-quinone methides for cycloaddition with unactivated alkenes. These include conventional[17] as well as electrochemical synthesis[18] performed on the surface of PTFE-fibre coated electrode and with the use of metallated 2-alkenyl sulfoximines.[19] We synthesized four natural euglobals and 16 new analogues using biomimetic approach and the structures were established by extensive spectral data viz., $^1$H NMR, MS and IR. The point of attachment of terpene moiety onto the pyran ring has been determined by the DEPT analysis and location of formyl and acyl functionalities was established by extensive 2D NMR spectral data, viz., HSQC and HMBC.

Diels–Alder reaction of **1** with β-pinene in the presence of DDQ in different solvents viz., dioxane, tetrahydrofuran, dimethyl formamide, dimethyl sulfoxide and methanol has been attempted, however, none of these yielded the expected products. Products were obtained only with nitromethane as a solvent. Reactions were also performed under microwave conditions. Reaction of **1** with β-pinene under microwave conditions (750 W, 1 min) using nitromethane as a solvent resulted in the formation of **4a** and **4b** in a yield of 15%. However, same reaction when performed using conventional heating, products **4a** and **4b** were formed in a yield of 30%.

Diels–Alder reaction of **1** and its acetyl analogue **28** with terpenes resulted in the formation of a pair of regioisomers which differ from each other in the location of formyl and acyl moieties on the aromatic ring. These regioisomers were separated by preparative TLC (hexane/EtOAc = 98: 2). The separated regioisomeric pairs, **13a/13b** and **14a/14b**, were characterized by 2D NMR experiments in which the position of isovaleryl and formyl group was confirmed by HMBC correlations.

When the separation was attempted on reverse-phase high performance liquid chromatography coupled with mass spectrometry (RPHPLC–MS), it has been noticed that, the isomer possessing acyl moiety β- to the pyran oxygen gets eluted earlier as compared to the other isomer. We also observed a typical pattern of chemical shift values for formyl group and the hydroxyl group between formyl and acyl in $^1$H NMR spectrum as shown in Table 1. $^1$H NMR spectra of euglobals (**3a**, **4a**) and analogues (**13a**–**18a**) in which formyl group is β- to the pyran oxygen showed peaks ranging from $\delta$ 14.30 to $\delta$ 14.47 for $OH_A$ and peaks ranging from $\delta$ 9.93 to $\delta$ 10.06 for formyl group, while regioisomers (**3b**, **4b**, **13b**–**18b**) of these, in which acyl functionality is at β-position to the pyran oxygen showed peaks ranging from $\delta$ 13.10 to 13.45 and $\delta$ 10.17 to 10.20 for $OH_A$ and formyl group, respectively. The chemical shifts for $OH_B$ are fairly identical in all the regioisomers ranging from $\delta$ 15.10 to 15.45. These observations can be generalized as this has been the case for almost 30 naturally occurring[14] as well as 16 synthetic compounds reported in this paper. This observation makes the structure elucidation of this class of compounds simpler without the need of 2D NMR experiments.

**Table 1.** Parameters to distinguish pair of regioisomers

| Entry | RP-LCMS $t_R$[a] | $^1$H NMR ($\delta$ values)[b] | | |
|---|---|---|---|---|
| | | CHO | $OH_A$ | $OH_B$ |
| **3a/3b** | 10.54/8.42 | 9.93/10.20 | 14.36/13.14 | 15.34/15.43 |
| **4a/4b** | 12.60/10.08 | 10.01/10.18 | 14.43/13.18 | 15.37/15.37 |
| **13a/13b** | 11.63/8.97 | 10.06/10.19 | 14.47/13.21 | 15.40/15.36 |
| **14a/14b** | 13.56/10.85 | 10.00/10.20 | 14.38/13.15 | 15.36/15.38 |
| **15a/15b** | 6.59/5.68 | 9.94/10.20 | 14.28/13.16 | 15.13/15.20 |
| **16a/16b** | 7.72/6.46 | 10.02/10.17 | 14.35/13.20 | 15.13/15.31 |
| **17a/17b** | 6.75/5.76 | 10.06/10.19 | 14.39/13.22 | 15.15/15.17 |
| **18a/18b** | 7.66/6.17 | 10.01/10.19 | 14.30/13.19 | 15.12/15.44 |

[a] Retention time in minutes.
[b] $^1$H NMR chemical shift values for formyl and hydroxyl groups ($OH_A$ and $OH_B$) on aromatic ring.

In general, the Diels–Alder reaction between unsymmetrical dienophile and unsymmetrical diene takes place in two ways to give two isomeric products. However, formation of one of the isomer, is strongly favoured depending upon the electronic nature of substituent. In case of [4 + 2] Diels–Alder reaction between *O*-quinone methide and 3-carene, presence of the methyl group onto the double bond results in the formation of isomer **13a-I** rather than **13a-II**. Similarly in case of Diels–Alder reaction of *O*-quinone methide with camphene, presence of the ring residues onto double bond favours the formation of 1,2-adduct (**14a-I**) rather than 1,3-adduct (**14a-II**).[20a]

The point of attachment of the terpene moiety onto the chroman ring has been identified on the basis of $^1$H NMR chemical shifts. In the case of Diels–Alder reaction of **1** with 3-carene, two possibilities arise with respect to orientation of terpene moiety. Structures **13a-I** and **13a-II** as shown in Figure 3 can be formed. In **13a-II**, triplet for 2a-H around $\delta$ 4.0 in the $^1$H NMR spectrum should have been present. Absence of any signal around $\delta$ 4.0 ruled out the possibility of **13a-II**. This was confirmed by DEPT experiments which showed C-2a as a singlet at $\delta$ 76.9 indicating quaternary nature of this oxycarbon. In **13a-II**, this oxycarbon should have been a doublet.

Similarly in the case of Diels–Alder reaction of **1** with camphene, the alternative structure **14a-II** has been ruled out based on $^1$H NMR data. In **14a-II**, singlet for methylenoxy protons around $\delta$ 4.0 in $^1$H NMR spectrum should have been present. Absence of any signal around $\delta$ 4.0 ruled out the possibility of **14a-II**. This was also confirmed by the DEPT experiment which showed C-2 as singlet indicating its quaternary nature. In **14a-II**, C-2 should have been a triplet (Fig. 4).

It has been reported that in Diels–Alder reaction, *cis*-product is the main product at moderate temperature, but the proportion of *trans*-product increases with rise in temperature.[20a] As we carried out Diels–Alder reactions at 50 °C, the *cis*-products are expected to be formed in Diels–Alder reactions of α-pinene and 3-carene. This is supported by our previous work[20b] as well as *cis* stereochemistry reported for all naturally occurring monoterpene adducts.[14c]

The essential oil of *Eucalyptus* is a rich source of monoterpenes which mainly include 1,8-cineole (50–90%), α-pinene (15-50%) and α-terpeneol (1–8%) in most of the species but the percentage of these varies from species to species. Apart from these major monoterpenes, eucalyptus oil also contains a number of other terpenoids such as limonene, 3-carene, camphene, and citronellal, citronellol in minor amounts. 3-Carene occurs in *Eucalyptus radiata* (2.7%), *Eucalyptus dives* (0.6%), *Eucalyptus bakeri* (0.32%), *Eucalyptus smithi* (0.2%), *Eucalyptus polybractea* (0.09%) and also in *Eucalyptus globulus.* Camphene occurs in *Eucalyptus gigantangion* (2.2%) and also in *Eucalyptus polybractea* (0.065%), *Eucalyptus smithi* (0.053%) and *Eucalyptus bakeri* (0.012%).[21] As euglobals are proposed to be formed biogenetically from Diels–Alder cycloaddition between

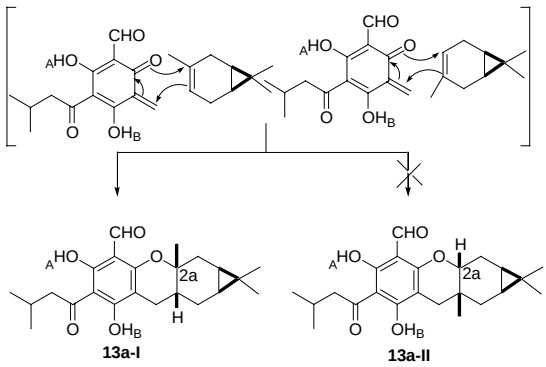



**Figure 3.** Diels–Alder cycloaddition of grandinol (**1**) with 3-carene.

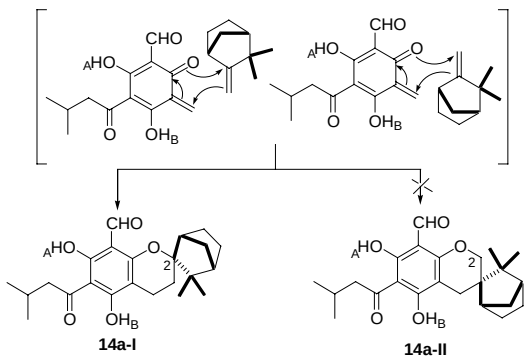



**Figure 4.** Diels–Alder cycloaddition of grandinol (**1**) with camphene.

*O*-quinone methides generated from phloroglucinol derivatives, grandinol or jensenone and monoterpenes, the possibility of **13a/b** and **14a/b** being discovered at some stage from *Eucalyptus* containing these terpenes (3-carene and camphene) in a major amount is not ruled out, and hence these may be hitherto undiscovered natural products. Compounds **13a/b** and **14a/b** are structurally related to natural euglobals, however these have new skeleton that have not been reported so far, so we propose to call these unnatural euglobals as *S*-euglobals (*S*-for synthetic).

### 2.2. Biological evaluation

Susceptibility of *S. aureus* and methicillin-resistant *S. aureus* to test compounds was determined according to the procedure as described by the National Committee for Clinical Laboratory Standards (NCCLS).[22] Susceptibility of *M. intracellulare* was done using the modified Alamar Blue procedure of Franzblau et al.[23] As shown in Table 2, euglobal G4 (**4b**) and analogues **14b** and **17b** exhibited good antibacterial activity against methicillin-resistant *S. aureus* with $IC_{50}$s of 10, 15 and 3 μg/mL, respectively, while none of the compounds possessed activity against *M. intracellulare* (data not shown).Ciprofloxacin was used as positive control for comparison.

The antifungal activities against pathogenic fungi associated with opportunistic infections (*Candida albicans*, *Candida glabrata, Candida krusei, Cryptococcus neoformans* and *Aspergillus fumigatus*) were also evaluated. Most of the compounds were active against *C. glabrata*, a few against *C. krusei*, while none showed activity against *C. albicans*, *C. neoformans* and *A. fumigatus*. Diformylated euglobal analogues, **19**–**22**, exhibited most potent antifungal activity against *C. glabrata* with $IC_{50}$s of 2.5, 1.5, 1.5 and 2.5 μg/mL, respectively, while amongst acyl-formyl euglobal analogues, only **16a** and **3a** exhibited antifungal activity against *C. glabrata* with $IC_{50}$s of 3.0 and 8.0 μg/ml, respectively. Analogues **20** and **21** also exhibited activity against *C. krusei* at much higher concentrations. Amphotericin B was included, as a standard drug, for comparison.

Antileishmanial activity against *L. donovani* promastigotes was determined by the Alamar Blue assay.[24] All the compounds exhibited antileishmanial activity with $IC_{50}$ and $IC_{90}$ values ranging from 3.6 to 24 μg/ml and 7.8 to 40 μg/ml, respectively, as shown in Table 3. Two important structure-activity correlations were observed from the results. The compounds possessing an isovaleryl functionality on the aromatic ring (**3a/b** and **4a/b** and **13a/b**–**14a/b**) showed activity with $IC_{50}$s ranging from 3.6 to 14 μg/mL compared to analogues with an acetyl functionality (**15a/b**–**18a/b**) which showed $IC_{50}$s ranging from 4.3 to 18 μg/mL. Another interesting difference in activity was noticed between the pair of regioisomers. The regioisomers in which formyl functionality is at β- to the pyran oxygen (**3a**–**4a** and **13a**–**18a**) showed better antileishmanial activity than those in which acyl functionality is at the β-position to pyran oxygen (**3b**–**4b** and **13b**–**18b**). Thus, compounds (**3a**, **4a**, **13a** and **14a**) comprising a combination of these two favorable structural requirements (presence of isovaleryl moiety and formyl β- to the pyran oxygen) are the most potent with $IC_{50}$s ranging from 2.8 to 3.9 μg/mL and $IC_{90}$s ranging from 7.8 to 24 μg/ml. Another important aspect is the position of double bond in monoterpene prior to cycloaddition. Terpenoids (α-pinene and 3-carene) containing

**Table 2.** In vitro antibacterial and antifungal activities of euglobals and their analogues

| Entry | $IC_{50}$[a,b] | | |
|---|---|---|---|
| | Antibacterial activity Methicillin-resistant *S. aureus* | Antifungal activity | |
| | | *C. glabrata* | *C. krusei* |
| **3a** | NA | 8 | NA |
| **4a** | NA | 16 | NA |
| **4b** | 10 | NA | NA |
| **13a** | NA | 20 | NA |
| **14b** | 15 | NA | NA |
| **15b** | NA | 10 | NA |
| **16a** | NA | 3 | NA |
| **17a** | NA | 15 | NA |
| **17b** | 3 | NA | NA |
| **18a** | NA | 3.5 | NA |
| **19** | NA | 2.5 | NA |
| **20** | NA | 1.5 | 15 |
| **21** | NA | 1.5 | 10 |
| **22** | NA | 2.5 | NA |
| Amphotericin B | — | 0.20 | 0.70 |
| Ciprofloxacin | 0.10 | — | — |

NA, not active.
[a] $IC_{50}$: The concentration that affords 50% inhibition of bacterial/fungal growth.
[b] Values expressed in terms of μg/mL.

**Table 3.** In vitro antileishmanial activities of euglobals and their analogues

| Entry | $IC_{50}$[a](μg/mL) | $IC_{90}$[a] (μg/mL) |
|---|---|---|
| **3a** | 3.6 | 24 |
| **3b** | 7.1 | 29 |
| **4a** | 3.9 | 24 |
| **4b** | 12 | 32 |
| **13a** | 2.8 | 7.8 |
| **13b** | 6.2 | 27 |
| **14a** | 3.6 | 15 |
| **14b** | 14 | 33 |
| **15a** | 9.4 | 30 |
| **15b** | 17 | 34 |
| **16a** | 16 | >40 |
| **16b** | 16 | 34 |
| **17a** | 9.5 | 30 |
| **17b** | 14 | 33 |
| **18a** | 4.3 | 20 |
| **18b** | 18 | 37 |
| **19** | 24 | >40 |
| **20** | 21 | 40 |
| **21** | 19 | 36 |
| **22** | 14 | 34 |
| Pentamidine | 0.9 | 3.0 |
| Amphoterecin B | 0.35 | 0.8 |

[a] $IC_{50}$ and $IC_{90}$, sample concentrations that kill 50% and 90% cells compared to solvent controls.

endocyclic double bond lead to the formation of adducts with xanthan skeleton (e.g., **3a/b**) in which terpenoid is attached in a linear fashion. Adducts formed by terpenes containing exocyclic double bond (β-pinene and camphene) contain spiroskeleton. Euglobals with xanthan skeleton **3a/b** showed a comparatively higher activity with spiro-euglobals **4a/b**. Phenolic compounds, such as chalcones, aurones and hydroxynaphthoquinones, are reported to affect ultrastructure and function of the parasite mitochondria, thus exerting their effect on parasite respiratory chain.[2b] Euglobals contain both phenolic and formyl groups and may be acting through a similar mechanism.

Since, phloroglucinol class of compounds have never been evaluated for their antileishmanial activity, interesting activity profile of natural, as well as synthetic phloroglucinol-monoterpene adducts, opens up a new class of antileishmanial compounds.

In vitro antimalarial activity was evaluated against chloroquine sensitive (D6) and chloroquine resistant (W2) clones of *P. falciparum.* Analogue **16a** exhibited antimalarial activity against D6 clone of *P. falciparum* with an $IC_{50}$ of 2.3 μg/mL. Rest of the analogues were inactive. Determination of in vitro antimalarial activity was based on the determination of plasmodial LDH activity.[25]

None of the compounds were found to have any cytotoxic effects towards mammalian kidney fibroblasts (vero cells) up to a concentration of 4.76 μg/ml (data not shown).[26,27]

More importantly, from this work euglobals have emerged as a promising new class of antileishmanial compounds and further work to synthesize and evaluate more compounds from this class using monocyclic and bicyclic monoterpenes is in progress.

## 3. Conclusion

In conclusion, euglobals G1–G4 (**3b/a**–**4a/b**), *S*-euglobals (**13a/b**–**14a/b**) and other structural analogues were synthesized in four steps from phloroglucinol. The *S*-euglobals were designed and synthesized so as to incorporate those bicyclic monoterpenes not encountered in natural euglobals. Also compounds were designed and synthesized by varying the acyl and formyl functionalities. Euglobals and their analogues possessed in vitro antileishmanial activity against *L. donovani* promastigotes. Amongst these, **3a**, **4a**, **13a** and **14a** showed the most potent activity. The presence of isovaleryl moiety and formyl functionality β- to the pyran oxygen seems to be necessary for potent activity, as analogues with acetyl functionality and acyl moiety β- to the pyran oxygen showed a comparatively weaker activity. Decrease in the acyl chain length from isovaleryl to acetyl to formyl resulted in decrease in antileishmanial activity. Adducts formed by Diels–Alder cycloaddition of *O*-quinone methide with monoterpenes containing an endocyclic double bond (α-pinene and 3-carene) showed better antileishmanial activity compared to adducts formed from monoterpenes with exocyclic double bond (β-pinene and camphene). Euglobal analogues bearing a diformyl functionality possessed potent antifungal activity, while other analogues bearing an acyl–formyl functionality possessed weaker or were devoid of antifungal activity, indicating the role of diformyl functionality for antifungal activity. Thus, it can be concluded that euglobal analogues bearing long chain acyl and formyl functionalities at δ and β- to the pyran oxygen, respectively, provide an interesting lead in our search for newer antileishmanial agents, while diformyl euglobal analogues are the promising leads for antifungal drug discovery.

## 4. Experimental

Melting points were recorded with a capillary melting point apparatus and are uncorrected. $^1$H NMR spectra are recorded on a 300 MHz Bruker FT-NMR (Avance DPX300) spectrometer using tetramethylsilane as internal standard and the chemical shifts are reported in $\delta$ units. Mass spectra were recorded on either GCMS (Shimadzu QP 5000 spectrometer) autosampler/direct injection (EI/CI) or LCMS (APCI/ ESI). Elemental analyses were recorded on Elementar Vario EL spectrometer. The HPLC analysis was carried out on Phenomenex C18 column (250 × 4.6 mm) connected to a Shimadzu (USA manufacturing Inc) HPLC system consisting of a model LC-10AT VP fitted with 20 μl injection loop and a model SPD-M10A VP photodiode array detector. The analysis was carried out using MeOH/water/acetic acid—100:5:3 as a mobile phase with the flow rate of 1.7 ml/min. Class-VP software (Shimadzu) was used for data collection. All chromatographic purifications were performed with silica gel (60–120 mesh), whereas all TLC (silica gel) development was performed on silica gel coated (Merck Kiesel $60F_{254}$, 0.2 mm thickness) sheets. All chemicals were purchased from Sigma–Aldrich, SD fine chemicals, Lancaster and CDH. Solvents used for the chemical synthesis purchased from commercial sources were of analytical grade and were used without further purification unless otherwise stated.

### 4.1. General method for Friedel–Crafts acylation: synthesis of 24–25

A solution of phloroglucinol (**23**, 1 mmol) and anhydrous aluminium chloride (3.17 g, 3 mmol) in carbon disulfide (10 mL) was stirred at room temperature for 20 min. Nitrobenzene (15 mL) was added and the temperature of the reaction mixture was allowed to increase up to 50 °C. Acyl chloride (3 mmol) was added and the reaction mixture was stirred further for 30 min. Nitrobenzene was removed from the reaction mixture under reduced pressure and crude reaction mixture was purified by silica gel column chromatography using hexane/EtOAc (50:50) as eluent to yield acyl phloroglucinols (**24**–**25**). Compounds were characterized by comparing their spectral data and melting point with literature values.[15]

### 4.2. General method for Vilsmeier–Haack formylation: synthesis of 29, 26 and 27

To the solution of phloroglucinol or acyl phloroglucinols (**23**–**25**) (1 mmol) in ethyl acetate (15 mL) were added dimethylformamide (1 mmol) and phosphoryl chloride (1.1 mmol) at room temperature. The reaction mixture was further stirred for 30 min. Water was added to the reaction mixture and extracted with ethyl acetate. Ethyl acetate layer was washed with brine solution and dried over $Na_2SO_4$ and concentrated to afford the crude product. Column chromatography over silica gel using hexane/EtOAc (70:30) as eluent provided formyl phloroglucinol (**29**) or formyl acyl phloroglucinols (**26**–**27**). Compounds were characterized by comparing their spectral data and melting point with literature values.[15,28]

### 4.3. General method for methylation: synthesis of 1 and 28

To the solution of formylated acyl phloroglucinols (**26** and **27**, 1 mmol) and potassium hydroxide (2 mmol) in methanol (10 mL) was added methyl iodide (5 mmol). The reaction mixture was refluxed for 2 h. Solvent was evaporated and the crude product was purified by silica gel column chromatography using hexane/EtOAc (80:20) as eluent to afford **1, 28**. Compounds were characterized by comparing their spectral data and melting point with literature values.[15]

### 4.4. General method for deoxygenation: synthesis of 30

To the solution of 1-formyl-2,4,6-trihydroxy benzene (**29**, 1 mmol) in tetrahydrofuran (20 mL) was added methyl orange (1–2 drops) followed by addition of sodium cyanoborohydride (3 mmol). The pH of the reaction mixture was maintained at 4.0 throughout the reaction by addition of 1 N HCl. The reaction mixture was stirred at room temperature for 12 h and then extracted with ethyl acetate. Ethyl acetate layer was washed with brine solution and finally dried over $Na_2SO_4$. Solvent was evaporated to get the crude product and the column chromatography over silica using hexane/EtOAc (50:50) as eluent afforded 2,4,6-trihydroxy toluene (**30**). The compound was characterized by comparing its spectral data and melting point with literature values.[29]

### 4.5. General method for Duff formylation: synthesis of 31

A mixture of 2,4,6-trihydroxy toluene (**30**, 1 mmol) and hexamethylene tetramine (2 mmol) in anhydrous trifluoroacetic acid (5 mL) was refluxed for 20 h. The reaction mixture was cooled to room temperature and then poured into 4 N HCl (10 mL) and stirred for 10 min. The product was extracted with ethyl acetate. The combined ethyl acetate layer was washed with 4 N HCl, brine solution and then dried over $Na_2SO_4$. The solvent evaporated under reduced pressure and the crude product was purified by silica gel column chromatography using hexane/EtOAc (90:10) as eluent to afford 3,5-diformyl-2,4,6-trihydroxy toluene (**31**).

**4.5.1. 3,5-Diformyl-2,4,6-trihydroxy toluene (31).** Yield: 43%; light yellow solid; mp 188–190 °C; $^1$H NMR (300 MHz, $CDCl_3$+$CD_3OD$): $\delta$ 10.09 (s, 2H), 2.01 (S, 3H); IR (KBr) $\nu_{max}$: 3119, 1622, 1257, 1182 cm$^{-1}$; CIMS: *m*/*z* 197 $[M+1]^+$.

### 4.6. General method for Diels–Alder cycloaddition: synthesis of 3a/b–4a/b, 13a/b–18a/b and 19–22

To the solution of **4**, **28** or **31** (1 mmol) and DDQ (1 mmol) in nitromethane (10 mL) was added monoterpene (1 mmol) and the reaction mixture was stirred at 50 °C for 2 h. The solvent was evaporated on rotavapor and the crude product was purified by silica gel (# 60–120) column chromatography to get a viscous liquid as a mixture of two isomers. These isomers were separated by preparative thin layer chromatography (hexane/EtOAc = 98:2) to afford **3a/b** and **4a/b** and **13a/b**–**18a/b**. In case of reaction of monoterpenes with **31**, a single product was obtained and was purified by column chromatography on silica gel using hexane/EtOAc (95:5) to afford **19**–**22**. Euglobals G1–G4 (**3b/a** and **4a/b**) were characterized by comparing their spectral data with literature values.[14]

**4.6.1. 5,7-Dihydroxy-1,1,2a-trimethyl-6-(3-methyl-butanoyl)-1,1a,2,2a,8,8a,9,9a-octahydro-3-oxa-cyclopropa[*b*]-anthracene-4-carbaldehyde (13a).** Yield: 19%; brownish yellow oil; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 15.40 (s, 1H, $OH_B$), 14.47 (s, 1H, $OH_A$), 10.06 (s, 1H, CHO), 2.99 (d, $J$ = 6.6 Hz, 2H), 2.58 (dd, $J$ = 5.8, 16.7 Hz, 1H), 2.39–2.24 (m, 3H), 2.16 (m, 1H), 2.04–1.88 (m, 1H), 1.76–1.48 (m, 3H), 1.41–1.28 (m, 2H), 1.26 (s, 3H), 1.21 and 1.00 (s, 3H each), 0.98 (d, $J$ = 7.7 Hz, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 206.4, 191.9, 172.4, 168.2, 162.4, 104.2, 103.6, 99.0, 76.9, 52.7, 32.2, 31.5, 29.7, 28.6, 25.1, 24.8, 23.2, 22.8, 19.1, 16.5; IR (neat) $\nu_{max}$: 3620, 3020, 2400, 1619, 1424, 1216, 1046 cm$^{-1}$; CIMS: *m*/*z* 387 $[M+1]^+$, 251 $[M-C_{10}H_{16}]$; analysis for $C_{23}H_{30}O_5$ (386.2), calcd: C, 71.48; H, 7.82; found: C, 71.37; H, 7.98.

**4.6.2. 5,7-Dihydroxy-1,1,2a-trimethyl-4-(3-methyl-butanoyl)-1,1a,2,2a,8,8a,9,9a-octahydro-3-oxa-cyclopropa[*b*]-anthracene-6-carbaldehyde (13b).** Yield: 17%; yellow sticky solid; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 15.36 (s, 1H, $OH_B$), 13.21 (s, 1H, $OH_A$), 10.19 (s, 1H, CHO), 2.97 (d, $J$ = 9.8 Hz, 2H), 2.63 (dd, $J$ = 5.8, 16.8 Hz, 1H), 2.42–2.20 (m, 4H), 2.01 (m, 1H), 1.78–1.68 (m, 2H), 1.57–1.53 (m, 1H), 1.44–1.25 (m, 2H), 1.26 (s, 3H), 1.24 and 1.02 (s, 3H each), 0.98 (d, $J$ = 7.0 Hz, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 206.0, 192.4, 169.9, 168.0, 163.5, 104.5, 98.0, 76.6, 53.1, 32.4, 31.0, 29.7, 28.6, 25.3, 24.8, 23.3, 23.0, 22.9, 22.7, 19.0, 16.7; IR (neat) $\nu_{max}$: 3401, 2926, 2856, 1613, 1447, 1419, 1313, 1192, 1156 cm$^{-1}$; CIMS: *m*/*z* 387 $[M+1]^+$, 251 $[M-C_{10}H_{16}]$; analysis for $C_{23}H_{30}O_5$ (386.2), calcd, C, 71.48; H, 7.82; found, C, 71.21; H, 7.55.

**4.6.3. 3,4-Dihydro-5,7-dihydroxy-3′,3′-dimethyl-6-(3-methyl-butanoyl)-spiro-[2*H*-1-benzopyran-2,2′-bicyclo-[2.2.1]heptane]-8-carboxaldehyde (14a).** Yield: 14%; yellow oil; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 15.36 (s,

1H, $OH_B$), 14.38 (s, 1H, $OH_A$), 10.00 (s, 1H, CHO), 2.97 (d, $J = 6.7$ Hz, 2H), 2.73–2.53 (m, 1H), 2.42–2.32 (m, 1H), 2.31–2.20 (m, 2H), 2.18–2.08 (m, 1H), 2.06–1.98 (m, 1H), 1.96–1.73 (m, 2H), 1.72–1.53 (m, 1H), 1.51–1.41 (m, 1H), 1.38–1.31 (m , 1H), 1.26 and 1.07 (s, 3H each), 0.98 (d, $J = 6.6$ Hz, 6H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$ 206.4, 191.6, 171.4, 168.2, 162.1, 104.5, 103.3, 101.5, 90.1, 52.7, 49.4, 45.1, 34.4, 29.7, 25.2, 25.1, 23.7, 22.8, 22.5, 22.3, 16.8; IR (neat) $\nu_{max}$: 3373, 2928, 1621, 1425, 1293, 1216, 1178, 1124, 1038 $cm^{-1}$; CIMS: $m/z$ 387 $[M+1]^+$, 251 $[M-C_{10}H_{16}]$; analysis for $C_{23}H_{30}O_5$ (386.2) calcd: C, 71.48; H, 7.82; found: C, 71.21; H, 7.62.

**4.6.4. 3,4-Dihydro-5,7-dihydroxy-3′,3′-dimethyl-8-(3-methyl-butanoyl)-spiro-[2*H*-1-benzopyran-2,2′-bicyclo[2.2.1]heptane]-6-carboxaldehyde (14b).** Yield: 13%; yellow oil; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 15.38 (s, 1H, $OH_B$), 13.15 (s, 1H, $OH_A$), 10.20 (s, 1H, CHO), 3.06–2.95 (m, 2H), 2.70–2.58 (m, 1H), 2.53–2.38 (m, 1H), 2.40–2.22 (m, 2H), 2.12–1.93 (m, 2H), 1.92–1.72 (m, 3H), 1.70–1.58 (m, 2H), 1.55–1.45 (m, 1H), 1.44–1.36 (m, 1H), 1.26 (s, 3H), 1.06 (s, 3H), 0.98 (d, $J = 4.3$ Hz, 6H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$ 205.4, 192.5, 169.7, 166.8, 164.0, 105.0, 104.3, 101.2, 93.0, 52.9, 50.1, 46.3, 35.0, 29.7, 27.2, 24.4, 23.5, 23.4, 22.8, 22.6, 22.5, 16.5; IR (neat) $\nu_{max}$: 3620, 3026, 2400, 1602, 1522, 1476, 1423, 1323, 1214, 1046 $cm^{-1}$; CIMS: $m/z$ 387 $[M+1]^+$, 251 $[M-C_{10}H_{16}]$; analysis for $C_{23}H_{30}O_5$ (386.2) calcd: C, 71.48; H, 7.82; found: C, 71.10; H, 7.52.

**4.6.5. 7-Acetyl-2,3,4,4a,9,9a-hexahydro-6,8-dihydroxy-3,3,4a-trimethyl-2,4-methano-1*H*-xanthene-5-carboxaldehyde (15a).** Yield: 14%; reddish brown viscous oil; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 15.13 (s, 1H, $OH_B$), 14.28 (s, 1H, $OH_A$), 9.94 (s, 1H, CHO), 2.71 (s, 3H), 2.72–2.68 (m, 2H), 2.48–2.41 (dd, $J = 6.5$, 15.9 Hz, 1H), 2.25 (t, $J = 5.4$ Hz, 1H), 2.19–2.06 (m, 2H), 1.98 (m, 1H), 1.90 (m, 1H), 1.47 (s, 3H), 1.32–1.22 (m, 1H), 1.31 and 1.10 (s, 3H each); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 203.9, 191.7, 170.9, 168.4, 164.6, 103.8, 103.6, 100.8, 87.7, 54.9, 44.2, 40.6, 34.5, 32.7, 32.0, 28.8, 28.2, 27.9, 22.7, 19.7; IR (neat) $\nu_{max}$: 3401, 2922, 2849, 1625, 1449, 1377, 1299, 1205, 1119, 1020 $cm^{-1}$; CIMS: $m/z$ 345 $[M+1]^+$, 209 $[M-C_{10}H_{16}]$; analysis for $C_{20}H_{24}O_5$ (344.2) calcd: C, 69.75; H, 7.02; found: C, 69.24; H, 7.42.

**4.6.6. 5-Acetyl-2,3,4,4a,9,9a-hexahydro-6,8-dihydroxy-3,3,4a-trimethyl-2,4-methano-1*H*-xanthene-7-carboxaldehyde (15b).** Yield: 12%; yellow sticky solid; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 15.20 (s, 1H, $OH_B$), 13.16 (s, 1H, $OH_A$), 10.20 (s, 1H, CHO), 2.80–2.60 (m, 2H), 2.56 (s, 3H), 2.40 (dd, $J = 5.9$, 15.3 Hz, 1H), 2.29 (t, $J = 5.4$ Hz, 1H), 2.15 (m, 2H), 2.02 (m, 1H), 1.90 (m, 1H), 1.51 (s, 3H), 1.32–1.24 (m, 1H), 1.31 and 1.10 (s, 3H each); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 203.4, 192.3, 170.1, 167.2, 166.3, 104.5, 103.9, 100.6, 89.2, 55.3, 44.1, 40.3, 33.8, 32.2, 31.8, 29.2, 28.1, 27.5, 22.7, 19.8; IR (neat) $\nu_{max}$: 3402, 2924, 2849, 2325, 1628, 1559, 1447, 1252, 1049, 1021 $cm^{-1}$; CIMS: $m/z$ 345 $[M+1]^+$, 209 $[M-C_{10}H_{16}]$; analysis for $C_{20}H_{24}O_5$ (344.2) calcd: C, 69.75; H, 7.02; found: C, 69.51; H, 6.89.

**4.6.7. 6-Acetyl-3,4-dihydro-5,7-dihydroxy-6′,6′-dimethyl-spiro-[2*H*-1-benzopyran-2,2′-bicyclo[3.1.1]-heptane]-8-carboxaldehyde (16a).** Yield: 12%; brown solid; mp 107–109 °C; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 15.13 (s, 1H, $OH_B$), 14.35 (s, 1H, $OH_A$), 10.02 (s, 1H, CHO), 2.70 (s, 3H), 2.55 (t, $J = 6.5$ Hz, 2H), 2.26 (m, 1H), 2.16 (t, $J = 4.5$ Hz, 1H), 2.04–1.95 (m, 5H), 1.91–1.81 (m, 2H), 1.62 (d, $J = 10$ Hz, 1H), 1.29 and 1.02 (s, 3H each); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 203.8, 191.7, 170.8, 168.4, 162.1, 104.3, 103.3, 101.0, 85.0, 49.6, 40.6, 38.2, 32.6, 31.8, 28.5, 27.5, 26.5, 24.7, 23.2, 15.3; IR (KBr) $\nu_{max}$: 3568, 2923, 1621, 1444, 1295, 1203, 1105 $cm^{-1}$; CIMS: $m/z$ 345 $[M+1]^+$, 209 $[M-C_{10}H_{16}]$; analysis for $C_{20}H_{24}O_5$ (344.2) calcd: C, 69.75; H, 7.02; found; C, 69.40; H, 6.92.

**4.6.8. 8-Acetyl-3,4-dihydro-5,7-dihydroxy-6′,6′-dimethyl-spiro-[2*H*-1-benzopyran-2,2′-bicyclo[3.1.1]heptane]-6-carboxaldehyde (16b).** Yield: 10%; cream colored solid; mp 137–140 °C; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 15.31 (s, 1H, $OH_B$), 13.20 (s, 1H, $OH_A$), 10.17 (s, 1H, CHO), 2.65 (s, 3H), 2.57 (m, 2H), 2.28 (m, 1H), 2.19 (t, $J = 4.7$ Hz, 1H), 2.06–1.88 (m, 7H), 1.57 (d, $J = 6.6$ Hz, 1H), 1.29 and 1.03 (s, 3H each); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 203.3, 192.4, 170.0, 167.2, 163.5, 104.7, 104.1, 100.3, 86.8, 48.9, 40.4, 38.3, 33.0, 31.3, 28.9, 27.5, 27.0, 24.7, 23.3, 15.2; IR (KBr) $\nu_{max}$: 3568, 2915, 2869, 1630, 1425, 1320, 1172, 1146 $cm^{-1}$; CIMS: $m/z$ 345 $[M+1]^+$, 209 $[M-C_{10}H_{16}]$; analysis for $C_{20}H_{24}O_5$ (344.2) calcd: C, 69.75; H, 7.02; found: C, 68.87; H, 6.74.

**4.6.9. 6-Acetyl-5,7-dihydroxy-1,1,2a-trimethyl-1,1a,2,2a,8,8a,9,9a-octahydro-3-oxa-cyclopropa[*b*]anthracene-4-carbaldehyde (17a).** Yield: 16%; yellow sticky solid; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 15.15 (s, 1H, $OH_B$), 14.39 (s, 1H, $OH_A$), 10.06 (s, 1H, CHO), 2.71 (s, 3H), 2.71–2.69 (m, 1H), 2.58 (dd, $J = 5.6$, 16.7 Hz, 1H), 2.39–2.28 (m, 3H), 2.04 (br s, 1H), 1.73 (m ,2H), 1.42–1.21 (m, 1H), 1.21 (s, 3H), 1.01 and 0.95 (s, 3H each); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 203.9, 192.0, 172.0, 168.4, 162.6, 104.2, 103.7, 98.9, 77.2, 32.7, 32.2, 31.5, 30.2, 28.6, 24.8, 23.1, 22.8, 19.1, 16.5; IR (KBr) $\nu_{max}$: 3403, 2924, 1624, 1443, 1281, 1204, 1108, 1021 $cm^{-1}$; CIMS: $m/z$ 345 $[M+1]^+$, 209 $[M-C_{10}H_{16}]$; analysis for $C_{20}H_{24}O_5$ (344.2) calcd: C, 69.75; H, 7.02; found: C, 69.51; H, 6.93.

**4.6.10. 4-Acetyl-5,7-dihydroxy-1,1,2a-trimethyl-1,1a,2,2a,8,8a,9,9a-octahydro-3-oxa-cyclopropa[*b*] anthracene-6-carbaldehyde (17b).** Yield: 15%; yellow sticky solid; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 15.17 (s, 1H, $OH_B$), 13.22 (s, 1H, $OH_A$), 10.19 (s, 1H, CHO), 2.71 (s, 3H), 2.70–2.66 (m, 1H), 2.62 (dd, $J = 5.6$, 16.4 Hz, 1H), 2.41–2.31 (m, 3H), 2.02 (br s, 1H), 1.78–1.68 (m ,2H), 1.44–1.24 (m, 1H), 1.24 (s, 3H), 1.02 and 0.96 (s, 3H each); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 203.5, 192.3, 169.7, 168.4, 163.8, 104.4, 98.1, 77.2, 33.1, 32.4, 31.0, 29.7, 28.5, 25.0, 23.0, 22.6, 18.9, 16.6; IR (Neat) $\nu_{max}$: 3401, 2924, 2856, 1618, 1454, 1312, 1200, 1112, 1020 $cm^{-1}$; CIMS: $m/z$ 345 $[M+1]^+$, 209 $[M-C_{10}H_{16}]$; analysis for $C_{20}H_{24}O_5$ (344.2) calcd: C, 69.75; H, 7.02; found: C, 69.62; H, 6.90.

**4.6.11. 6-Acetyl-3,4-dihydro-5,7-dihydroxy-3′,3′-dimethyl-spiro-[2*H*-1-benzopyran-2,2′-bicyclo[2.2.1]heptane]-8-carboxaldehyde (18a).** Yield: 16%; yellow oil; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 15.12 (s, 1H, $OH_B$), 14.30 (s, 1H, $OH_A$), 10.01 (s, 1H, CHO), 2.70 (s, 3H), 2.66 (dd, $J$ = 5.6, 16.7 Hz, 1H), 2.50–2.26 (m, 1H), 2.24 (d, $J$ = 4.6 Hz, 1H), 2.10–2.01 (m, 2H), 1.89 (m, 1H), 1.75–1.60 (m, 3H), 1.50–1.24 (m, 2H), 1.12 and 1.02 (s, 3H each); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 203.9, 191.9, 170.9, 169.2, 162.4, 104.6, 103.3, 101.9, 90.2, 49.4, 45.2, 34.4, 32.9, 29.7, 25.2, 23.6, 22.5, 22.3, 16.67; IR (Neat) $\nu_{max}$: 3435, 2963, 1628, 1446, 1372, 1296, 1198, 1107 cm$^{-1}$; CIMS: $m/z$ 345 $[M+1]^+$, 209 $[M-C_{10}H_{16}]$; analysis for $C_{20}H_{24}O_5$ (344.2) calcd: C, 69.75; H, 7.02; found: C, 69.61; H, 7.00.

**4.6.12. 8-Acetyl-3,4-dihydro-5,7-dihydroxy-3′,3′-dimethyl-spiro-[2*H*-1-benzopyran-2,2′-bicyclo[2.2.1]heptane]-6-carboxaldehyde (18b).** Yield: 15%; yellow solid; mp 135–138 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 15.44 (s, 1H, $OH_B$), 13.19 (s, 1H, $OH_A$), 10.19 (s, 1H, CHO), 2.71 (s, 3H), 2.66 (m, 1H), 2.48 (m, 1H), 2.26 (d, $J$ = 4.6 Hz, 1H), 2.09–1.99 (m, 2H), 1.90–1.87 (m, 2H), 1.86–1.81 (m, 1H), 1.66–1.61 (m, 2H), 1.51–1.46 (m, 1H), 1.43–1.37 (m, 1H), 1.10 and 1.06 (s, 3H each); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 203.1, 192.4, 170.0, 167.2, 164.1, 104.7, 104.2, 101.5, 93.1, 50.1, 46.8, 35.0, 33.2, 29.7, 27.0, 23.5, 23.3, 22.9, 22.5, 16.4; IR (KBr) $\nu_{max}$: 3568, 2939, 2881, 1627, 1421, 1376, 1317, 1142 cm$^{-1}$; CIMS: $m/z$ 345 $[M+1]^+$, 209 $[M-C_{10}H_{16}]$; analysis for $C_{20}H_{24}O_5$ (344.2) calcd: C, 69.75; H, 7.02; found: C, 69.83; H, 6.91.

**4.6.13. 2,3,4,4a,9,9a-Hexahydro-6,8-dihydroxy-3,3,4a-trimethyl-2,4-methano-1*H*-xanthene-5,7-dicarboxaldehyde (19).** Yield: 17%; brown viscous oil, $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 13.36 and 13.20 (s, 1H each, $OH_A$ and $OH_B$), 10.15 and 9.95 (s, 1H each, CHO), 2.71 (m, 1H), 2.66 (m, 1H), 2.45 (dd, $J$ = 5.6, 15.7 Hz, 1H), 2.26 (t, $J$ = 4.5 Hz, 1H), 2.15 (m, 2H), 1.90 (m, 1H), 1.80 (d, $J$ = 15.0 Hz, 1H),1.48 (s, 3H), 1.32–1.22 (m, 1H), 1.31 and 1.10 (s, 3H each); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 192.0, 191.7, 168.6, 168.1, 166.0, 103.7, 100.4, 88.3, 54.7, 43.9, 40.5, 34.2, 31.7, 28.6, 28.0, 27.8, 22.6, 19.4; IR (Neat) $\nu_{max}$: 3402, 2925, 1632, 1558, 1446, 1306, 1255, 1181 cm$^{-1}$; CIMS: $m/z$ 331 $[M+1]^+$, 195 $[M-C_{10}H_{16}]$; analysis for $C_{19}H_{22}O_5$ (330.1) calcd: C, 69.07; H, 6.71; found: C, 69.00; H, 6.67.

**4.6.14. 3,4-Dihydro-5,7-dihydroxy-6′,6′-dimethyl-spiro-[2*H*-1-benzopyran-2,2′-bicyclo[3.1.1]heptane]-6,8-dicarboxaldehyde (20).** Yield: 25%, light brown solid; mp 109–111 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 13.42 and 13.21 (s, 1H each, $OH_A$ and $OH_B$), 10.13 and 10.02 (s, 1H each, CHO), 2.55 (t, $J$ = 6.7 Hz, 2H), 2.26 (m, 1H), 2.16 (t, $J$ = 6.0 Hz, 1H), 2.05–1.96 (m, 5H), 1.91–1.84 (m, 2H), 1.60 (d, $J$ = 10 Hz, 1H), 1.30 and 1.02 (s, 3H each); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 191.9, 191.5, 168.6, 168.1, 163.5, 104.4, 103.5, 100.6, 85.5, 49.6, 40.5, 38.2, 31.5, 28.5, 27.4, 26.4, 24.6, 23.2, 14.7; IR (KBr) $\nu_{max}$: 3401, 2923, 1637, 1547, 1444, 1306, 1181, 1018 cm$^{-1}$; CIMS: $m/z$ 331 $[M+1]^+$, 195 $[M-C_{10}H_{16}]$; analysis for $C_{19}H_{22}O_5$ (330.1), calcd; C, 69.07; H, 6.71; found: C, 68.96; H, 6.59.

**4.6.15. 5,7-Dihydroxy-1,1,2a-trimethyl-1,1a,2,2a,8,8a,9,9a-octahydro-3-oxa-cyclopropa[*b*]anthracene-4,6-dicarbaldehyde (21).** Yield: 20%; yellow brown oil; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 13.47 and 13.24 (s, 1H each, $OH_A$ and $OH_B$), 10.16 and 10.08 (s, 1H each, CHO), 2.57 (dd, $J$ = 5.8, 16.6 Hz, 1H), 2.36–2.28 (m, 2H), 2.04–1.95 (m, 2H), 1.74–1.56 (m, 3H), 1.39–1.25 (m, 1H), 1.23 (s, 3H), 1.01 and 0.96 (s, 3H each); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 192.1, 191.6, 169.9, 168.1, 164.1, 104.3, 103.9, 98.6, 79.1, 32.2, 31.3, 29.7, 28.6, 27.6, 24.9, 22.7, 22.6, 19.0, 16.4; IR (Neat) $\nu_{max}$: 3343, 3019, 2930, 2401, 1632, 1521, 1426, 1216, 1046 cm$^{-1}$; CIMS: $m/z$ 331 $[M+1]^+$, 195 $[M-C_{10}H_{16}]$; analysis for $C_{19}H_{22}O_5$ (330.1) calcd: C, 69.07; H, 6.71; found: C, 69.01; H, 6.62.

**4.6.16. 3,4-Dihydro-5,7-dihydroxy-3′,3′-dimethyl-spiro-[2*H*-1-benzopyran-2,2′-bicyclo[2.2.1]heptane]-6,8-dicarboxaldehyde (22).** Yield: 21%; yellow solid; mp 107–109 °C, $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 13.38 and 13.22 (s, 1H each, $OH_A$ and $OH_B$), 10.15 and 10.03 (s, 1H each), 2.72–2.57 (m, 2H), 2.46–2.30 (m, 1H), 2.25 (m, 1H), 2.20–2.09 (m, 1H), 2.05–1.95 (m, 1H), 1.96–1.73 (m, 2H), 1.72–1.51 (m, 3H), 1.56–1.38 (m, 1H), 1.38–1.22 (m, 1H), 1.12 and 1.02 (s, 3H each); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 192.2, 191.4, 168.8, 168.1, 163.8, 104.7, 103.6, 101.1, 90.7, 49.4, 45.2, 34.4, 29.7, 27.2, 25.2, 23.6, 22.5, 22.5, 16.3; IR (KBr) $\nu_{max}$: 3742, 2941, 1640, 1440, 1377, 1303, 1169, 1071 cm$^{-1}$; CIMS: $m/z$ 331 $[M+1]^+$,195 $[M-C_{10}H_{16}]$; analysis for $C_{19}H_{22}O_5$ (330.1) calcd: C, 69.07; H, 6.71; found: C, 68.87; H, 6.51.

### 4.7. Assay for in vitro antimicrobial activity

Antibacterial and antifungal activities of all the synthesized natural and unnatural euglobals against pathogenic bacteria and fungi were evaluated in the similar way as described earlier.[30]

### 4.8. Assay for in vitro antileishmanial activity

Antileishmanial activity was tested in vitro against a culture of *L. donovani* promastigotes as described earlier.[30] Pentamidine and amphotericin B were used as the standard antileishmanial agents. $IC_{50}$ and $IC_{90}$ values were computed from dose–response curves generated by plotting the percent growth versus drug concentration.

### 4.9. Assay for in vitro antimalarial activity

In vitro antimalarial activity was determined against chloroquine sensitive and resistant (D6 and W2) clones of *P. falciparum* as described.[30]

### 4.10. Assay for in vitro cytotoxicity to vero cells

Cytotoxicity was determined against mammalian kidney fibroblasts (vero cells) as described earlier.[30]

## Acknowledgments

S.B.B. is thankful to Director, NIPER, for the fellowship. Financial assistance from NIPER for synthesis of compounds is gratefully acknowledged.

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