http://dx.doi.org/10.5935/0103-5053.20150287

J. Braz. Chem. Soc., Vol. 27, No. 3, 551-565, 2016.
Printed in Brazil - ©2016 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Synthesis, *in vitro* Antimalarial Activity and *in silico* Studies of Hybrid Kauranoid 1,2,3-Triazoles Derived from Naturally Occurring Diterpenes

Juliana de O. Santos,[a] Guilherme R. Pereira,[b] Geraldo C. Brandão,[c] Tatiane F. Borgati,[a] Lucas M. Arantes,[d] Renata C. de Paula,[a] Luciana F. Soares,[a] Maria F. A. do Nascimento,[a] Márlia R. C. Ferreira,[e] Alex G. Taranto,[f] Fernando P. Varotti[g] and Alaíde B. de Oliveira**,[a]

[a]Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Campus Pampulha, 31270-901 Belo Horizonte-MG, Brazil

[b]Departamento de Engenharia Química, Pontifícia Universidade Católica de Minas Gerais, Av. Dom José Gaspar, 500, Campus Coração Eucarístico, 30535-901 Belo Horizonte-MG, Brazil

[c]Faculdade de Farmácia, Universidade Federal de Ouro Preto (UFOP), Rua Costa Sena, 171, 35400-000 Ouro Preto-MG, Brazil

[d]Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Campus Pampulha, 31270-901 Belo Horizonte-MG, Brazil

[e]Departamento de Botânica, Museu Paraense Emilio Goeldi, Av. Perimetral, 1901, Terra Firme, 66077-530 Belém-PA, Brazil

[f]Laboratório de Química Farmacêutica Medicinal and [g]Núcleo de Pesquisa em Química Biológica, Universidade Federal de São João Del-Rey, Praça Frei Orlando, 170, 36307-352 Divinópolis-MG, Brazil

We herein report the synthesis of hybrid kauranoid molecules of type 1,2,3-triazole-1,4-disubstituted aiming to improve the antimalarial activity of kaurenoic and xylopic acids. The Cu^{I}-catalyzed cycloaddition of azides and kauranoid terminal alkynes was explored as a hybridization strategy. Kauranoid terminal alkynes were prepared from kaurenoic and xylopic acids that were isolated from *Wedelia paludosa* D. C. (Asteraceae) and *Xylopia frutescens* Aubl. (Annonaceae). A total of 15 kauranoid derivatives, including nine new triazoles, were obtained and five out of these were more active than the original diterpenes. Interestingly, an increased activity was observed for a kauranoid propargyl ether. Interaction between *ent*-kaurane diterpene derivatives and Ca^{2+}-ATPase (PfATP6) was investigated. Synthesis of diterpene derivatives emerges as a possible route to be explored in the quest of potentially new inhibitors of PfATP6.

Keywords: kaurenoic acid, xylopic acid, click chemistry, antiplasmodial activity, PfATP6 inhibitors

Introduction

Malaria remains a major concern in public health because of its morbidity, mortality and drug resistance of its infective agents. The protozoa *Plasmodium falciparum*, that is transmitted by *Anopheles* mosquitoes[1] is among the most prevalent species infecting humans and killing more than two million people every year. It is estimated that, globally, 3.3 billion people are at risk of malaria which represents approximately half of the world's population.[2] In Brazil, malaria is restricted to the Amazonian region, including the states of Acre, Amapá, Amazonas, Pará, Rondônia, Roraima, Tocantins, Mato Grosso and Maranhão, where 99% of malaria cases were registered.[3]

Historically, plants have proved to be an important source of antimalarial drugs,[4] such as quinine (**1**) (Figure 1), an alkaloid extracted from the bark of Peruvian *Cinchona* sp., and artemisinin (**5**) (Figure 2), a sesquiterpene lactone with a 1,2,4-trioxane unit, that was isolated from *Artemisia annua* L. (Asteraceae), a plant of the traditional

*e-mail: alaidebraga@terra.com.br

Chinese medicine with millennial use to treat fever in China. Structure simplification of quinine led to new efficient drugs such as mefloquine (**2**), chloroquine (**3**) and primaquine (**4**) (Figure 1).[5] However, due to clinically significant parasite resistance to antimalarial drugs, there is an urgent need to promote the quest for new efficacious drugs to fight against malaria.[6]

We have recently demonstrated the *in vitro* antimalarial activity of kaurenoic acid (**6**) (Figure 2) against the chloroquine-resistant W2 strain of *P. falciparum* with a 50% inhibitory concentration (IC_{50}) of 21.1 ± 3.3 μM, and a low cytotoxicity against HepG2 cells (50% cytotoxic concentration (CC_{50}) of 192.4 ± 25.5 μM), resulting in a favorable selectivity index (SI = 9.1).[7] Kaurenoic acid (**6**) is a widely occurring diterpenoid.[7] Chemical modifications of kaurenoic acid afforded more effective derivatives such as epoxides what has motivated the application of a patent.[8] Kaurenoic acid is found in plant species of different families and we obtained it in gram quantities from *Wedelia paludosa* D. C. (Asteraceae).[9]

Another kaurane diterpene that is a convenient substrate for chemical transformations aiming to synthesize potentially bioactive compounds is xylopic acid (**7**) (Figure 2) that occurs in *Xylopia frutescens* Aubl. (Annonaceae).[10]

The antiplasmodial activity of kaurenoic acid derivatives[7] has motivated a project aiming to explore the potential of abundantly occurring kaurane diterpenes as starting compounds for the synthesis of hybrid molecules having a kauranoid core linked to a 1,2,3-triazole unit (Scheme 1). Molecular hybridization is an interesting strategy for the rational development of drugs. Recently, it has been explored by different groups to generate antimalarial hits.[11,12] This technique combines two "chemical structures" to generate a hybrid molecule that incorporate characteristics of the two original ones. One possible approach is the combination of a terminal alkyne and an organic azide via copper-catalyzed azide-alkyne cycloaddition (CuAAC) reactions,[13,14] that have been explored for the synthesis of different molecules affording new analogues of chloroquine, chalcones, naphthoquinones and several other hybrid antimalarial molecules.[12] We have previously explored this strategy by combining the antimalarial pharmacophore 7-chloroquinoline with different 1,2,3-triazole moieties.[15]

Our research group has recently demonstrated the antiplasmodial activity of oxidized/epoxidized *ent*-kaurane diterpene derivatives obtained from naturally occurring *ent*-kaurenes.[7] Due to the selective antimalarial activity exhibited by these compounds, and knowing that diterpenes induce calcium overload in myocytes, the interaction between *ent*-kaurane diterpene derivatives and Ca^{2+}-ATPase (PfATP6) was investigated.[16] The results indicated a stronger interaction of some kaurane derivatives with the targets of thapsigargin (TG), a sesquiterpene that is a natural inhibitor of PfATP6. These data suggest PfATP6 as a potential target for antimalarial kaurane diterpenes and motivated docking studies with compounds here described.[17]

In the present paper, we report the synthesis of nine kauranoid-1,2,3-triazole hybrid molecules, starting with

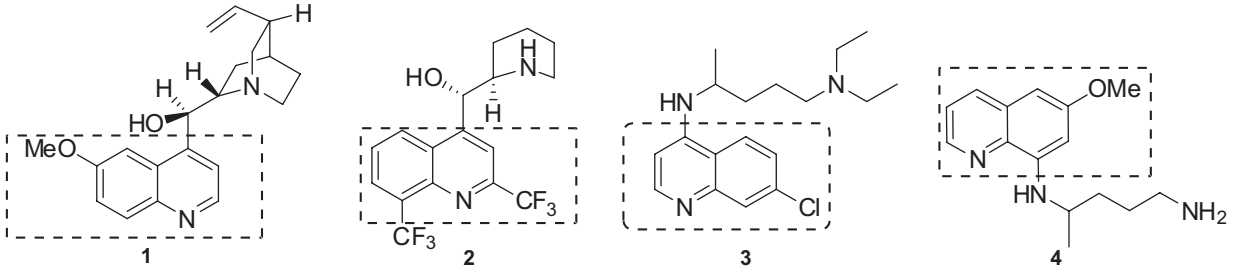


Figure 1. Chemical structures of quinine (**1**), mefloquine (**2**), chloroquine (**3**) and primaquine (**4**).

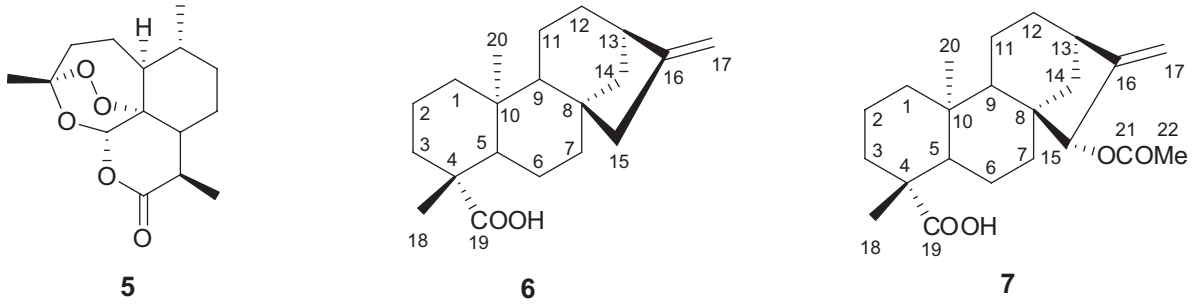
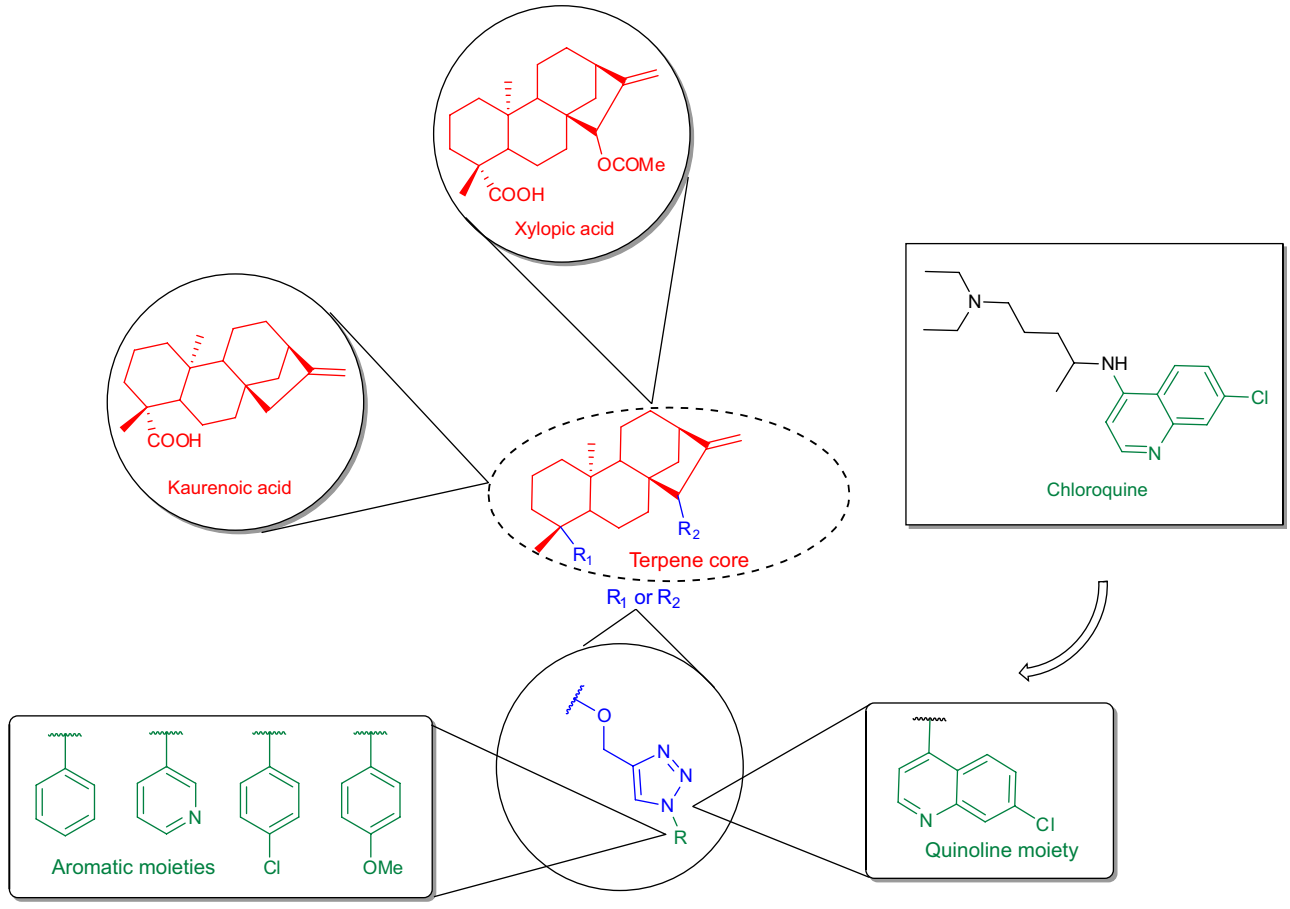


Figure 2. Chemical structures of the terpenoids artemisinin (**5**), kaurenoic acid (**6**) and xylopic acid (**7**).



Scheme 1. Origin of the kauranoid core and the substituents in the novel 1,2,3-triazole-1,4-disubstituted hybrid molecules synthesized by CuAAC.

kaurenoic and xylopic acids, by CuAAC (Scheme 1), the antiplasmodial activity against *P. falciparum* W2 strain, the HepG2 cytotoxicity against HepG2 cells and docking studies towards PfATP6.

Experimental

General

Melting points (m.p.) were measured in a model MQAPF-307 (Microquímica) melting point apparatus and are uncorrected. Chemicals and reagents were purchased from commercial suppliers and used as received unless noted otherwise. Reactions were monitored by thin layer chromatography (TLC) with silica gel 60 with fluorescent indicator (e.g., silica gel F-254 or IB-F, Merck) and activated previously with heating at 100 ºC overnight and visualized in several ways with an ultraviolet light source at 254 nm, by spraying with Hanessian reagent (ceric ammonium molibidate (CAM)), anisaldehyde sulfuric acid, Dragendorff reagent and/or exposure to iodine. All reactions were performed in standard dry glassware without inert atmosphere. Evaporation and concentration were carried out under vacuum in a Büchi rotavapor. Products purification was conducted using silica column chromatography with Merck 60 (0.063-0.200 mm; 70-230 mesh American Society for Testing and Materials (ASTM)). UV absorptions were recorded by a Fourier transform infrared (FTIR) spectrophotometer (Spectrum One, Perkin-Elmer), using an attenuated total reflectance (ATR) system. High resolution mass spectrometry (HRMS) data were recorded on a Shimadzu liquid chromatography-mass spectrometry ion trap and time-of-flight (LCMS-IT-TOF) spectrometer using electrospray ionization (ESI) and Waters ACQUITY® tandem quadrupole detector (TQD). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured on Bruker Avance DPX 200 and DRX400 with FT analysis. Chemical shifts (δ, in ppm) are reported relative to tetramethylsilane (TMS). Coupling constants (J) are given in Hz. Used deuterated solvent were CD_3OD, $CDCl_3$ or deuterated dimethylsulfoxide (DMSO-d_6). All 2D NMR data were recorded at 400 MHz (Bruker DRX400); heteronuclear single quantum coherence (HSQC) using J 145 Hz, and heteronuclear multiple-bond correlation (HMBC) using J 8 Hz.

Plant material

Aerial parts of *W. paludosa* were collected by Guilherme R. Pereira, at Av. Afonso Pena, Belo Horizonte-MG, in November 2011. The plant species was taxonomically identified by PhD João Renato Stehmann, Departamento de Botânica, Instituto de Ciências Biológicas (ICB), Universidade Federal de Minas Gerais (UFMG), Belo Horizonte-MG, Brazil. A voucher specimen was deposited at the Herbarium of ICB-UFMG (BHCB). Plant material was dried in an air circulating oven (ca. 40 ºC) and then ground in a knife mill. Unripe fruits of *X. frutescens* were collected by PhD Márlia R. Coelho-Ferreira, Museu Paraense Emílio Goeldi, Belém-PA, Brazil, in the Reserva Florestal do Gumma, municipality of Santa Bárbara, state of Pará, in May and June 2011. The botanical species was taxonomically identified by the collector and the identification was confirmed by MSc Jorge Oliveira, specialist on the taxonomy of the Annonaceae family. Voucher specimens were deposited in the Museu Paraense Emílio Goeldi, under the numbers MG 203912 and MG 207249.

Extraction and isolation of kaurane diterpene acids

Wedelia paludosa

Pulverized plant material (450 g) was percolated with hexane at room temperature. The solvent was removed in a rotavapor and the dark residue obtained (34 g) was chromatographed in a silica gel column. Fractions eluted with hexane and mixtures of hexane/dichloromethane (DCM) were combined and recrystallized affording 1.6 g of kaurenoic acid (**6**)[9] that was characterized by spectroscopic analyses and comparison with literature data.[18]

Xylopia frutescens

Pulverized dried unripe fruit material (2.5 kg) was exhaustively extracted by percolation with hexane at room temperature. The solvent was removed in a rotavapor and the residue obtained (5 g) was chromatographed in a silica gel column. Fractions eluted with hexane, mixtures of hexane/dichloromethane and finally with ethyl acetate, were combined affording 2.3 g of xylopic acid (**7**), whose identification was confirmed on the basis of spectroscopic analyses and comparison with literature data.[10]

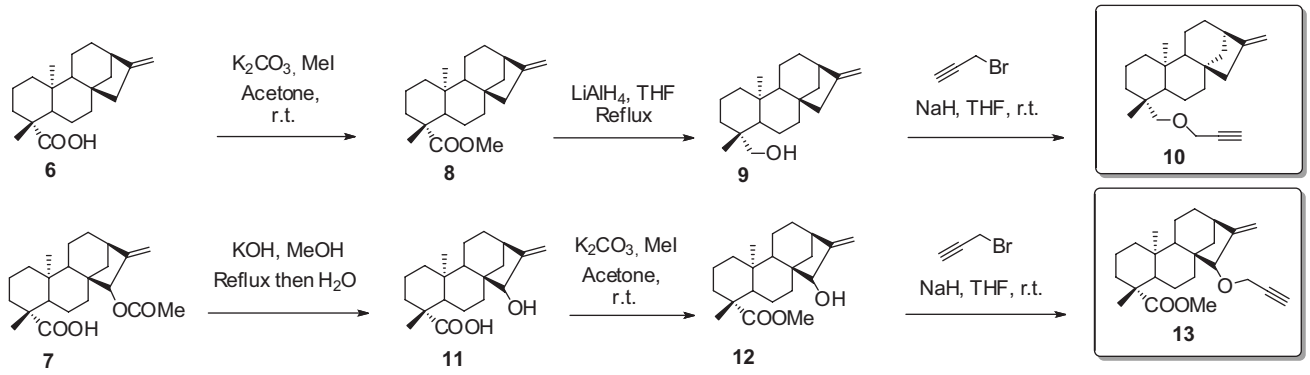
Synthesis and characterization of the products

Methyl *ent*-kaur-16-en-19-oate (**8**)[19]

To a round bottom flask containing K_2CO_3 (1.175 g, 8.5 mmol) and anhydrous acetone (100 mL), under nitrogen atmosphere, kaurenoic acid (**6**) (500 mg, 1.65 mmol) was added. The resulting mixture was vigorously stirred and after 30 min, methyl iodide was added. After reaction completion (6 h), the mixture was worked up as usually.[19] The crude reaction product was purified by silica gel column chromatography eluted with a mixture of hexane/dichloromethane (1:1), affording compound **8** (Scheme 2) with 73% yield (408 mg, 1.29 mmol). m.p. 79.6-80.5 ºC;[18] IR (ATR) ν_{max} / cm^{-1} 2930, 2852, 1722, 1656; ^1H NMR (200 MHz, $CDCl_3$) δ 4.78 (bs, 1H, H-17a), 4.72 (bs, 1H, H-17b), 3.63 (s, 3H, H-21), 2.62 (sl, 1H, H-13), 2.19-1.06 (m, 20H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12, 2H-14 and 2H-15), 1.16 (s, 3H, H-18), 0.82 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178 (C-19), 155.7 (C-16), 102.9 (C-17), 57 (C-5), 55 (C-9), 51 (C-21), 48.9 (C-15), 44.1 (C-8), 43.7 (C-13 and C-4), 41.2 (C-7), 40.7 (C-1), 39.6 (C-14), 39.3 (C-10), 38 (C-3), 33 (C-12), 28.7 (C-18), 21.9 (C-6), 19.1 (C-2), 18.3 (C-11), 15.3 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{21}H_{33}O_2$ $[M + H]^+$: 317.2436; found: 317.2288.

ent-Kaur-16-en-19-ol (**9**)[20]

To a round bottom flask containing a solution of compound **8** (78 mg, 0.24 mmol) in anhydrous tetrahydrofuran (THF, 10 mL), $LiAlH_4$ (150 mg, 3.95 mmol) was added. The resulting mixture was vigorously stirred and



Scheme 2. Synthetic routes for preparation of the kaurane derivatives **10** and **13** bearing a terminal alkyne moiety.

heated under reflux. After completion of the reaction (3 h), the crude product was separated by filtration following purification by silica gel column chromatography eluted with mixtures of hexane/dichloromethane (9:1, 8:2, 7:3, 6:4 and 1:1), affording compound **9** (Scheme 2) with 90% yield (64 mg, 0.21 mmol). m.p. 124-125 ºC;[20] IR (ATR) ν_{max} / cm^{-1} 3459, 2923, 2853, 1656; ^1H NMR (200 MHz, $CDCl_3$,) δ 4.78 (bs, 1H, H-17a), 4.72 (bs, 1H, H-17b), 3.74 (d, 1H, *J* 10.9 Hz, H-19a), 3.42 (d, 1H, *J* 10.9 Hz, H-19b), 2.04-1.07 (m, 20H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12, 2H-14 and 2H-15), 2.63 (bs, 1H, H-13), 1 (s, 3H, H-18), 0.95 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.8 (C-16), 102.9 (C-17), 65.4 (C-19), 56.8 (C-5), 56.1 (C-9), 49 (C-15), 44.1 (C-8), 43.9 (C-4), 41.6 (C-7), 40.4 (C-1), 39.6 (C-14), 39.1 (C-10), 38.6 (C-3), 35.5 (C-13), 33.1 (C-12), 27 (C-18), 20.4 (C-6), 18.2 (C-2), 18.1 (C-11), 18 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{20}H_{33}O$ $[M + H]^+$: 289.2487; found: 289.2229.

3'(*ent*-Kaur-16-en-19-oxy) prop-1'-yne (**10**)

To a 50 mL flask was added sodium hydride (63 mg, 2.62 mmol) and pretreated THF (5 mL) and the system was allowed to stir at room temperature for 15 min under nitrogen atmosphere. After this time was added compound **9** (185 mg, 0.64 mmol) solubilized in anhydrous THF (10 mL). After 30 min, 0.5 mL propargyl bromide solution (80% in toluene) was added to the reaction mixture that was kept under stirring at room temperature for 24 h. Afterwards, the mixture was extracted with ethyl acetate, followed by extraction with 20% aqueous NaOH and brine. The crude reaction product obtained after solvent removal was purified by silica gel column chromatography eluted with mixtures of hexane/dichloromethane (8:2, 7:3 and 6:4), affording compound **10** with 57% yield (119.4 mg, 0.36 mmol). IR (ATR) ν_{max} / cm^{-1} 3292, 2921, 2851, 2165, 1641; ^1H NMR (200 MHz, $CDCl_3$) δ 4.8 (bs, 1H, H-17a), 4.74 (bs, 1H, H-17b), 4.11 (s, 2H, H-3'), 3.60 (d, 1H, *J* 8.7 Hz, H-19a), 3.28 (d, 1H, *J* 8.7 Hz, H-19b), 2.64 (bs, 1H, H-13), 2.40 (s, 1H, H-1'), 2.05-1.26 (m, 20H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12, 2H-14 and 2H-15), 1.03 (s, 3H, H-18), 0.95 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.9 (C-16), 102.9 (C-17), 80.5 (C-2'), 73.7 (C-1'), 73.1 (C-19), 58.5 (C-3'), 56.9 (C-5), 56.2 (C-9), 49.1 (C-15), 44.2 (C-8), 44 (C-4), 41.6 (C-7), 40.5 (C-1), 39.6 (C-14), 39.2 (C-10), 37.8 (C-3), 36.3 (C-13), 33.2 (C-12), 29.7 (C-18), 20.5 (C-6), 18.2 (C-2), 18.4 (C-11), 18.2 (C-20); LCMS-IT-TOF calcd. for $C_{23}H_{35}O$ $[M + H]^+$: 327.2610; found: 271.2299.

ent-15α-Hydroxy-kaur-16-en-19-oic acid (**11**)[10]

To a round bottom flask containing a solution of xylopic acid (**7**) (500 mg) in methanol a solution of 10% KOH in methanol was added (5 mL). The resulting mixture was vigorously stirred and heated under reflux. After completion of the reaction (6 h), the mixture was acidified with 10% aqueous HCl, methanol was removed in a rotavapor, the aqueous residue was extracted with ethyl acetate that was washed with saturated aqueous $NaHCO_3$ and satured aqueous NaCl, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. This procedure afforded compound **11** in 86% yield (379 mg, 1.2 mmol). m.p. 202-205 ºC; IR (ATR) ν_{max} / cm^{-1} 3392, 2923, 2853, 1683; ^1H NMR (200 MHz, $CDCl_3$) δ 5.10 (bs, 1H, H-17a), 4.96 (d, 1H, *J* 2.2 Hz, H-17b), 3.77 (s, 1H, H-15), 2.66 (bs, 1H, H-13), 2.19-1.32 (m, 18H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12 and 2H-14), 1.24 (s, 3H, H-18), 0.97 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 184.1 (C-19), 158.2 (C-16), 104.8 (C-17), 82.5 (C-15), 56.3 (C-5), 45.7 (C-9), 45.4 (C-8), 43.7 (C-4), 40.5 (C-1), 40 (C-13), 39.2 (C-10), 38.8 (C-7), 37.7 (C-3), 36.2 (C-14), 33 (C-12), 28.9 (C-18), 21.4 (C-6), 19 (C-2), 18.2 (C-11), 15.6 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{20}H_{31}O_3$ $[M + H]^+$: 319.2228; found: 319.2245.

Methyl *ent*-15α-hidroxy-kaur-16-en-19-oate (**12**)[10]

Compound **12** was prepared by a procedure similar to the one described for **8**. m.p. 202-205 ºC;[10] IR (ATR) ν_{max} / cm^{-1} 3459, 2923, 2853, 1739, 1656; ^1H NMR (200 MHz, $CDCl_3$) δ 5.07 (sl, 1H, H-17a), 4.93 (bs, 1H, H-17b), 3.74 (s, 1H, H-15), 3.63 (s, 3H, H-21), 2.63 (bs, 1H, H-13), 2.18-1.30 (m, 18H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12 and 2H-14), 1.16 (s, 3H, H-18), 0.83 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.3 (C-19), 158.5 (C-16), 105 (C-17), 82.7 (C-15), 56.6 (C-5), 51.3 (C-21), 45.9 (C-9), 45.7 (C-8), 44 (C-4), 40.8 (C-1), 40.2 (C-13), 39.2 (C-10), 39.1 (C-7), 38.2 (C-3), 36.5 (C-14), 33.3 (C-12), 28.9 (C-18), 21.7 (C-6), 19.3 (C-2), 18.5 (C-11), 15.7 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{21}H_{31}O_3$ $[M - H]^-$: 331.4694; found: 331.2163.

Methyl (*ent*-15α-prop-2-ynyloxy-kaur-16-en-19-ate) (**13**)

Compound **13** was prepared by a procedure similar to the one described for **10**. IR (ATR) ν_{max} / cm^{-1} 3306, 2933, 2855, 2116, 1721, 1660; ^1H NMR (200 MHz, $CDCl_3$) δ 5.14 (sl, 1H, H-17a), 4.94 (sl, 1H, H-17b), 4.43 (bs, 2H, H-3'), 3.75 (s, 1H, H-15), 3.63 (s, 3H, H-21), 2.60 (bs, 1H, H-13), 2.46 (bs, 1H, H-1'), 2.20-1.45 (m, 18H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12 and 2H-14), 1.17 (s, 3H, H-18), 0.83 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.3 (C-19), 156.7 (C-16), 105.8 (C-17), 88.8 (C-15), 81.0 (C-2'), 74.5 (C-1'), 60.1 (C-3'), 56.7 (C-5), 51.3 (C-21), 46.6 (C-9), 46.4

(C-8), 44 (C-4), 40.8 (C-1), 40.6 (C-13), 39.8 (C-10), 39.3 (C-7), 38.2 (C-3), 36.6 (C-14), 33.5 (C-12), 28.9 (C-18), 21.8 (C-6), 19.3 (C-2), 18.2 (C-11), 15.8 (C-20); LCMS-IT-TOF calcd. for $C_{24}H_{35}O_3$ $[M + H]^+$: 371.2508; found: 315.2394.

4-Azido-7-chloroquinoline (**14**)[21]

NaN_3 (1.306 g, 20.1 mmol) was added to a solution of 4,7-dichloroquinoline (1 g, 5.07 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF, 5 mL). The reaction mixture was stirred and heated under reflux at 90 ºC for 24 h. The reaction mixture was then allowed to cool down to room temperature and then it was diluted with 100 mL CH_2Cl_2, washed with water (3 × 20 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography eluted with hexane/dichloromethane mixture (9:1, 7:3 and 6:4) affording compound **14** with 86% yield (890 mg, 4.36 mmol). m.p. 112-113 ºC;[21] IR (ATR) ν_{max} / cm^{-1} 3036, 2090, 1572; ^1H NMR (200 MHz, $CDCl_3$) δ 8.73 (d, 1H, *J* 4.8 Hz, H-2), 7.97 (d, 1H, *J* 1.9 Hz, H-8), 7.85 (d, 1H, *J* 8.9 Hz, H-5), 7.37 (dd, 1H, *J* 8.9, 1.7 Hz, H-6), 7,01 (d, 1H, *J* 4.8 Hz, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 151.37 (C-2), 149.66 (C-10), 146.38 (C-4), 136.53 (C-7), 128.27 (C-8), 127.56 (C-5), 123.81 (C-6), 119.98 (C-9), 108.77 (C-3); HRMS-ESI-IT-TOF calcd. for $C_9H_6ClN_4$ $[M + H]^+$: 205.0281; found: 205.0485.

3-Azidopyridine (**15**)[22]

A solution of $NaNO_2$ (4.4 g, 0.064 mol) in water (10 mL) was slowly added to a round bottom flask containing 10% aqueous HCl (6 mL) and 3-aminopyridine (5 g, 0.053 mol). Subsequently, a solution of NaN_3 (2.68 g, 0.064 mol) in water (10 mL) at 0 ºC was added to the reaction mixture that was stirred at room temperature for 45 min. The mixture was alkalinized with a saturated solution of Na_2CO_3 and extracted with dichloromethane. The solvent was removed in a rotavapor and **15** was obtained with 78% yield (5 g, 0.041 mol). IR (ATR) ν_{max} / cm^{-1} 3408, 3034, 2090, 1571, 1474, 1421; ^1H NMR (200 MHz, $CDCl_3$) δ 8.35-8.39 (m, 2H, H-2 and H-4), 7.28-7.37 (m, 2H, H-5 and H-6); ^{13}C NMR (50 MHz, $CDCl_3$) δ 146.04 (C-2), 141.34 (C-6), 137.17 (C-3), 125.97 (C-4), 124.19 (C-5); HRMS-ESI-IT-TOF calcd. for $C_5H_5N_4$ $[M + H]^+$: 121.0514; found: 121.0596.

Azidobenzene (**16**)

Compound **16** was prepared from the corresponding azide by a procedure similar to the one described for compound **15**. IR (ATR) ν_{max} / cm^{-1} 3247, 3065, 3036, 2090, 1593, 1506, 1491, 1455; ^1H NMR (200 MHz, $CDCl_3$) δ 7.38 (t, 2H, *J* 7.6 Hz, H-3), 7.18 (d, 1H, *J* 7.6 Hz, H-4), 7.05 (d, 2H, *J* 7.6 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 140.21 (C-1), 129.96 (C-3), 125.08 (C-4), 119.23 (C-2); HRMS-ESI-IT-TOF calcd. for $C_6H_6N_3$ $[M + H]^+$: 120.0562; found: 120.0687.

1-Azido-4-chlorobenzene (**17**)

Compound **17** was prepared from the corresponding azide by a procedure similar to the one described for compound **15**. IR (ATR) ν_{max} / cm^{-1} 3062, 2091, 1592, 1491, 1455; ^1H NMR (200 MHz, $CDCl_3$) δ 7.31 (d, 2H, *J* 8.7 Hz, H-3), 6.95 (d, 2H, *J* 8.7 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 138.92 (C-1), 130.47 (C-4), 130.10 (C-2), 120.52 (C-3); HRMS-ESI-IT-TOF calcd. for $C_6H_5ClN_3$ $[M + H]^+$: 154.0172; found 154.0254.

1-Azido-4-methoxybenzene (**18**)

Compound **18** was prepared from the corresponding azide by a procedure similar to the one described for compound **15**. IR (ATR) ν_{max} / cm^{-1} 3394, 3225, 2100, 1587, 1455, 1440; ^1H NMR (200 MHz, $CDCl_3$) δ 6.96 (d, 2H, *J* 9.0 Hz, H-2), 6.88 (d, 2H, *J* 9.0 Hz, H-3), 3.79 (s, 3H, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.95 (C-4), 132.29 (C-1), 119.93 (C-2), 115.07 (C-3), 55.50 (C-5); HRMS-ESI-IT-TOF calcd. for $C_7H_8N_3O$ $[M + H]^+$: 150.0667; found: 150.0765.

General procedure for the synthesis of **19-27**

To a round bottom flask containing a terminal alkyne (0.11 mmol) and an organic azide (0.11 mmol) in dichloromethane (2 mL) and water (2 mL), $CuSO_4.5H_2O$ (0.004 mmol) and sodium ascorbate (0.20 mmol) were added. The resulting mixture was vigorously stirred at room temperature for 24 h. Subsequently, the mixture was extracted with dichloromethane, the solvent was removed and the residue was purified by silica gel column chromatography. The product was eluted with mixtures of hexane/dichloromethane (9:1; 8:2; 6:4 and 1:1).

7''-Chloro-4''-(4'-*ent*-kaur-16-en-19-oxymethyl)-1*H*-1,2,3-triazol-1'-yl)quinoline (**19**)

m.p. 146-148 ºC; IR (ATR) ν_{max} / cm^{-1} 3078, 2922, 2849, 1656, 1593, 1562, 1117; ^1H NMR (400 MHz, $CDCl_3$) δ 9.06 (d, 1H, *J* 4.5 Hz, H-2''), 8.25 (d, 1H, *J* 1.95 Hz, H-8''), 8.00 (d, 1H, *J* 8.9 Hz, H-5''), 7.97 (bs, 1H, H-5'), 7.60 (dd, 1H, *J* 8.9, 1.95 Hz, H-6''), 7.50 (d, 1H, *J* 4.5 Hz, H-3''), 4.79 (bs, 1H, H-17a), 4.73 (bs, 1H, H-17b), 4.75 (bs, 2H, H-6'), 3.68 (d, 1H, *J* 8.8 Hz, H-19a), 3.40 (d, 1H, *J* 8.8 Hz, H-19b), 2.63 (bs, 1H, H-13), 2.05-1.06 (m, 20H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12, 2H-14 and 2H-15), 1.01 (s, 3H, H-18), 0.99 (s,

3H, H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8 (C-16), 151.3 (C-2''), 150.2 (C-10''), 147.1 (C-4'), 141.1 (C-4''), 136.9 (C-7''), 129.4 (C-6''), 129.0 (C-8''), 124.5 (C-5''), 123.8 (C-5'), 120.7 (C-9''), 116 (C-3''), 102.9 (C-17), 74.1 (C-19), 65.0 (C-6'), 56.8 (C-9), 56.2 (C-5), 49.0 (C-15), 44.1 (C-8), 43.9 (C-13), 41.6 (C-7), 40.4 (C-1), 39.6 (C-14), 39.2 (C-10), 38.0 (C-4), 36.4 (C-3), 33.1 (C-12), 27.9 (C-18), 20.6 (C-6), 18.4 (C-2), 18.2 (C-11), 18.1 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{32}H_{40}ClN_4O$ $[M + H]^+$: 531.2846; found: 532.0730.

3''-(4'-(*ent*-Kaur-16-en-19-oxymethyl)-1*H*-1,2,3-triazol-1'-yl) pyridine (**20**)

m.p. 102-105 ºC; IR (ATR) ν_{max} / cm^{-1} 3063, 2923, 2855, 1659, 1586, 1108; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.0 (d, 1H, *J* 1.6 Hz, H-2''), 8.7 (d, 1H, *J* 4.5 Hz, H-6''), 8.14 (dd, 1H, *J* 8.2, 1.6 Hz, H-4''), 8 (bs, 1H, H-5'), 7.5 (dd, 1H, *J* 8.2, 4.5 Hz, H-5''), 4.77 (bs, 1H, H-17a), 4.71 (bs, 1H, H-17b), 4.68 (bs, 2H, H-6'), 3.63 (d, 1H, *J* 8.9 Hz, H-19a), 3.34 (d, 1H, *J* 8.9 Hz, H-19b), 2.61 (bs, 1H, H-13), 2.02-1.24 (m, 20H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12, 2H-14 and 2H-15), 0.98 (s, 3H, H-18), 0.96 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.7 (C-16), 149.8 (C-2''), 147.4 (C-4'), 141.5 (C-6''), 133.6 (C-3''), 128.0 (C5''), 124.1 (C4''), 120.0 (C-5'), 102.8 (C-17), 73.9 (C-19), 64.9 (C-6'), 56.8 (C-9), 56.1 (C-5), 48.9 (C-15), 44.0 (C-8), 43.9 (C-13), 41.5 (C-7), 40.3 (C-1), 39.5 (C-14), 39.1 (C-10), 37.9 (C-4), 36.3 (C-3), 33.1 (C-12), 27.8 (C-18), 20.5 (C-6), 18.3 (C-2), 18.1 (C-11), 18.0 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{28}H_{39}N_4O$ $[M + H]^+$: 447.3079; found: 447.3653.

4'-(*ent*-Kaur-16-en-19-oxymethyl)-1'-phenyl-1*H*-1,2,3-triazole (**21**)

m.p. 116-119 ºC; IR (ATR) ν_{max} / cm^{-1} 3065, 2925, 2856, 1658, 1597, 1095; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.94 (s, 1H, H-5'), 7.74 (d, 2H, *J* 7.4 Hz, H-2''), 7.43-7.56 (m, 3H, H-3'' and H-4''), 4.78 (bs, 1H, H-17a), 4.72 (bs, 1H, H-17b), 4.68 (s, 2H, H-6'), 3.63 (d, 1H, *J* 9.1 Hz, H-19a), 3.34 (d, 1H, *J* 9.1 Hz, H-19b), 2.63 (bs, 1H, H-13), 2.04-1.06 (m, 20H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12, 2H-14 and 2H-15), 0.99 (s, 3H, H-18), 0.98 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.8 (C-16), 146.8 (C-4'), 137.1 (C-1''), 129.6 (C-3''), 128.6 (C-4''), 120.5 (C-2''), 120.2 (C-5'), 102.8 (C-17), 73.7 (C-19), 65.0 (C-6'), 56.8 (C-9), 56.1 (C-5), 49.0 (C-15), 44.1 (C-8), 43.9 (C-13), 41.5 (C-7), 40.4 (C-1), 39.6 (C-14), 39.1 (C-10), 37.9 (C-4), 36.3 (C-3), 33.1 (C-12), 27.9 (C-18), 20.5 (C-6), 18.3 (C-2), 18.1 (C-11), 18.0 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{29}H_{40}N_3O$ $[M + H]^+$: 446.3127; found: 446.2508.

4'-(*ent*-Kaur-16-en-19-oxymethyl)-1'-(4''-chlorophenyl)-1*H*-1,2,3-triazole (**22**)

m.p. 155-158 ºC; IR (ATR) ν_{max} / cm^{-1} 3105, 2922, 2851, 1660, 1596, 1115; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.91 (s, 1H, H-5'), 7.70 (d, 2H, *J* 8.6 Hz, H-2''), 7.50 (d, 2H, *J* 8.6 Hz, H-3''), 4.79 (bs, 1H, H-17a), 4.73 (bs, 1H, H-17b), 4.67 (s, 2H, H-6'), 3.64 (d, 1H, *J* 9.1 Hz, H-19a), 3.34 (d, 1H, *J* 9.1 Hz, H-19b), 2.63 (bs, 1H, H-13), 2.04-1.25 (m, 20H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12, 2H-14 and 2H-15), 0.99 (s, 3H, H-18), 0.97 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.8 (C-16), 147.2 (C-4'), 135.6 (C-1''), 134.4 (C-4''), 129.9 (C-3''), 121.6 (C-2''), 120.1 (C-5'), 102.9 (C-17), 73.8 (C-19), 65.1 (C-6'), 56.9 (C-9), 56.1 (C-5), 49.0 (C-15), 44.1 (C-8), 43.9 (C-13), 41.6 (C-7), 40.4 (C-1), 39.6 (C-14), 39.2 (C-10), 38.0 (C-4), 36.3 (C-3), 33.1 (C-12), 27.9 (C-18), 20.5 (C-6), 18.4 (C-2), 18.1 (C-11), 18.0 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{29}H_{37}ClN_3O$ $[M - H]^-$: 478.2703; found: 478.3000.

4'-(*ent*-Kaur-16-en-19-oxymethyl)-1'-(4''-methoxyphenyl)-1*H*-1,2,3-triazole (**23**)

m.p. 108-112 ºC; IR (ATR) ν_{max} / cm^{-1} 2927, 2851, 1657, 1595, 1098; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.85 (s, 1H, H-5'), 7.63 (d, 2H, *J* 8.9 Hz, H-2''), 7.02 (d, 2H, *J* 8.9 Hz, H-3''), 4.78 (bs, 1H, H-17a), 4.72 (bs, 1H, H-17b), 4.66 (s, 2H, H-6'), 3.86 (s, 3H, H-30), 3.63 (d, 1H, *J* 9.1 Hz, H-19a), 3.34 (d, 1H, *J* 9.1 Hz, H-19b), 2.62 (bs, 1H, H-13), 2.03-1.05 (m, 20H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12, 2H-14 and 2H-15), 0.99 (s, 3H, H-18), 0.97 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 158.7 (C-4''), 155.8 (C-16), 146.6 (C-4'), 130.5 (C-1''), 122.1 (C-2''), 114.7 (C-3''), 120.1 (C-5'), 102.8 (C-17), 73.6 (C-19), 65.0 (C-6'), 56.8 (C-5''), 56.1 (C-9), 55.5 (C-5), 49.0 (C-15), 44.1 (C-8), 43.9 (C-13), 41.5 (C-7), 40.4 (C-1), 39.6 (C-14), 39.1 (C-10), 37.9 (C-4), 36.3 (C-3), 33.1 (C-12), 27.9 (C-18), 20.5 (C-6), 18.3 (C-2), 18.1 (C-11), 18.0 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{30}H_{42}N_3O_2$ $[M + H]^+$: 476.3232; found: 476.3324.

7''-Chloro-4''-(methyl *ent*-kaur-16-en-15-oxymethyl-19-ate)-1*H*-1,2,3-triazol-1'-yl) quinoline (**24**)

m.p. 133-136 ºC; IR (ATR) ν_{max} / cm^{-1} 3073, 2936, 2855, 1720, 1660, 1587, 1552, 1110; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.08 (d, 1H, *J* 4.6 Hz, H-2''), 8.25 (d, 1H, *J* 1.2 Hz, H-8''), 8.09 (bs, 1H, H-5'), 8.02 (d, 1H, *J* 8.9 Hz, H-5''), 7.61 (dd, 1H, *J* 8.9, 1.2 Hz, H-6''), 7.53 (d, 1H, *J* 4.6 Hz, H-3''), 5.21-4.94 (m, 4H, H-17a, H17-b, H6'a and H6'b), 3.82 (s, 1H, H-15), 3.65 (s, 3H, H-21), 2.62 (bs, 1H, H-13), 2.20-1.37 (m, 18H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12 and 2H-14), 1.17 (s, 3H, H-18), 0.85 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 177.9 (C-19), 156.7 (C-16), 151.3 (C-2''), 150.0 (C-10''), 147.1 (C-4'),

141.0 (C-4''), 136.9 (C-7''), 129.4 (C-6''), 128.8 (C-8''), 124.5 (C-5''), 124.1 (C-5'), 120.6 (C-9''), 115.9 (C-3''), 105.2 (C-17), 90.1 (C-15), 66.5 (C-6'), 56.6 (C-5), 51.1 (C-21), 46.6 (C-9), 46.3 (C-8), 43.7 (C-4), 40.7 (C-1), 40.3 (C-13), 39.9 (C-10), 39.0 (C-7), 37.9 (C-3), 36.3 (C-14), 33.2 (C-12), 28.6 (C-18), 21.6 (C-6), 19.0 (C-2), 18.0 (C-11), 15.5 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{33}H_{40}ClN_4O_3$ $[M + H]^+$: 575.2744; found: 575.4535.

3''-(4'-(Methyl *ent*-kaur-16-en-15-oxymethyl-19-ate)-1*H*-1,2,3-triazol-1'-yl) pyridine (**25**)

m.p. 112-115 ºC; IR (ATR) ν_{max} / cm^{-1} 2939, 1721, 1611, 1562, 1114; ^1H NMR (200 MHz, $CDCl_3$) δ 8.99 (d, 1H, *J* 1.9 Hz, H-2''), 8.66 (d, 1H, *J* 3.9 Hz, H-6''), 8.15-8.07 (m, 2H, H-4'' and H-5'), 7.51-7.44 (m, 1H, H-5''), 5.09-4.88 (m, 4H, H-17a, H17-b, H6' and H6'b), 3.73 (s, 1H, H-15), 3.59 (s, 3H, H-21), 2.56 (bs, 1H, H-13), 2.15-1.41 (m, 18H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12 and 2H-14), 1.12 (s, 3H, H-18), 0.85 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 177.9 (C-19), 156.6 (C-16), 149.7 (C-2''), 147.4 (C-4'), 141.4 (C-6''), 133.6 (C-3''), 128.0 (C5''), 124.2 (C4''), 120.4 (C-5'), 105.3 (C-17), 90.0 (C-15), 66.5 (C-6'), 56.5 (C-5), 51.0 (C-21), 46.5 (C-9), 43.6 (C-8), 40.6 (C-4), 40.3 (C-1), 39.8 (C-10), 39.0 (C-13), 37.9 (C-7), 36.3 (C-3), 33.2 (C-14), 28.6 (C-12), 21.6 (C-18), 20.9 (C-6), 19.0 (C-2), 18.0 (C-11), 15.5 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{29}H_{39}N_4O_3$ $[M + H]^+$: 491.3022; found: 491.2542.

4'-(Methyl *ent*-kaur-16-en-15-oxymethyl-19-ate)-1'-phenyl-1*H*-1,2,3-triazole (**26**)

m.p. 127-129 ºC; IR (ATR) ν_{max} / cm^{-1} 2934, 2854, 1720, 1660, 1598, 1109; ^1H NMR (200 MHz, $CDCl_3$) δ 8.02 (s, 1H, H-5'), 7.75 (d, 2H, *J* 7.2 Hz, H-2''), 7.40-7.56 (m, 3H, H-3'' and H-4''), 4.91-5.11 (m, 4H, H-17a, H17-b, H6'a and H6'b), 3.77 (s, 1H, H-15), 3.63 (s, 3H, H-21), 2.59 (bs, 1H, H-13), 2.19-1.28 (m, 18H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12 and 2H-14), 1.16 (s, 3H, H-18), 0.83 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.0 (C-19), 156.7 (C-16), 137.0 (C-1''), 129.6 (C-3''), 128.6 (C-4''), 120.5 (C-2''), 105.2 (C-17), 89.9 (C-15), 66.7 (C-6'), 56.5 (C-5), 51.0 (C-21), 46.5 (C-9), 46.3 (C-8), 43.7 (C-4), 40.7 (C-1), 40.3 (C-13), 39.8 (C-10), 39.0 (C-7), 37.9 (C-3), 36.3 (C-14), 33.3 (C-12), 28.6 (C-18), 21.6 (C-6), 19.0 (C-2), 18.0 (C-11), 15.5 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{30}H_{40}N_3O_3$ $[M + H]^+$: 490.3025; found: 490.4860.

4'-(Methyl *ent*-kaur-16-en-15-oxymethyl-19-ate)-1'-(4''-chlorophenyl)-1*H*-1,2,3-triazole (**27**)

m.p. 141-144 ºC; IR (ATR) ν_{max} / cm^{-1} 2922, 2853, 1721, 1599, 1110; ^1H NMR (200 MHz, $CDCl_3$) δ 7.99 (bs, 1H, H-5'), 7.70 (d, 2H, *J* 8.7 Hz, H-2''), 7.50 (d, 2H, *J* 8.7 Hz, H-3''), 4.92-5.11 (m, 4H, H-17a, H17-b, H6'a and H6'b), 3.77 (s, 1H, H-15), 3.64 (s, 3H, H-21), 2.60 (bs, 1H, H-13), 2.01-1.44 (m, 18H, 2H-1, 2H-2, 2H-3, 1H-5, 2H-6, 2H-7, 1H-9, 2H-11, 2H-12 and 2H-14), 1.17 (s, 3H, H-18), 0.84 (s, 3H, H-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.0 (C-19), 156.8 (C-16), 147.2 (C-4'), 135.6 (C-1''), 134.5 (C-4''), 129.9 (C-3''), 121.7 (C-2''), 120.4 (C-5'), 105.3 (C-17), 90.0 (C-15), 66.7 (C-6'), 56.6 (C-5), 51.1 (C-21), 46.6 (C-9), 46.4 (C-8), 43.8 (C-4), 40.7 (C-1), 40.4 (C-13), 39.9 (C-10), 39.1 (C-7), 37.9 (C-3), 36.4 (C-14), 33.3 (C-12), 28.7 (C-18), 21.6 (C-6), 19.1 (C-2), 18.1 (C-11), 15.6 (C-20); HRMS-ESI-IT-TOF calcd. for $C_{30}H_{38}ClN_3O_3$: 523.2602; found: 523.9615.

Biological assays

Plasmodium falciparum continuous culture

P. falciparum W2 strain was maintained in continuous culture on human erythrocytes (blood group A+) in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% human plasma (complete medium), as previously described.[23] Synchronization of the parasites was achieved by sorbitol treatment and the parasitemia was determined microscopically in Giemsa-stained smears.[24]

In vitro growth inhibition of *Plasmodium falciparum* by the *p*LDH assay

The antimalarial effects of the compounds and of the controls were measured by the lactate dehydrogenase of *Plasmodium falciparum* (*p*LDH) assay as previously described,[25] with minor modifications. Briefly, ring-stage parasites in sorbitol-synchronized blood cultures were added to 96-well culture plates at 2% parasitemia and 1% hematocrit and then incubated with the test drugs that were diluted in complete medium, from 50 mg mL^{-1} stock solutions in DMSO, at a final concentration of 0.002% (v/v) and stored at –20 ºC. After a 48 h incubation period the plates were frozen (–20 ºC for 24 h) and thawed for the *p*LDH assay. The hemolyzed cultures were transferred to another 96-well culture plate, and Malstat® and nitroblue tetrazolium salt and phenazine ethosulphate (NBT/PES) reagents were added. After 1 h of incubation at 37 ºC in the dark the absorbance was read at 570 nm in a spectrophotometer (Infinite®200 PRO, Tecan). The results were evaluated with the software Microcal Origin 8.5 for determination of the dose-response curves plotted with sigmoidal fit.[26] The IC_{50} was determined by comparison with controls with standard drug and without drugs.

Cytotoxicity in human hepatome cell cultures (Hep G2A16)

Hepatome cells Hep G2A16 were maintained at 37 ºC, 5% CO_2 in 75 cm^2 sterile culture flasks (Corning®) with RPMI 1640 culture medium supplemented with 5% fetal bovine serum (FBS), penicillin (10 U mL^{-1}), and streptomycin (100 g mL^{-1}), with changes of medium twice a week. The cells were maintained in weekly passages (at 1:3 dilutions in sterile culture flasks) and grown to 80%.[26] They were used for experiments after being trypsinized (0.05% trypsin/0.5 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA)) and plated on 96-well microplates.[27] When confluent, the monolayers were trypsinized, washed, counted, diluted in complete medium, distributed in 96-well microplates (4×10^3 cells *per* well), then incubated for another 24 h at 37 ºC. The test samples and controls were diluted to a final concentration of 0.02% DMSO in culture medium to yield four concentrations in serial dilutions starting at 1,000 mg mL^{-1}. After a period of 24 h incubation at 37 ºC, 18 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL^{-1} in phosphate buffered saline (PBS)) were added to each well, followed by another 1 h 30 min incubation at 37 ºC. The supernatant was then removed, and 180 µL of DMSO were added to each well. The culture plates were read in a spectrophotometer with a 570 nm filter.[28] The minimum cytotoxicity concentration was determined as described with slight modifications. Each test was performed in duplicate; the CC_{50} were determined.[29,30] The SI for the antimalarial activity was then calculated based on the rate between CC_{50} and IC_{50} for the *in vitro* activity against *P. falciparum* as described.[31]

Molecular docking studies

Initially, the 3D structures of kauranoic and xylopic acid derivatives were generated and checked in relation to the protonated state in pH 7.4, and the tautomers conformers through Marvin Sketch.[32] Next, refinement of all structures was performed by the semi-empirical method PM7[33] and the default optimization method implanted in MOPAC2012 software.[34,35] Following that, the refined structures were converted into Protein Data Bank, partial charge (Q), and atom type (T) (PDBQT) files, in which the net atomic Gasteiger charges were assigned for each compound.[36] On the other hand, the preparation of the PDBQT file of the receptor, PfATP6, was previously reported, and the redocking process showed a good superposition between the crystallographic ligand and docked conformations with root-mean-square deviation (RMSD) value of 1.12 Å.[17] All PDBQT files were generated through Autodock tools.[37] The grid box was centered at the ligand covering the entire binding site. The rigid docking process was carried out by Autodock Vina[38] with the exhaustiveness set to 8. In addition, the lipophilicity was calculated using ALOGPS 2.1 program.[39] Finally, Discovery Studio v. 4.0[40] was used to analyze the binding conformations.

Results and Discussion

The naturally occurring diterpenoids kaurenoic acid (**6**) and xylopic acid (**7**) were isolated from hexane extracts of *Wedelia paludosa* and *Xylopia frutescens*, respectively. Both compounds were identified by spectroscopic methods and were used for chemical modifications in the present work. Two different routes were explored from these diterpenoids. In the first route, kaurenoic acid (**6**) was esterified with methyl iodide and potassium carbonate in acetone to give methyl *ent*-kaur-16-en-19-oate (**8**) that was reduced with lithium aluminum hydride in THF under reflux followed by etherification of the kaurenol (**9**) with propargyl bromide and sodium hydride affording an *O*-tethered terminal alkyne (**10**) (Scheme 2) that was spectroscopically characterized. In the IR spectrum a typical alkyne band (2165 cm^{-1}) was observed besides an intense band relative to ether C–O bond (1087 cm^{-1}). A broad singlet at δ 2.40 ppm in its ^{1}H NMR spectrum was ascribed to H-1', the terminal alkyne hydrogen, and the propargylic methylene group (2H-3') appear as a broad singlet at δ 4.11 ppm. Representative signals related to the diterpene scaffold are those of the C-19 methylene group (H-19a and H-19b) and the olefinic hydrogens (H-17a and H-17b) which are represented by two doublets (J 8.7 Hz) at δ 3.6 ppm (H-19a) and 3.28 ppm (H-19a) and two broad singlets at 4.80 ppm (H-17a) and 4.74 ppm (H-17b), respectively. Diagnostc signals in the ^{13}C NMR spectrum of **10** are those of the propargylic carbons: C-1' (δ 73.7 ppm), C-2' (δ 80.5 ppm) and C-3' (δ 58.5 ppm) together with those assigned to C-19 (δ 73.1 ppm), C-17 (δ 102. 9 ppm) and C-16 (δ 155.9 ppm). The mass spectrum of **10** was registered by LCMS-IT-TOF and showed the pseudomolecular ion peak at *m/z* 327 [M + H] which loses a C_3H_4O neutral molecule to give the base peak at *m/z* at 271 u.

In the second route, xylopic acid (**7**) was taken as the starting material. The ester moiety was hydrolyzed with potassium hydroxide, in methanol, under reflux, giving the hydroxy acid derivative (**11**) that was submitted to esterification with methyl iodide, affording methyl *ent*-15α-hydroxy-kaur-16-en-19-oate (**12**). This compound, bearing a free hydroxy group, was also converted to the propargyl ether (**13**) by reaction with sodium hydride and propargyl bromide, in THF (Scheme 2). In the IR spectrum of compound **13**, the presence of the propargyl ether is evidenced by the bands at

3306 and 2116 cm^{-1} related to the stretching of the terminal alkyne C–H bond and to the C≡C bond, respectively; an intense band (1087 cm^{-1}) corresponding to the stretching of the C–O ether bond in addition to the absorption band (1721 cm^{-1}) related to the stretching of the C=O ester are also observed. Its ^{1}H NMR spectrum showed signals for the propargyl moiety at 4.43 and 2.46 ppm relative to H-3' and H-1', respectively. Diagnostic signals in the ^{13}C NMR spectrum of **10** are those of the propargylic carbons: δ 81, 74.5 and 60.1 ppm, concerning C-2', C-1' and C-3', respectively. The mass spetrum of **13** was registered in a LCMS-IT-TOF and showed the pseudomolecular ion peak at *m/z* 371 [M + H] which loses a C_3H_4O neutral molecule to give the base peak at *m/z* at 315 u.

Aromatic azides were prepared by Sandmeyer-type reactions and nucleophilic aromatic substitution, as shown in Scheme 3. In the first case, each one of the different aromatic amines was treated with sodium nitrite in aqueous acidic solution at low temperature (0 to 5 ºC), generating its respective diazonium salt that was simultaneously converted to the respective azide by addition of sodium azide to the reaction mixture. 4,7-Dichloroquinoline was converted into 4-azido7-chloroquinoline (**14**) by heating with sodium azide in DMF, in anhydrous conditions.[21] The five different azide moieties were further used in convergent synthetic approaches, as shown in Scheme 3.

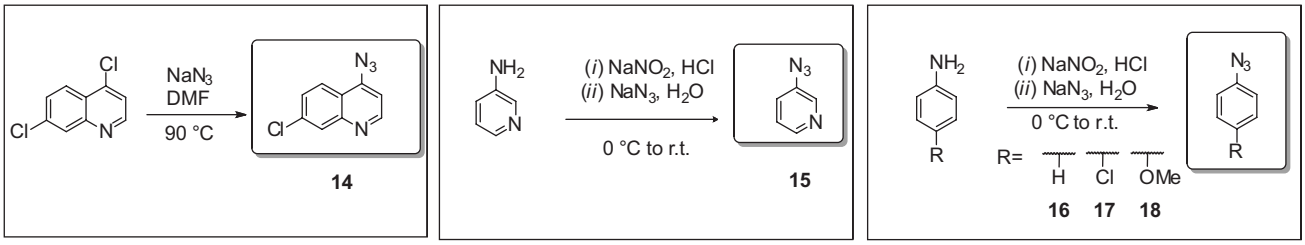
The methodology used for the synthesis of 1,2,3-triazole hybrids **19**-**27** (Scheme 4) is described by Rostovtsev *et al.*[41] as "a stepwise Huisgen cycloaddition process in which a copper(I)-catalyzed regioselective ligation of azides and terminal alkynes occurs in, copper(II) being a catalyst, *in situ*, to give 1,4-substituted triazoles". Once copper sulfate is taken with sodium ascorbate, the metal is reduced *in situ* and the cycloaddition reaction takes place.[12,14] The solvent used in this kind of reaction is a biphasic system composed of dichloromethane and water (1:1) that is stirred at room temperature while the development of the reaction is monitored by chromatographic methods, as described by Lee *et al.*,[42] and Pereira *et al.*[21] At the end, the triazoles are isolated by recrystallization or purification by silica column chromatography. The products were obtained with yields ranging from 25 to 78%.

The ^{1}H NMR spectra of the click reaction products **19**-**27** (Scheme 4) showed signals at δ 7-8 ppm for H-5', the only hydrogen of the triazole ring, and at δ 4.6-5.0 ppm for H-6', in addition to signals corresponding to the kauranoid moiety hydrogens. Thus, at δ 4.0-5.0 ppm two broad singlets were observed corresponding to H-17a and H-17b of the exocyclic double bond, and two singlets at δ 1.01 and 0.99 ppm, relative to the hydrogens H-18 and H-20 of the corresponding methyl groups, respectively. The triazole ring system was characterized by ^{13}C NMR spectra with signals for the ring carbons at δ 120-124 ppm (C-5') and 146-147.5 ppm (C-4') as well as for the methylene group at δ 65-67 ppm (C-6'), besides those expected for the kauranoid scaffold.

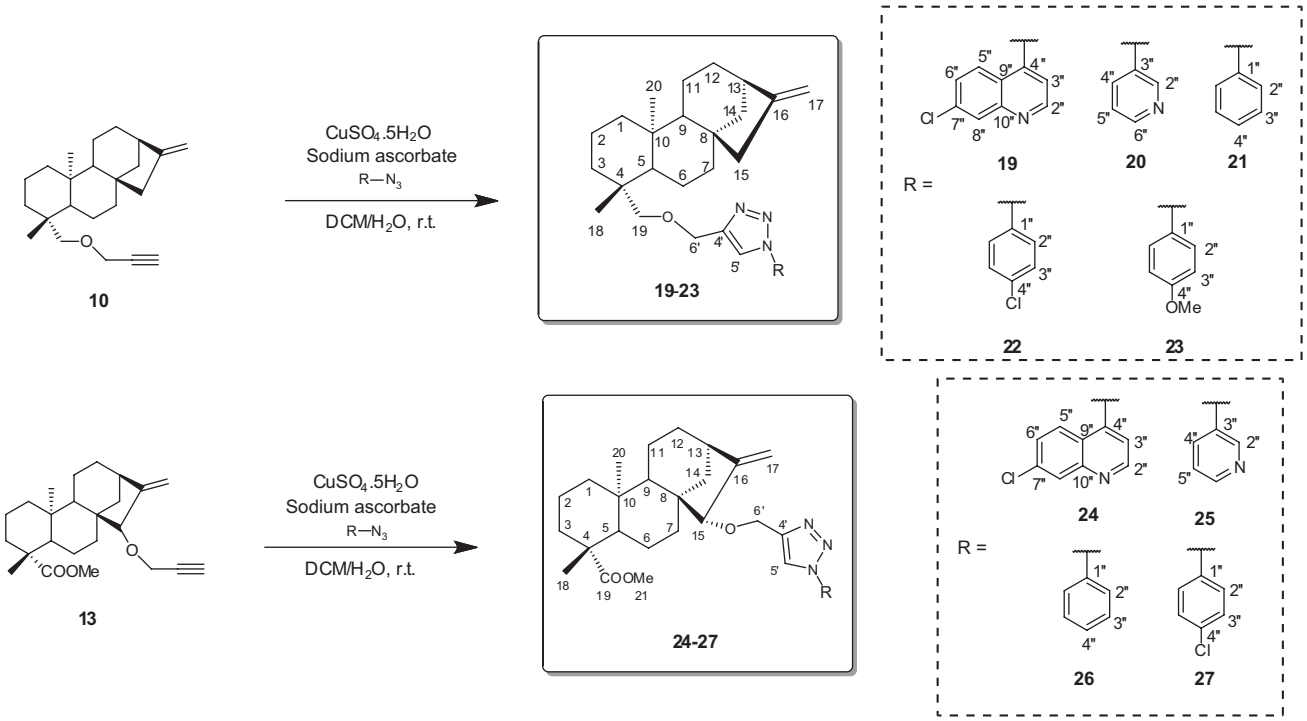
The two natural *ent*-kaurane diterpenes, kaurenoic (**6**) and xylopic (**7**) acids, the modified diterpene intermediates, as well as the final hybrid molecules were evaluated for their *in vitro* antimalarial activity against *P. falciparum* W2 strain that is chloroquine-resistant and mefloquine-sensitive. Quantitative assessment of the antiplasmodial activity was determined via the parasite lactate dehydrogenase assay (*p*LDH).[25]

For each tested sample, the percentage of parasite growth inhibition was determined in two different concentrations (25 and 50 μg mL^{-1}). Then, the most active compounds, in which the parasite growth inhibition was superior to 50%, has had the IC_{50} and the CC_{50} experimentally determined. Table 1 shows the values of IC_{50} for the *in vitro* antimalarial assay, the CC_{50} values for the cytotoxicity (Hep G2A16 cells) and the selective indexes ($SI = CC_{50} / IC_{50}$) for these compounds. Chloroquine (CQ) disclosed a CC_{50} value of 543.6 ± 71.4 μM and the SI equals to 1308. Kaurenoic acid (**6**) and the derivatives **20** and **23** showed CC_{50} values greater than CQ, in other words, the cytotoxicity of these substances tested in HepG2 cells was significantly lower when compared to CQ. However, the SI value of chloroquine was higher than the values found for our compounds, because it is much more active and, consequently, its IC_{50} is much lower.

Kaurenoic (**6**) and xylopic (**7**) acids disclosed low *in vitro* activity (IC_{50} of 115.6 and > 138.7 μM, respectively) (Table 1). As far as we are concerned, this is the first report on the *in vitro* antimalarial activity of xylopic acid (**7**).



Scheme 3. Aromatic azides obtained via Sandmeyer-type reaction and nucleophilic aromatic substitution.



Scheme 4. Final products synthesized via CuAAC reactions between the diterpenoid propargyl ethers **10** and **13** and the organic azides **14**-**18** leading to nine distinct hybrid molecules **19**-**27**.

Table 1. Mean percentage reduction growth (%Red) at concentrations of 25 and 50 μg mL^{-1}, *in vitro* antimalarial activity (IC_{50}) against *Plasmodium falciparum* (W2), cytotoxicity (CC_{50}, Hep G2A16 cells) and selectivity index (SI) for the kauranoid derivatives and kauranoid triazoles

Compound	%Red[a] (25 μg mL^{-1})	%Red[a] (50 μg mL^{-1})	IC_{50}[b] / μM	CC_{50}[c] / μM	SI	Binding energy / (kcal mol^{-1})	XLogP3
6	59.0 (82.7 μM)	74.0 (165.4 μM)	115 ± 2.4	945.2 ± 58.8	8.2	–8.5	5.43
8	32.0 (79.0 μM)	20.0 (158.0 μM)	> 158.1	N.D.	–	–7.7	5.75
9	33.0 (86.7 μM)	47.0 (173.4 μM)	> 173.5	N.D.	–	–8.1	5.50
10	90.0 (80.0 μM)	98.0 (160.1 μM)	19.7 ± 3.8	109.9 ± 23.0	5.6	–7.8	6.15
7	41.0 (69.4 μM)	67.0 (138.8 μM)	> 138.7	N.D.	–	–8.9	4.52
11	20.0 (78.5 μM)	30.0 (157.1 μM)	> 157.1	N.D.	–	–7.7	3.95
12	22.5 (77.5 μM)	27.0 (155.1 μM)	> 150.4	N.D.	–	–7.7	4.27
13	62.0 (67.5 μM)	81.5 (135.0 μM)	83.4 ± 3.8	181.8 ± 22.2	2.2	–7.5	4.92
19	21.0 (47.1 μM)	31.0 (94.3 μM)	> 94.1	N.D.	–	–10.5	7.63
20	56.1 (56.0 μM)	62.0 (112.0 μM)	53.0 ± 5.1	774.2 ± 48.1	14.5	–10.4	5.92
21	13.0 (56.1 μM)	35.0 (112.3 μM)	> 112.2	N.D.	–	–10.7	6.99
22	20.0 (52.1 μM)	33.0 (104.3 μM)	> 104.1	N.D.	–	–10.0	7.36
23	51.0 (52.6 μM)	60.0 (105.2 μM)	94.5 ± 2.7	> 2102.3	22.2	–9.4	6.97
24	31.0 (43.5 μM)	44.0 (87.0 μM)	> 86.9	N.D.	–	–9.3	6.40
25	47.0 (50.9 μM)	71.0 (102.0 μM)	56.3 ± 8.1	60.9	1.1	–8.7	4.69
26	28.6 (51.1 μM)	52.3 (102,2 μM)	> 102.1	N.D.	–	–9.3	5.76
27	27.5 (47.8 μM)	49.0 (95.5 μM)	> 95.4	N.D.	–	–8.7	6.13
Chloroquine	100 (78.1 μM)	90 (156.3 μM)	0.42 ± 0.09	543.6 ± 71.4	1308	–	–

[a]Percentage reduction growth (%Red) of *P. falciparum* chloroquine-resistant (W2) determined by the *p*LDH method at concentrations of 25 or 50 μg mL^{-1}; [b]IC_{50}: concentration that inhibits 50% of the parasite growth determined by the the *p*LDH in relation to control cultures with no drugs; [c]CC_{50}: concentration that kills 50% of HepG2cells 24 h after incubation with the compounds determined by the MTT method. SI: Selectivity index = CC_{50} / IC_{50}; N.D. = not determined; XLogP3: calculated coefficient of lipophilicity.

Kaurenoic acid (**6**) occurs in several plant species.[18] We have previously reported on its antiplasmodial activity against *P. falciparum* W2 strain (IC_{50} of 21.1 ± 3.3 μM).[7] The IC_{50} value for kaurenoic acid determined in the present work was higher than that reported previously.[7] A possible explanation for this difference may lie in the methodology used to assess the *in vitro* antimalarial activity of this substance. Despite using similar incubation time, parasitemia and hematocrit, in both experiments, the determination of parasitemia after exposure of parasites to drugs was based on different parameters, DNA synthesis, by incorporation of tritiated hypoxanthine, as reported by Batista *et al.*,[7] and determination of *p*LDH enzyme,[25] in the present work.

Recently, kaurenoic acid (**6**) was isolated after bioguided fractionation of *Schefflera umbellifera* (Sond.) Baill. extracts, a plant used to treat malaria in Malawi, Mozambique and Zimbabwe, as well as in South Africa, and a moderate antiplasmodial activity (IC_{50} 32.2 μg mL^{-1} = 106.5 μM) against the chloroquine-sensitive strain D10 was observed.[43] A close IC_{50} value (31.77 μg mL^{-1} = 105.1 μM) was shown against the D10 strain for this diterpene acid that is the major compound in the hexane and dichloromethane extracts from the stem bark of *Croton pseudopulchellus* Pax.[44] In both cases the antiplasmodial activity was determined by the *p*LDH technique.[25] The different strains of *P. falciparum* used, D10 by these authors, and W2 in our work, might explain the difference in the IC_{50} values.

All the kaurenoic (**8**-**10**, **19**-**23**) and xylopic (**11**-**13**, **24**-**27**) acids derivatives here described showed weaker antiplasmodial activity than the natural diterpene acids **6** and **7**, excepting the propargylic ethers **10** and **13** with IC_{50} of 19.7 ± 3.8 and 83.4 ± 3.8 μM, respectively, as well as the hybrid molecules **20** and **25** with IC_{50} of 53.0 ± 5.1 and 56.3 ± 8.1 μM, respectively (Table 1). Interestingly, good antimalarial activity was reported previously for some alkynes.[45] These results might lead one to think that the presence of an oxygenated group at position C19 would favor the antiplasmodial effect in comparison to the C16 position but this is not valid for **20** and **25** that showed statistically equivalent IC_{50}. However, in this case, the presence of nitrogens, and possibly of protonated forms, would certainly influence in receptors interaction and, consequently, in the antiplasmodial effect. Aiming to shed some light on these results, molecular docking studies were performed.

Molecular docking is a methodology which resolves two questions, the binding energy and pharmacophore conformation. The advantages and limitations of this methodology have been reported in previous reviews.[46] In general, all methodologies are very efficient to predict the binding mode of ligands, however, they need to improve the binding energy estimation. Figure 3 shows the docking studies between PfATP6 and kaurane derivatives. The binding site of this enzyme is located in the transmembrane region (Figure 3a). As can be seen, the most active compound **10** complexes with Lys250, Ile251, Phe254, Gln257, Asn814, Leu815, Ile816, Leu821 and Ile825 aminoacids, essentially through van der Waals interactions (Figure 3b). This finding can explain differences among the activity of the compounds, even though compound **10** could not reach two extra binding sites (Figure 3c). In addition, in general, this compound showed a high lipophilicity with XLogP value of 6.15. Significantly, compound **10** has few flexible bonds. Consequently, there is a minimum loss of entropy energy resulting in a better overall activity.[47] On the other hand, for **23**, for instance, the biological activity decreased in relation to compound **10**; maybe by the protonation of the triazole moiety due to the acid environment of parasite and its lower lipophilicity (5.92). However, the activity increased among other triazole derivatives, such as **20** and **25**, possibly because of reaching two extra binding sites and thus carrying out two hydrogen bonds with Gln257 and Leu815 with 2.95 and 3.57 Å, respectively (Figure 3d). In general, the biological activity can be improved by chain extension strategy, permitting the ligand to reach the two extra binding sites. Figures 3c and 3d show hydrogen bond donor and acceptor aminoacids in the cavity.

Conclusions

Structure modification of natural products is a valid strategy to create new hits for antimalarial drug research. This approach is particularly viable in the case of abundant ones, as we have demonstrated by the conversion of kaurenoic acid (**6**) into several more active derivatives.[7] These results have supported our proposal of extending the exploration to xylopic acid (**7**), another abundant kaurane diterpene. This time, a total of nine novel hybrid kauranoid-1,2,3-triazole derivatives were obtained via CuAAC reaction, even though the products were much less active than the starting diterpene acids. Docking studies described the intermolecular forces for the molecular recognition between diterpenes and PfATP6 and the kauranoid 1,2,3-triazole hybrid molecules were generally less active, although disclosing extra binding sites in relation to the starting diterpene acids. In addition, as can be observed, the lipophilicity and hydrogen bonds formed with receptor could explain the biological activity of kauranoid derivatives. The most active compound of

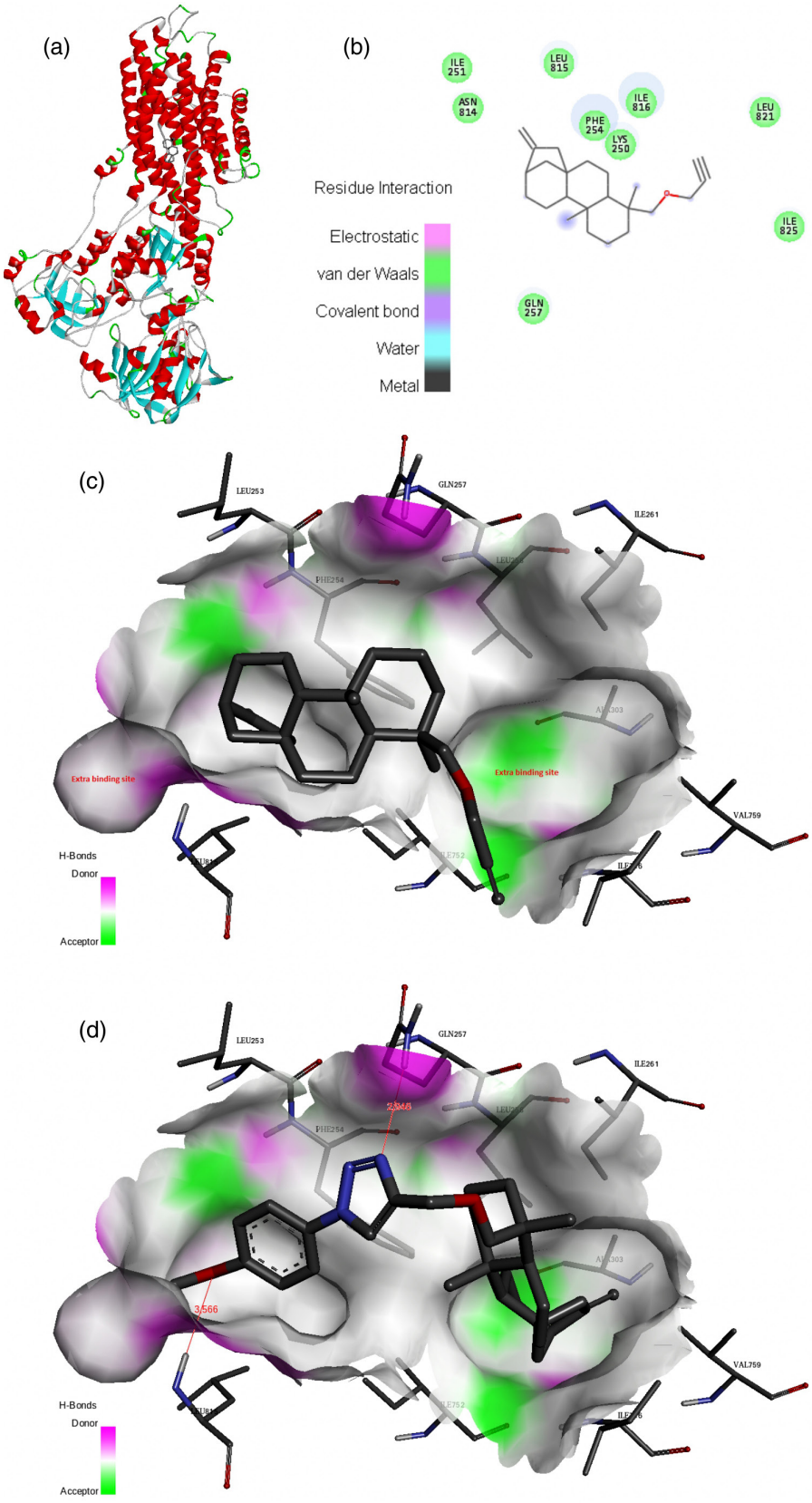


Figure 3. Docking results between PfATP6 and kaurane derivatives. (a) The PfATP6 model, in which the colors blue, red and gray represent β-sheets, α-helix and loop region, respectively. (b) 2D Diagram of pharmacophore map between **10** and the binding site of enzyme. (c) and (d) Hydrogen bond receptor surface with **10** and **23** derivatives, respectively. Pink and green colors show donor and acceptor regions of PfATP6, respectively. The hydrogens were omitted for better visualization.

those herein described is an *O*-propagyl ether (**10**) derived from kaurenoic acid disclosing the synthesis of *O*-tethered kaurane diterpenes as a possible route to be explored in the quest of new inhibitors of PfATP6.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Process No. 555655/2009-1) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Process No. CDS APQ 01129-10). Fellowships to J. O. S. (D FAPEMIG), G. R. P. (PDJ FAPEMIG), G. C. B. (PDJ CNPq), T. F. B. (D FAPEMIG), R. C. P. (D CNPq), L. F. S. (DTI2 CNPq) and M. F. A. N. (DTI2 FAPEMIG) are also acknowledged.

References

1. Oliveira, A. B.; Dolabela, M. F.; Braga, F. C.; Jácome, R. L. R. P.; Varotti, F. P.; Póvoa, M. M.; *An. Acad. Bras. Cienc.* **2009**, *81*, 715.
2. http://www.who.int/malaria/publications/world_malaria_report_2014/report/en/ accessed in October 2015.
3. http://bvsms.saude.gov.br/bvs/periodicos/boletim_epidemiologico_numero_1_2013.pdf accessed in October 2015.
4. Kumar, V.; Mahajan, A.; Chibale, K.; *Bioorg. Med. Chem.* **2009**, *17*, 2236.
5. Vale, N. F. S.; Moreira, R.; Gomes, P.; *Quim., Bol. Soc. Port. Quim.* **2005**, *99*, 57.
6. Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, N.; Socheat, D.; White, N. J.; *N. Engl. J. Med.* **2009**, *361*, 455.
7. Batista, R.; García, P. A.; Castro, M. A.; Miguel Del Corral, J. M.; Speziali, N. L.; Varotti, F. P.; de Paula, R. C.; García-Fernández, L. F.; Francesch, A.; San Feliciano, A.; Oliveira, A. B.; *Eur. J. Med. Chem.* **2013**, *62*, 168.
8. Oliveira, A. B.; Varotti, F. P.; Batista, R.; *WO pat. 2012083408 A8* **2012**.
9. Batista, R.; Garcia, P. A.; Castro, M. A.; Miguel, J. M. D. C.; Feliciano, A.; Oliveira, A. B.; *An. Acad. Bras. Cienc.* **2010**, *82*, 823.
10. Takahashi, J. A.; Boaventura, M. A. D.; Bayma, J. C.; Oliveira, A. B.; *Phytochemistry* **1995**, *40*, 607.
11. Feng, T. S.; Guantai, E. M.; Nell, M.; Van Rensburg, C. E. J.; Ncokazi, K.; Egan, T. J.; Hoppe, H. C.; Chibale, K.; *Biochem. Pharmacol.* **2011**, *82*, 236.
12. Guantai, E. M.; Ncokazi, K.; Egan, T. J.; Gut, J.; Rosenthal, P. J.; Smith, P. J.; Chibale, K.; *Bioorg. Med. Chem.* **2010**, *18*, 8243.
13. Kolb, H. C.; Finn, M. G.; Sharpless, K. B.; *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 2004.
14. Tornøe, C. W.; Christensen, C.; Meldal, M. J.; *Org. Chem.* **2002**, *67*, 3057.
15. Pereira, G. R.; Santos, L. J.; Luduvico, I.; Alves, R. B.; Freitas, R. P.; *Tetrahedron Lett.* **2010**, *51*, 1022.
16. Sun, G. B.; Sun, H.; Meng, X.-B.; Hu, J.; Zhang, Q.; Liu, B.; Wang, M.; Xu, H.-B.; Sun, X.-B.; *Toxicol. Appl. Pharmacol.* **2014**, *279*, 8.
17. Guimarães, D. S. M.; da Fonseca, A. L.; Batista, R.; Comar Jr., M.; de Oliveira, A. B.; Taranto, A. G.; Varotti, F. de P.; *Mem. Inst. Oswaldo Cruz* **2015**, *110*, 255.
18. Batista, R.; Braga, F. C.; Oliveira, A. B.; *Braz. J. Pharmacogn.* **2005**, *15*, 119.
19. Boeck, P.; Sá, M. M.; Souza, B. S.; Cercená, R.; Escalante, A. M.; *J. Braz. Chem. Soc.* **2005**, *16*, 1360.
20. Batista, R.; Humberto, J. L.; Chiari, E.; Oliveira, A. B.; *Bioorg. Med. Chem.* **2007**, *15*, 381.
21. Pereira, G. R.; Brandão, G. C.; Arantes, L. M.; de Oliveira, H. A.; de Paula, R. C.; Nascimento, M. F. A.; Santos, F. M.; Rocha, R. K.; Lopes, J. C. D.; Oliveira, A. B.; *Eur. J. Med. Chem.* **2014**, *73*, 295.
22. Ito, S.; Satoh, A.; Nagatomi, Y.; Hirata, Y.; Suzuki, G.; Kimura, T.; Satow, A.; Maehara, S.; Hikichi, H.; Hata, M.; Kawamoto, H.; Ohta, H.; *Bioorg. Med. Chem.* **2008**, *16*, 9817.
23. Trager, W.; Jensen, J.; *Science* **1976**, *193*, 673.
24. Lambros, C.; Vanderberg, J. P.; *J. Parasitol.* **1979**, *65*, 418.
25. Makler, M. T.; Ries, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbins, B. L.; Hinrichs, D. J.; *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739.
26. Varotti, F. P.; Botelho, A. C. C.; Andrade, A. A.; de Paula, R. C.; Fagundes, E. M. S.; Valverde, A.; Mayer, L. M. U.; Mendonça, J. S.; de Souza, M. V. N.; Boechat, N.; Krettli, A. U.; *Antimicrob. Agents Chemother.* **2008**, *52*, 3868.
27. Calvocalle, J. M.; Moreno, A.; Eling, W. M. C.; Nardin, E. H.; *Exp. Parasitol.* **1994**, *79*, 362.
28. Mosmann, T.; *J. Immunol. Methods* **1983**, *65*, 55.
29. Madureira, M. C.; Martins, A. P.; Gomes, M.; Paiva, J.; Cunha, A. P.; Rosário, V. L.; *J. Ethnopharmacol.* **2002**, *81*, 23.
30. Sá, M. S.; Costa, J. F. O.; Krettli, A. U.; Zalis, M. G.; Maia, G. L. D. A.; Sette, I. M. F.; Câmara, C. A.; Filho, J. M. B.; Giulietti-Harley, A. M.; dos Santos, R. R.; Soares, M. B. P.; *Parasitol. Res.* **2009**, *105*, 275.
31. Bézivin, C.; Tomasi, S.; Lohézic-Le Dévéhat, F.; Boustie, J.; *Phytomedicine* **2003**, *10*, 499.

32. ChemAxon; *MarvinSketch 14.9.22.0*; ChemAxon, Budapest, 2014.
33. Stewart, J. J.; *J. Mol. Model*. **2013**, *19*, 1.
34. Baker, J.; *J. Comput. Chem*. **1986**, *7*, 385.
35. Stewart, J. J. P.; *MOPAC2009*; Stewart Computational Chemistry, Colorado Springs, 2008.
36. Gasteiger, J.; Marsili, M.; *Tetrahedron* **1980**, *36*, 3219.
37. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J.; *J. Comput. Chem*. **2009**, *30*, 2785.
38. Trott, O.; Olson, A. J.; *J. Comput. Chem*. **2010**, *31*, 455.
39. Tetko, I. V.; Poda, G. I.; *J. Med. Chem*. **2004**, *47*, 5601.
40. Accelrys Software Inc.; *Discovery Studio Modeling Environment, Release 3.5*; Accelrys Software Inc., San Diego, 2012.
41. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B.; *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 2596.
42. Lee, B.-Y.; Park, S. R.; Jeon, H. B.; Kim, K. S.; *Tetrahedron Lett.* **2006**, *47*, 5105.
43. Mthembu, X.; Van Heerden, F.; Fouché, G.; *S. Afr. J. Bot*. **2010**, *76*, 82.
44. Langat, M. K.; Crouch, N. R.; Pohjala, L.; Tammela, P.; Smith, P. J.; Mulholland, D. A.; *Phytochem. Lett.* **2012**, *5*, 414.
45. Olagnier, D.; Costes, P.; Berry, A.; Linas, M. D.; Urrutigoity, M.; Dechy-Cabaret, O.; Benoit-Vical, F.; *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6075.
46. Elokely, K. M.; Doerksen, R. J.; *J. Chem. Inf. Model*. **2013**, *53*, 1934.
47. Klebe, G.; *Nat. Rev. Drug Discovery* **2015**, *14*, 95.

Submitted: September 16, 2015
Published online: October 30, 2015