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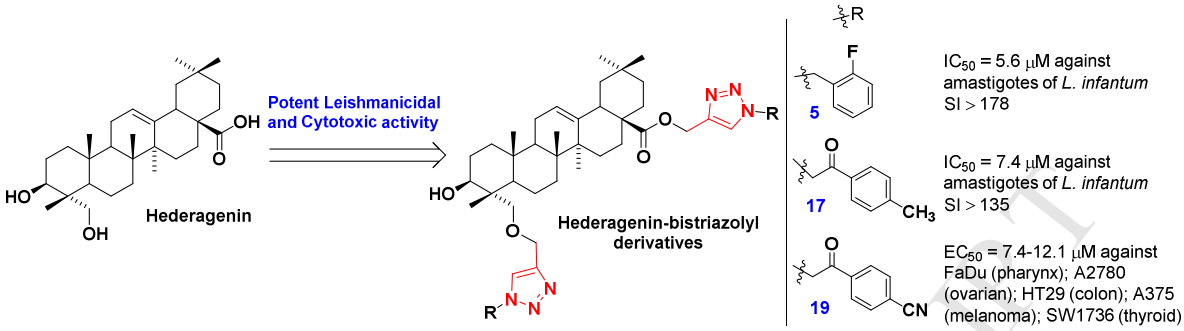
Graphical abstract



Leishmanicidal and cytotoxic activity of hederagenin-bistriazolyl derivatives

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Abstract

Aiming to obtain new potent leishmanicidal and cytotoxic compounds from natural sources, the triterpene hederagenin was converted into several new 1,2,3-triazolyl derivatives tethered at C-23 and C-28. For this work hederagenin was isolated from fruits of *Sapindus saponaria* and reacted with propargyl bromide to afford as a major product bis-propargylic derivative **1** in 74%. Submitting this compound to Huisgen 1,3-dipolar cycloaddition reactions with several azides afforded the derivatives **2-19** with yields in the range of 40-87%. All compounds have been screened for *in vitro* cytotoxic activity in a panel of five human cancer cell lines by a SRB assay. The bioassays showed that compound **19** was the most cytotoxic against all human cancer cell lines with EC_{50} = 7.4-12.1 μM. Moreover, leishmanicidal activity was evaluated through the *in vitro* effect in the growth of *Leishmania infantum*, and derivatives **1**, **2**, **5** and **17** were highly effective preventing proliferation of intracellular amastigote forms of *L. infantum* (IC_{50} = 28.8, 25.9, 5.6 and 7.4 μM, respectively). All these compounds showed a higher selectivity index and low toxicity against two strains of kidney BGM and liver HepG2 cells. Compound **5** has higher selectivity (1780 times) in comparison with the commercial antimony drug and is around 8 times more selective than the most active compound previously reported hederagenin derivative. Such high activity associated with low toxicities make the new bis-traiazolyl derivatives promising candidates for the treatment of leishmaniasis. In addition, hederagenin and some derivatives (**2**, **5** and **17**) showed interaction in the binding site of the enzyme $CYP51_{Li}$.

Keywords: Hederagenin, tryazol, *Leishmania infantum*, Cytotoxicity, $CYP51_{Li}$

1. Introduction

Natural pentacyclic triterpenes are widely found in several plant species like those from the Celastraceae, Lamiaceae, Sapindaceae, Fabaceae family [1-3]. These compounds are also found in olives, apples, figs, and cranberries [2-4], as well as in some oriental and traditional medicine herbs widely distributed all over the world [5-6]. They often exhibit a wide spectrum of biological activities such as anti-inflammatory [7], cytotoxic [8], antiprotozoal [9], anti-hypoglycemic [10], antibacterial [11], antiviral [12], and larvicidal [13].

Among the numerous terpenoids found in nature, we highlight Hederagenin (Fig. 1), a pentacyclic oleane-type triterpenoid found in large quantities in the pericarps of fruits of *Sapindus saponaria* (Sapindaceae) [14]. Several biological activities have been reported for hederagenin, including anti-inflammatory [15], antifungal [16], antimicrobial [17], anti-leishmanial [18] and antitumoral [14]. Due to such reports and our continued interest in the search for bioactive compounds based on natural products [19-24] we have recently started investigating the potential of hederagenin as a source of new anti-leishmanial [18] and antitumor [14, 25] drugs.

During this endeavor, we have synthesized a set of hederagenin derivatives modified at the carbonyl-28 via introduction of different groups such as benzylic, amine or heterocycles triazolyl (Fig. 1) [14, 18, 25]. Some derivatives holding a triazolyl moiety showed activity against several cancer cell lines being much higher than natural hederagenin [25]. In addition, some hederagenin derivatives are known for the ability to inhibit the proliferation of amastigote forms of *Leishmania infantum* (BH46) (Fig. 1), being active at micromolar level, presenting good selectivity index and low toxicity against two strains of kidney BGM and liver HepG2 cells. Thus, these results lead

hederagenin derivatives to be more potent than a commercial positive drug control (*potassium antimonyl tartrate trihydrate;* IC_{50} = 80 μM) [18].

[Insert Figure 1]

At the moment, the molecular target(s) or the mechanism(s) of action of hederagenin triazolyl derivatives against amastigote form of *L. infantum* remains unclear and is yet to be elucidated. Despite that, some studies report the role of triazolyl derivatives as effective antiparasitic drugs, potentially blocking sterol biosynthesis by inhibiting the enzymatic activity of a sterol 14α-demethylase (CYP51). This inhibition can lead to the accumulation of toxic methylated sterols, which in turn leads to parasitic death [26]. Sterol 14α-demethylase (CYP51) is an essential enzyme involved in the survival and virulence of *Trypanosoma* and *Leishmania* species, acting mainly in the biosynthesis of ergosterol. The disruption of this biosynthesis affects cytokinesis, stops cell growth, and can lead to the collapse of the cellular membrane of these parasites [26-27].

Crystal structures of protozoan sterol 14α-demethylases provide an opportunity for the structure-directed development of such inhibitors [26]. Hargrove et al. [28] determined the crystal structure of sterol 14α-demethylase ($CYP51_{Li}$) from *L. infantum* bound to fluconazole (PDB ID: 3L4D). In this context, the enzyme CYP51 has emerged as a promising target for antiprotozoal chemotherapy [26]. There are reports involving virtual screening of oleanolic acid, a pentacyclic triterpene that shows affinity for this enzyme $CYP51_{Li}$ [28-29].

In continuation of our studies aiming to obtain more active compounds based on the hederagenin scaffold, we report here a series of new bis-triazolyl derivatives modifying the C-23 and C-28 positions simultaneously. For all new compounds, the cytotoxic profiles against several cancer cell lines and the *in vitro* effect on the

proliferation of intracellular amastigote forms of *L. infantum* were investigated. An approach of the binding interactions of the most active compounds with sterol 14α-demethylase enzyme CYP51 from *L. infantum* (PDB ID: 3L4D) were explored through molecular docking.

2. Results and Discussion

2.1 Chemistry

The strategy envisaged in the present study involved the preparation of 18 new 1,2,3-triazolyl derivatives of hederagenin employing Huisgen 1,3-dipolar cycloaddition reactions [30]. The essential hederagenin was isolated from the pericarp of *S. saponaria* as previously described [14]. Subsequently, the bis-propargyl derivative **1** was prepared in 74% by the reaction of hederagenin with propargyl bromide in the presence of NaH (Scheme 1). A small quantity of the propargylic ester, not alkylated at the alcohols OH, was isolated as a side product and was identical with a previously reported ester [14].

The required substituted alkylazides (**a**-**r**) were prepared via the reaction of sodium azide and the corresponding alkylbromides (for details see Scheme S1 in the Supplementary material). The reaction of **1** with azides (**a**-**r**), catalyzed by copper (I) in the presence sodium ascorbate as reducing agent, afforded 18 new bis-triazolyl-hederagenin derivatives **2-19**. The overall chemical yields of the synthesized derivatives ranged from 41-87% (Scheme 1).

[Insert Scheme 1]

The purity of the compounds was proven by HRMS and microanalysis, and their identity confirmed by extensive NMR analyses. Thus, in the ^{1}H NMR spectra, the formation of the heterocyclic unit was confirmed by the signal around δ = 7.45-7.75 corresponding to the hydrogen of the triazolyl ring. The corresponding signals of the

aromatic hydrogen atoms were detected between δ = 6.80 and 8.25. Concerning to ^{13}C NMR, all signals for the triterpenoic skeleton in this series of compounds showed similar shifts to the parent hederagenin with exception of the signals for C-23 and C-28 and the groups attached at these positions. For the ester carbon C-28 a shift to low frequency was observed when compared with parent (δ = 180.7 for hederagenin to δ = 177.4 ± 0.4 for the esters carbonyl-28). Regarding to C-23 a shift to high frequency was observed as compared to natural triterpene (δ = 68.7 for hederagenin to δ = 75 ± 0.5 for the carbon-23). The signals of the aromatic carbons were detected at high frequency at δ = 163.5-104.5. For compounds, **5** and **15** the occurrence of ^{19}F-^{13}C couplings were detected in the ^{13}C NMR spectra. A detailed assignment of the NMR spectra (^{1}H and ^{13}C) for all compounds is presented in the Supplementary material associated to this paper. The assignments were possible by means of 2D NMR techniques when required, and for ^{13}C NMR the assignments were also supported by the literature [31].

2.2 Biological assays – cytotoxicity

The cytotoxic activity of the hederagenin derivatives (**2**-**19**) was investigated *in vitro* employing five human tumor cell lines (pharynx carcinoma - FaDu; ovarian carcinoma - A2780; colon adenocarcinoma - HT29; malignant melanoma - A375; thyroid carcinoma - SW1736); by using the well-established photometric sulforhodamine B assay (SRB) [32]. For comparison, betulinic acid was used as a positive standard control. The results of these assays are summarized in Table 1.

[Insert Table 1]

Amongst all new hederagenin derivatives, few were endowed with high cytotoxicity against the human tumor cell lines tested. Most were less active that the cut-off concentration of 30 μM. The derivatives **5**, **6** and **7** showed low cytotoxicity with

EC_{50} around 16.3-29.1 μM in the cell lines tested. However, compound **19** carrying in the triazolyl core a substituted 2-(*p*-cyanophenyl)-2-oxoethyl substituent, was the most cytotoxic against all human cancer cell lines showing EC_{50} in the range of 7.4-12.1 μM. The colon adenocarcinoma (HT29), a class of solid tumor, proved to be the most sensitive cell lines, even the compound **19** (EC_{50} = 7.4 μM) presented higher cytotoxic activity compared to standard compound (betulinic acid; EC_{50} = 18.4 μM) (Table 1).

To gain a deeper insight into the mode of action of compound **19** onto tumor cells HT29, an extra experiment was performed through fluorescence microscopic studies using acridine orange and propidium iodide staining (Fig. 2).

[Insert Figure 2]

The result for this assay have shown that no changes in cell morphology and cell membrane permeability occurred compared to the control. Hence, neither necrosis nor apoptosis occurred. Thus, the impact of the derivative **19** is likely the result from cytostatic effects rather than from being cytotoxic, since a significant inhibition of cell growth was observed. Furthermore, when comparing these results with those previously obtained by our group (Fig. 1) [25], we noticed that adding of a triazolyl core at carbon-23 has no influence on the cytotoxicity of this class of compounds.

2.3 Biological assays – antileishmania activity

The *in vitro* antileishmanial activity for all hederagenin derivatives **1**-**19** was evaluated in intracellular amastigote form of *L. infantum* (BH46) parasitized macrophages (DH82). Initially, all compounds were tested at 1000 μM (phase I) to select those with the most promising activity (see all results in Table S1 in the Supplementary material). The results revealed that hederagenin and seven derivatives

(**1**, **2**, **5**, **8**, **15**, **17** and **18**) reduced the multiplication of amastigotes of *L. infantum* by at least 50% (Table S1). Although no clear structure-activity relationship can be deduced at this stage, a comparison of the activity of **14** (Table S1) with those of other benzyl derivatives (**2**-**15**), shows that in general the most active compounds bear a substituent at *para* or *ortho* position. None of the compounds with a *meta*-substituent had a significant activity. On the other hand, among the four aryl-ketone derivatives (**16**-**19**), two were highly active at this phase I test. A comparison of the activities for the pairs **8**/**17** (with a *p*-methylphenyl group) and **14**/**16** (phenyl group), revealed no clear influence of the extra C=O moiety attached to the phenyl ring on the activity.

Following the initial assay, the most active compounds were then subjected to the same assay at lower concentrations as 0.1, 1.0, 10 and 100 μM (phase II). It was noted that although compounds **8** and **18** were able to reduce the growth of amastigotes in the phase 1 test, there was no clear correlation between the bioactivity and concentration, precluding the calculation of the IC_{50} values in the phase 2 test. Along with these tests, the hederagenin, selected compounds (**1**, **2**, **5**, **15** and **17**) and a positive drug control (*potassium antimonyl tartrate trihydrate*) were tested on cellular viability, using the canine macrophages cell line DH82, Monkey African green kidney BGM and human hepatocytes-like HepG2 cells, by microscopically monitoring and by using the colorimetric tetrazolium salt assay (MTT).

Hederagenin and compounds **1**, **2**, **5**, **15** and **17** showed good results, preventing the proliferation of intracellular amastigote forms of *L. infantum* (BH46), presenting IC_{50} values in the range of 5.4-154.8 μM (Table 2). All these calculated IC_{50} values were obtained from four replicates experiments. A representative sigmoidal dose-response curve (log [substance] versus percentage inhibition of intracellular amastigote

forms of *L. infantum*) is illustrated for compound **5** (Fig. 3). Evaluation of the curves allowed the determination of IC_{50} values.

[Insert Figure 3]

Among these, the derivatives **1**, **2**, **5** and **17** were more active than parent hederagenin, while compound **15** (holding a 2,4-difluorobenzyl moiety) was the only one with a reduced activity (with IC_{50} = 154.8 μM) compared to the commercial drug based on antimony (III) salt used as a positive control (IC_{50} = 80 μM) (Table 2). The hederagenin derivatives substituted with *o*-fluorobenzyl (**5**) and 2-(*p*-methylphenyl)-2-oxoethyl (**17**) moiety linked to a 1,2,3-triazolyl group were most potent showing IC_{50} values between 5.4 and 7.4 μM. These results represent an 11- and 8-fold higher activity than hederagenin and 14- and 10-fold higher than the antimonyl tartrate drug used as positive control, respectively.

Since it has been shown that interaction between some lupane derivatives and the antileishmanial drug miltefosine result in synergistic effect [33], we could expect that a similar interaction could also potentiate the hederagenin derivatives.

Having now selected the most active compounds, these were subjected to a MTT assay, and the results of the cytotoxic concentration to canine macrophages (DH82) are presented in Table 2. As the experiments revealed, hederagenin and derivatives **1**, **2**, **5** and **17** to exhibit a remarkably high selectivity index (ranging from 9 to >178) compared with that of antimony commercial drug (SI = 0.1). It is important to point out that although the most active bis-triazolyl derivative **5** had IC_{50} 2.8 times higher than the most active amide (Fig. 1, X = *o*-NO_2) previously reported (IC_{50} = 5.6 versus 2.0 μM), it was at least 8 times more selective (SI = >178 versus 22.5).

[Insert Table 2]

Following this selectivity study, the most active compounds were tested for their cytotoxicity against two strains of liver (HepG2) and kidney (BGM) cells to evaluate their effects on different parts of the organisms; the results are compiled in Table 3.

Concerning the kidney cells, the natural hederagenin and compounds **2**, **5** and **17** presented higher values of selective index (SI) than the corresponding value for the positive control. Thereby, the antimony drug presented SI = 1.6 (BGM) confirming its high toxicity as already reported [34]. In relation to this parameter the most selective compounds were **2** (SI = 38), **5** (SI = >178) and **17** (SI = >135). It is generally accepted that a drug candidate should have a selectivity index of at least 10 [35] to be regarded as a promising candidate for further testing.

In the same way, for the liver cells, the hederagenin derivatives **2**, **5** and **17** showed selective index equal to 38, >178 and >135, respectively (Table 3), much higher in comparison with the observed for the antimony commercial drug (SI = 0.3). Such results make these compounds 127 to 593 times more selective towards the human hepatocytes than the reference drug.

When comparing these results with those previously obtained by our group [19], we observed that adding a triazolyl core at carbon-23, the toxicity towards *L. infantum* parasite was approximately the same, however, the selectivity is highly improved in comparison with the most active amide derivatives previously reported (Table 1) [36].

Considering these results on the toxicity of some bis-tryazolyl derivatives towards macrophages, monkey kidney and human hepatocytes cells, these compounds seem to be promising candidates for further development of a new drug for the treatment of *L. infantum* infection.

[Insert Table 3]

2.4. *Prediction of the binding mode of hederagenin and bis-tryazolyl-derivatives (**2**, **5** and **17**) in the CYP51$_{Li}$ structure*

An approach of molecular modeling was used to verify a possible leishmanicidal mode of action via the ergosterol inhibition pathway. We employed the Autodock 4.2 program [37] that has a free energy scoring function, which uses an AMBER force field to estimate the free energy of binding of a ligand to its target [38]. Hederagenin and the most active derivatives (**2**, **5** and **17**) were docked into the crystallographic structure of CYP51 from *L. infantum* available in the Protein Data Bank (PDB code: 3L4D; resolution: 2.75 Å). These compounds showed satisfactory docking with acceptable statistics near the active site of CYP51$_{Li}$. Detailed results of analysis (free energy of binding values for each ligand along with the description of interacting residues) are presented in Table 4.

[Insert Table 4]

The residues involved in bonding and nonbonding interaction with ligands are as follows: Ile-167, Glu-204, Ser-205, Cys-206, Leu:-207, Pro-209, Ala-210, Phe-213, Gln-224, Phe-289, Ile-297, His-293, Arg-346, Met-405, Phe-412, Phe-415, Gly-416, Asn-455, His-457, Trh-458, Met-459, Gly-462, Pro-463 and Ala-465. The residues involved hydrogen bonding with the ligands are as follows: Glu-204, Ser-205, Arg-227, Lys-406, Glu-425, Met-459, Val-460. These residues are found near the active site (heme cofactor). These ligands (hederagenin and derivatives **2**, **5**, **17**) are surrounded by the above residues; it can be concluded that these residues are highly conserved and play important functional roles. Thus, interaction of the ligands with these residues might be changing the active site structure, which leads to an inhibition of the enzyme. In addition, these ligands show low free energy of binding against CYP51$_{Li}$; thus, they

bind with high affinity. For example, the most active compound (**5**) showed the best binding energy followed by **17**, **2** and hederagenin in that order. The interactions for this derivative **5** with the enzyme are represented in Fig. 4A. The figure shows that the lead molecules docks at a position near the active site of $CYP51_{Li}$, where also the cofactor heme (shown in green color) is bound.

[Insert Figure 4]

The protein contact potential was generated for the derivative **5** (Fig. 4B) depicting the whole surface of the protein. Red and blue colors show negative and positive charges, respectively. In this depiction, the triazolyl derivative **5** was shown to be bound inside a cavity formed at the interface of the enzyme. Although the docked conformation was different for each compound tested, the ligands are positioned at almost the same place, thus showing a consensus pattern of interactions with the residues near the active site. Figures showing interactions of hederagenin and derivatives **2** and **17** with the enzyme $CYP51_{Li}$ can be found in the Supplementary material. It can be predicted that the inhibition modes of all of these ligands could be similar, because of their interactions with almost the same residues.

3. Conclusion

We have described the synthesis and biological activities of 18 new triterpenoid derivatives of natural product hederagenin bearing two triazolyl-aromatic substituted moieties. Regarding the cytotoxic activity, the majority of the bis-triazolyl-derivatives were active in high micromolar ranges. Concerning antileishmanial activity, the most active bis-triazolyl derivatives presented very high selectivity index in relation to BGM and HepG2 line cells, revealing to be much less toxic in comparison with the antimonyl commercial drug used in the treatment of leishmaniasis. Thus, considering that

hederagenin can be obtained in large quantities from natural source, the new derivatives herein reported can be used as a lead for the development of the novel drug against *Leishmania*.

Experimental Section

4.1. General experimental procedures

Reagents were procured from Sigma Aldrich (Milwaukee, Wisconsin, USA) and were used without any purification. Solvents were supplied by Vetec (Rio de Janeiro, Brazil). Analytical thin layer chromatography (TLC) were performed on silica gel 60 F_{254} 0.2 mm thick plates (supplied by Merck, Rio de Janeiro, Brazil) and were visualized under UV-B light or by spraying with phosphomolybdic acid in 10% ethanol, followed by heating. Flash column chromatography (typical size of 20 cm length and 2 cm of diameter) was performed using silica gel 230-400 mesh. All compounds were fully characterized by IR, ESI-MS (or ASAP-MS), ^{1}H NMR and ^{13}C NMR spectroscopy. Infrared spectra were recorded on a Perkin Elmer Paragon 1000 FTIR spectrophotometer, preparing the samples as potassium bromide disks (1% w/w). Mass spectra were recorded on a Shimadzu GCMS-QP5050A instrument by direct insertion, using EI mode (70 eV). Mass spectra were recorded on an Advion express ion^{L} CMS instrument (ion source: ESI or ASAP, positive and negative mode). Elemental analyses were measured on a Foss-HeraeusVario EL unit. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance at 400 MHz and 100 MHz, using $CDCl_3$ as solvent (25°C). Chemicals shifts (δ) are reported from tetramethylsilane with the solvent resonance as the internal standard. Data are reported as follows: chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, coupling constants (J = Hz) and assignment. Melting points were measured

with on a MQAPF-301 apparatus. The ^{1}H NMR spectra for all compound were assigned for the signals that were clearly well defined as described for each compound. For all compounds a multiplet was observed in the range of 0.5-2.5 ppm, so the hydrogen atoms in such range could not be assigned.

*4.2. Isolation of Hederagenin (**He**)*

Fruits of *Sapindus saponaria* were collected in the municipality Tocantins (21°10′30″S and 43°01′04″W), Minas Gerais State, Brazil. Voucher specimens were dried and placed in the collection of the VIC Herbarium of the Plant Biology Department of Viçosa Federal University (UFV), under the register number VIC 35.403. Hederagenin was isolated from pericarp of *S. saponaria* L., using the method previously reported [36]. It was obtained as a white solid; m.p. 319-321 °C (lit.: 318-320 °C) [14]; R_f = 0.24 (hexane/ethyl acetate, 1:1 v/v). All spectroscopic data (IR, MS and NMR) were in agreement with the literature [31].

*4.3. Procedure for the synthesis of (3β)3-hydroxyolean-12-en-23,28-dipropargyl-28-oate (**1**).*

Hederagenin (1.0 g, 2.1 mmol) was allowed to react with NaH (80% in oil, 0.19 g, 8 mmol) in dry THF (20 mL) under nitrogen atmosphere for 2 hours before a solution of propargyl bromide (0.53 g; 4.5 mmol in 5 mL THF) was slowly added. The reaction mixture was stirred for another 24 h, and quenched by adding water (75 mL). The aqueous phase was extracted with ethyl acetate (3 x 50 mL) and the combined organic phases were dried over sodium sulfate (Na_2SO_4). The filtrate was evaporated under reduced pressure to afford a white residue that was purified by silica gel column chromatography (gradient elution: hexane/ethyl acetate 6:1 to hexane/ethyl acetate 1:1) to yield compound **1** as a white solid in 74% yield (767 mg; 1.40 mmol): R_f = 0.55

(hexane/ethyl acetate 1:1 v/v); m.p: 83.5-85.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ= 5.30 (brs, 1H, H-12); 4.67 (d, 1H, J = 15.4 Hz, H-31$_a$); 4.57 (d, 1H, J = 15.5 Hz, H-31$_b$); 4.17 (d, 1H, J = 16.0 Hz, H-34$_a$); 4.11 (d, 1H, J = 16.0 Hz, H-34$_b$); 3.61 (m, 1H, H-3); 3.56 (d, 1H, J = 8.6 Hz, H-23$_a$); 3.31 (d, 1H, J = 8.6 Hz, H-23$_b$); 2.86 (brd, 1H, J = 12.6 Hz, H-18); 2.44 (brs, 1H, H-33); 2.42 (brs, 1H, H-36); 1.13 (s, 3H, CH_3); 0.93 (s, 3H, CH_3); 0.92 (s, 3H, CH_3); 0.89 (s, 3H, CH_3); 0.86 (s, 3H, CH_3); 0.74 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 176.78 (C-28); 143.41 (C-13); 122.69 (C-12); 79.71 (C-32); 79.25 (C-35); 78.20 (C-3); 75.74 (C-23); 74.67 (C-33); 74.51 (C-36); 58.73 (C-31); 51.71 (C-34); 49.88 (C-9); 47.66 (C-17); 46.84 (C-5); 45.91 (C-19); 41.87 (C-4); 41.79 (C-14); 41.35 (C-18); 39.44 (C-8); 38.13 (C-1); 36.94 (C-10); 33.92 (C-21); 33.16 (C-29); 32.56 (C-7); 32.27 (C-22); 30.74 (C-20); 27.43 (C-15); 26.09 (C-27); 25.94 (C-2); 23.69 (C-30); 23.45 (C-11); 23.09 (C-16); 18.69 (C-6); 17.19 (C-26); 15.68 (C-25); 12.24 (C-24).

*4.4. General procedure for the synthesis of azides **a-r***

The azides **a**-r were prepared as indicated in Scheme S1 (Supplementary material) using methods previously published [25, 39].

*4.5. General procedure for the synthesis of bis-triazolyl-hederagenin derivatives **2-19**)*

A 50 mL round-bottomed flask was charged with compound **1** (44 mg; 0.08 mmol), the appropriate azide (0.50 mmol), $CuSO_4.5H_2O$ (126 mg; 0.50 mmol) and Na-L-ascorbate (200 mg; 1 mmol). To this mixture were added CH_2Cl_2 (8 mL) and H_2O (8 mL). The reaction mixture was stirred vigorously for 24 h at room temperature, until TLC analysis revealed a total consumption of the starting material **1**. The reaction mixture was quenched by addition of H_2O (20 mL) followed by extraction of the product with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with

brine (50 mL), dried over Na_2SO_4, filtered, and the solvent was removed in a rotary evaporator under reduced pressure. The crude product was purified by column chromatography on silica gel, using gradient elution: hexane/ethyl acetate 4:1 to hexane/ethyl acetate 1:2 to afford the products **2**-**19** each as a solid material, respectively.

*4.5.1 (p-Nitrobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(p nitrobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**2**)*

White solid (43 mg; 60 %): R_f = 0.39 (hexane/ethyl acetate 1:1 v/v); m.p: 114.3-116.1 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3408, 3154, 3152, 3086, 1720, 1528, 1162, 1058, 1034 807 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 8.18 (d, 4H, *J* = 8.4 Hz, H-37, H-37', H-45, H-45'); 7.59 (s, 1H, H-33); 7.55 (s, 1H, H-41); 7.38 (d, 4H, *J* = 8.4 Hz, H-36, H-36', H-44, H-44'); 5.62 (d, 4H, *J* = 13.6 Hz, H-34, H-42); 5.20 (brs, 1H, H-12); 5.17 (d, 1H, *J* = 12.9 Hz, H-31_a); 5.12 (d, 1H, *J* = 12.9 Hz, H-31_b); 4.62 (brt, 2H, *J* = 13.5 Hz, H-39); 3.56 (d, 1H, *J* = 8.6 Hz, H-23_a); 3.50 (m, 1H, H-3); 3.25 (d, 1H, *J* = 8.6 Hz, H-23_b); 2.79 (brd, 1H, *J* = 8.5 Hz, H-18); 1.02 (s, 3H, CH_3); 0.86 (s, 3H, CH_3); 0.85 (s, 3H, CH_3); 0.82 (s, 3H, CH_3); 0.79 (s, 3H, CH_3); 0.45 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.78 (C-28); 148.37 (C-38, C-46); 144.20 (C-13); 143.51 (C-32, C-40); 141.76 (C-43); 141.70 (C-35); 128.68 (C-36, C-36', C-44, C-44'); 124.37 (C-45, C-45'); 124.34 (C-37, C-37'); 122.62 (C-12); 122.48 (C-33, C-41); 80.02 (C-3); 75.89 (C-23); 64.91 (C-39); 57.42 (C-31); 53.25 (C-34); 53.11 (C-42); 49.87 (C-9); 47.56 (C-17); 46.81 (C-5); 45.91 (C-19); 41.90 (C-4); 41.77 (C-14); 41.34 (C-18); 39.30 (C-8); 38.10 (C-1); 36.87 (C-10); 33.88 (C-21); 33.07 (C-29); 32.48 (C-7); 32.42 (C-22); 30.70 (C-20); 27.68 (C-15); 26.03 (C-27); 25.78 (C-2); 23.61 (C-30); 23.35 (C-11); 23.05 (C-16); 18.63 (C-6); 16.82 (C-26); 15.65 (C-25); 12.16 (C-24); EIMS *m/z* 905.3 $[M+H]^+$; anal. C 66.03, H 7.27, N 12.00%, calcd for $C_{50}H_{64}N_8O_8$, C 66.35, H 7.13, N 12.38%.

*4.5.2 (p-Bromobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(p bromobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**3**)*

White solid (58 mg; 75%): R_f = 0.52 (hexane/ethyl acetate 1:1 v/v); m.p: 102.8-104.3 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3411, 3153, 3150, 1635, 1041, 1014 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 7.43 (d, 4H, *J* = 7.9 Hz, H-37, H-37', H-45, H-45'); 7.46 (s, 1H, H-33); 7.44 (s, 1H, H-41); 7.11 (d, 4H, *J* = 8.3 Hz, H-36, H-36', H-44, H-44'); 5.46 (brs, 2H, H-42); 5.45 (d, 2H, *J* = 15.0 Hz, H-34_a); 5.41 (d, 2H, *J* = 15.0 Hz H-34_b); 5.20 (brs, 1H, H-12); 5.13 (t, 1H, *J* = 12.9 Hz, H-31); 4.60 (brs, 2H, H-39); 3.57 (d, 1H, *J* = 8.7 Hz, H-23_a); 3.50 (m, 1H, H-3); 3.25 (d, 1H, *J* = 8.7 Hz, H-23_b); 2.79 (dd, 1H, *J* = 3.8, 13.9 Hz, H-18); 1.04 (s, 3H, CH_3); 0.87 (s, 3H, CH_3); 0.86 (s, 3H, CH_3); 0.85 (s, 3H, CH_3); 0.82 (s, 3H, CH_3); 0.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.73 (C-28); 145.87 (C-13); 143.90 (C-40); 143.61 (C-32); 133.68 (C-43); 133.59 (C-35); 132.39 (C-37, C-37', C-45, C-45'); 129.73 (C-44, C-44'); 129.70 (C-36, C-36'); 123.10 (C-38); 123.06 (C-46); 123.89 (C-12); 122.46 (C-33); 122.23 (C-41); 80.13 (C-3); 75.93 (C-23); 64.96 (C-39); 57.57 (C-31); 53.60 (C-34); 53.50 (C-42); 49.96 (C-9); 47.59 (C-17); 46.79 (C-5); 45.92 (C-19); 41.90 (C-4); 41.78 (C-14); 41.35 (C-18); 39.32 (C-8); 38.11 (C-1); 36.88 (C-10); 33.90 (C-21); 33.11 (C-29); 32.47 (C-7); 32.39 (C-22); 30.70 (C-20); 27.69 (C-15); 26.03 (C-27); 25.85 (C-2); 23.65 (C-30); 23.36 (C-11); 23.06 (C-16); 18.67 (C-6); 16.82 (C-26); 15.66 (C-25); 12.21 (C-24); EIMS *m/z* 995.2 $[M+Na]^+$; anal. C 61.52, H 6.91, N 8.54%, calcd for $C_{50}H_{64}Br_2N_6O_4$, C 61.73, H 6.63, N 8.64%.

*4.5.3 (p-Chlorobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(p chlorobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**4**)*

White solid (57 mg; 82%): R_f = 0.51 (hexane/ethyl acetate 1:1 v/v); m.p: 112.6-114.1 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3403, 3156, 3152, 3061, 1719, 1631, 1523, 1047, 1014, 731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 7.47 (s, 1H, H-33); 7.44 (s, 1H, H-41); 7.31 (d,

4H, *J* = 8.3 Hz, H-37, H-37', H-45, H-45'); 7.17 (d, 4H, *J* = 8.2 Hz, H-36, H-36', H-44, H-44'); 5.44 (m, 4H, *J* = 15.0 Hz H-34, H-42); 5.20 (brs, 1H, H-12); 5.13 (s, 1H, H-31); 4.59 (brs, 2H, H-39); 3.56 (d, 1H, *J* = 8.7 Hz, H-23_a); 3.50 (m, 1H, H-3); 3.25 (d, 1H, *J* = 8.7 Hz, H-23_b); 2.78 (dd, 1H, *J* = 3.1, 13.6 Hz, H-18); 1.04 (s, 3H, CH_3); 0.86 (s, 6H, 2xCH_3); 0.84 (s, 3H, CH_3); 0.81 (s, 3H, CH_3); 0.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): *δ*= 177.63 (C-28); 145.78 (C-13); 143.78 (C-40); 143.51 (C-32); 134.87 (C-46); 134.82 (C-38); 133.12 (C-35); 133.00 (C-43); 129.37 (C-45, C-45'); 129.32 (C-36, C-36', C-37, C-37', C-44, C-44'); 123.79 (C-12); 122.51 (C-33); 122.10 (C-41); 79.97 (C-3); 75.80 (C-23); 64.89 (C-39); 57.48 (C-31); 53.41 (C-34); 53.34 (C-42); 49.84 (C-9); 47.50 (C-17); 46.69 (C-5); 45.83 (C-19); 41.81 (C-4); 41.68 (C-14); 41.26 (C-18); 39.22 (C-8); 38.01 (C-1); 36.78 (C-10); 33.80 (C-21); 33.01 (C-29); 32.37 (C-7); 32.30 (C-22); 30.62 (C-20); 27.59 (C-15); 25.93 (C-27); 25.73 (C-2); 23.55 (C-30); 23.27 (C-11); 22.96 (C-16); 18.56 (C-6); 16.71 (C-26); 15.55 (C-25); 12.11 (C-24); EIMS *m/z* 905.2 $[M+Na]^+$; anal. C 67.69, H 7.58, N 9.39%, calcd for $C_{50}H_{64}Cl_2N_6O_4$, C 67.93, H 7.30, N 9.51%.

*4.5.4 (o-Fluorobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(o fluorobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**5**)*

White solid (45 mg; 66%): R_f = 0.48 (hexane/ethyl acetate 1:1 v/v); m.p: 84.6-85.8 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3404, 3152, 3150, 3052, 1637, 1524, 1233, 1048, 1005, 756 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): *δ*= 7.54 (s, 1H, H-33); 7.52 (s, 1H, H-43); 7.32 (m, 2H, H-40, H-50); 7.23 (m 2H, H-38, H-48); 7.10 (m 4H, H-37, H-39, H-47, H-49); 5.56 (brs, 2H, H-34); 5.53 (brs, 2H, H-44); 5.20 (t, 1H, *J* = 3.0 Hz, H-12); 5.12 (brs, 1H, H-31); 4.59 (brs, 2H, H-41); 3.56 (d, 1H, *J* = 8.7 Hz, H-23_a); 3.50 (m, 1H, H-3); 3.25 (d, 1H, *J* = 8.7 Hz, H-23_b); 2.79 (dd, 1H, *J* = 3.8, 13.8 Hz, H-18); 1.05 (s, 3H, CH_3); 0.86 (s, 9H, 3xCH_3); 0.81 (s, 3H, CH_3); 0.49 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): *δ*=

177.69 (C-28); 160.65 (d, *J* = 246.7 Hz, C-36); 160.62 (d, *J* = 246.3 Hz, C-46); 145.59 (C-13); 143.70 (C-32); 143.62 (C-42); 131.10 (d, *J* = 3.5 Hz, C-40); 131.02 (d, *J* = 3.6 Hz, C-50); 130.70 (d, *J* = 3.0 Hz, C-38); 130.61 (d, *J* = 12.6 Hz, C-48); 124.94 (d, *J* = 2.0 Hz, C-35); 124.91 (d, *J* = 2.1 Hz, C-45); 124.02 (d, *J* = 1.0 Hz, C-49); 122.48 (C-12); 122.01 (C-33); 121.88 (d, *J* = 1.5 Hz, C-39); 121.74 (C-43); 115.94 (d, *J* = 21.0 Hz, C-37, C-47); 80.16 (C-3); 75.96 (C-23); 64.92 (C-41); 57.58 (C-31); 49.99 (C-9); 47.84 (d, *J* = 4.2 Hz, C-34); 47.74 (d, *J* = 4.2 Hz, C-44); 47.60 (C-17); 46.78 (C-5); 45.94 (C-19); 41.88 (C-4); 41.79 (C-14); 41.37 (C-18); 39.33 (C-8); 38.12 (C-1); 36.90 (C-10); 33.91 (C-21); 33.13 (C-29); 32.45 (C-7); 32.39 (C-22); 30.72 (C-20); 27.68 (C-15); 26.00 (C-27); 25.86 (C-2); 23.66 (C-30); 23.39 (C-11); 23.05 (C-16); 18.69 (C-6); 16.76 (C-26); 15.66 (C-25); 12.19 (C-24); EIMS *m/z* 873.6 $[M+Na]^+$; anal. C 70.31, H 7.72, N 9.72%, calcd for $C_{50}H_{64}F_2N_6O_4$, C 70.56, H 7.58, N 9.87%.

*4.5.5 (o-Chlorobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(o chlorobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**6**)*

White solid (29 mg; 41%): R_f = 0.51 (hexane/ethyl acetate 1:1 v/v); m.p: 94.9-96.3 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3400, 3153, 3150, 3072, 1723, 1636, 1520, 1048, 751, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 7.57 (s, 1H, H-33); 7.55 (s, 1H, H-43); 7.43 (brd, 2H, *J* = 7.8 Hz, H-37, H-47); 7.32 (brt, 2H, *J* = 7.8 Hz, H-40, H-50); 7.26 (m, 2H, H-38, H-48); 7.19 (brt, 2H, *J* = 7.5 Hz, H-39, H-49); 5.66 (brs, 2H, H-44); 5.65 (d, 1H, *J* = 15.1 Hz, H-34_a); 5.61 (d, 1H, *J* = 15.1 Hz, H-34_b); 5.23 (brs, 1H, H-12); 5.18 (s, 1H, *J* = 13.1 Hz, H-31_a); 5.14 (s, 1H, *J* = 13.1 Hz, H-31_b); 4.63 (brs, 2H, H-41); 3.59 (d, 1H, *J* = 8.7 Hz, H-23_a); 3.53 (m, 1H, H-3); 3.26 (d, 1H, *J* = 8.7 Hz, H-23_b); 2.81 (brdd, 1H, *J* = 3.5, 13.9 Hz, H-18); 1.08 (s, 3H, CH_3); 0.89 (s, 9H, 3xCH_3); 0.84 (s, 3H, CH_3); 0.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.71 (C-28); 145.50 (C-13); 143.65 (C-32); 143.65 (C-42); 133.69 (C-35); 133.59 (C-45); 132.47 (C-36); 132.35 (C-

46); 130.55 (C-37); 130.42 (C-40, C-47); 130.40 (C-50); 130.07 (C-38); 130.05 (C-48); 127.71 (C-39); 127.68 (C-49); 124.18 (C-12); 122.68 (C-33); 122.49 (C-43); 80.12 (C-3); 75.98 (C-23); 64.90 (C-41); 57.61 (C-31); 51.60 (C-34); 51.60 (C-44); 50.01 (C-9); 47.62 (C-17); 46.80 (C-5); 45.94 (C-19); 41.90 (C-4); 41.80 (C-14); 41.38 (C-18); 39.34 (C-8); 38.14 (C-1); 36.91 (C-10); 33.93 (C-21); 33.14 (C-29); 32.47 (C-7); 32.40 (C-22); 30.73 (C-20); 27.70 (C-15); 26.02 (C-27); 25.88 (C-2); 23.68 (C-30); 23.41 (C-11); 23.05 (C-16); 18.71 (C-6); 16.83 (C-26); 15.67 (C-25); 12.20 (C-24); EIMS *m/z* 905.3 $[M+Na]^+$; anal. C 67.81, H 7.51, N 9.30%, calcd for $C_{50}H_{64}Cl_2N_6O_4$, C 67.93, H 7.30, N 9.51%.

*4.5.6 (p-Methylbenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(p methylbenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**7**)*

White solid (27 mg; 40%): R_f = 0.51 (hexane/ethyl acetate 1:1 v/v); m.p: 126.9-128.3 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3406, 3155, 3152, 3067, 1729, 1626, 1530, 1046, 749 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 7.46 (s, 1H, H-33); 7.43 (s, 1H, H-44); 7.17 (s, 8H, H-36, H-36', H-37, H-37', H-45, H-45', H-46, H-46'); 5.46 (brs, 2H, H-43); 5.45 (m, 2H, H-34); 5.22 (brs, 1H, H-12); 5.14 (brs, 2H, *J* = 13.1 Hz, H-31); 4.62 (brs, 2H, H-40); 3.59 (d, 1H, *J* = 8.6 Hz, H-23_a); 3.52 (m, 1H, H-3); 3.28 (d, 1H, *J* = 8.7 Hz, H-23_b); 2.81 (brdd, 1H, *J* = 3.2, 13.6 Hz, H-18); 2.35 (s, 6H, 2xCH_3); 1.08 (s, 3H, CH_3); 0.89 (s, 9H, 3xCH_3); 0.85 (s, 3H, CH_3); 0.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.74 (C-28); 145.49 (C-13); 143.67 (C-32); 143.61 (C-41); 138.87 (C-38); 138.85 (C-47); 131.56 (C-35); 131.46 (C-44); 129.92 (C-36, C-36'); 129.91 (C-45, C-45'); 128.26 (C-37, C-37'); 128.21 (C-46, C-46'); 123.78 (C-12); 122.48 (C-33); 122.20 (C-42); 80.39 (C-3); 76.10 (C-23); 64.97 (C-40); 57.65 (C-31); 54.20 (C-34); 54.09 (C-43); 50.09 (C-9); 47.63 (C-17); 46,80 (CH-5); 45,94 (CH-19); 41,90 (C-4); 41,80 (C-14); 41,38 (CH-18); 39,34 (C-8); 38,14 (CH_2-1); 36,92 (C-10); 33,94 (CH_2-21); 33,16 (CH_3-

29); 32,49 (CH_2-7); 32,40 (CH_2-22); 30,75 (C-20); 27,72 (CH_2-15); 26,00 (CH_3-27); 25,90 (CH_2-2); 23,69 (CH_3-30); 23,40 (CH_2-11); 23,06 (CH_2-16); 21.27 (C-38, C-48); 18,72 (CH_2-6); 16,84 (CH_3-26); 15,66 (CH_3-25); 12,21 (CH_3-24); EIMS *m/z* 843.4 $[M+H]^+$; anal. C 73.88, H 8.35, N 9.65%, calcd for $C_{52}H_{70}N_6O_4$, C 74.07, H 8.37, N 9.97%.

*4.5.7 (o-Bromobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(o bromobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**8**)*

White solid (36 mg; 47%): R_f = 0.50 (hexane/ethyl acetate 1:1 v/v); m.p: 97.8-98.9 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3386, 3153, 3150, 3070, 1720, 1639, 1522, 1047, 1033, 743, 731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 7.56 (m, 4H, H-33, H-37, H-43, H-47); 7.26 (brt, 2H, *J* = 6.8 Hz, H-39, H-49); 7.18 (brt, 2H, *J* = 7.4 Hz, H-40, H-50); 7.14 (m, 2H, H-38, H-48); 7.26 (m, 2H, H-38, H-48); 7.19 (brt, 2H, *J* = 7.5 Hz, H-39, H-49); 5.66 (brs, 2H, H-44); 5.60 (m, 4H, H-34, H-44); 5.18 (brs, 1H, H-12); 5.12 (brs, 2H, H-31); 4.59 (brs, 2H, H-41); 3.52 (m, 2H, H-3, H-23_a); 3.22 (d, 1H, *J* = 8.3 Hz, H-23_b); 2.77 (brd, 1H, *J* = 12.6 Hz, H-18); 1.03 (s, 3H, CH_3); 0.84 (s, 9H, 3xCH_3); 0.78 (s, 3H, CH_3); 0.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.52 (C-28); 145.34 (C-13); 143.46 (C-32); 143.41 (C-42); 134.06 (C-35); 133.88 (C-45); 133.20 (C-37); 133.16 (C-47); 130.45 (C-40); 130.39 (C-38, C-50); 130.22 (C-48); 128.18 (C-39); 128.16 (C-49); 123.50 (C-36); 123.36 (C-46); 124.13 (C-12); 122.65 (C-33); 122.31 (C-43); 79.52 (C-3); 75.55 (C-23); 64.70 (C-41); 57.45 (C-31); 53.79 (C-34); 53.74 (C-44); 49.67 (C-9); 47.42 (C-17); 46.60 (C-5); 45.75 (C-19); 41.73 (C-4); 41.61 (C-14); 41.19 (C-18); 39.15 (C-8); 37.96 (C-1); 36.72 (C-10); 33.74 (C-21); 33.01 (C-29); 32.28 (C-7); 32.24 (C-22); 30.58 (C-20); 27.53 (C-15); 25.86 (C-27); 25.74 (C-2); 23.54 (C-30); 23.25 (C-11); 22.87 (C-16); 18.51 (C-6); 16.67 (C-26); 15.55 (C-25); 12.12 (C-24); EIMS *m/z* 995.3

$[M+Na]^+$; anal. C 61.70, H 6.74, N 8.44%, calcd for $C_{50}H_{64}Br_2N_6O_4$, C 61.73, H 6.63, N 8.64%.

*4.5.8 (o-Nitrobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(o nitrobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**9**)*

White solid (59 mg; 81%): R_f = 0.39 (hexane/ethyl acetate 1:1 v/v); m.p: 94.8-96.4 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3046, 3158, 3155, 3064, 1718, 1528, 1162, 1048, 1006, 729 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 8.12 (d, 2H, *J* = 7.2 Hz H-37, H-47); 7.72 (s, 1H, H-33); 7.70 (brs, 1H, H-43); 7.59 (brt, 2H, *J* = 7.3 Hz, H-38, H-48); 7.52 (brt, 2H, *J* = 7.2 Hz H-39, H-49); 7.05 (brt, 2H, *J* = 6.3 Hz, H-40, H-50); 5.91 (d, 2H, *J* = 7.4 Hz, H-34, H-44); 5.23 (brs, 1H, H-12); 5.17 (brs, 2H, H-31); 4.64 (brs, 2H, H-41); 3.59 (d, 1H, *J* = 8.3 Hz, H-23_a); 3.53 (brt, 1H, *J* = 8.0 Hz, H-3); 3.28 (d, 1H, *J* = 8.2 Hz, H-23_b); 2.81 (brd, 1H, *J* = 12.6 Hz, H-18); 1.06 (s, 3H, CH_3); 0.87 (s, 9H, 3xCH_3); 0.82 (s, 3H, CH_3); 0.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.50 (C-28); 147.47 (C-36); 147.47 (C-46); 145.68 (C-13); 143.76 (C-32); 143.49 (C-42); 134.40 (C-35); 134.32 (C-45); 130.63 (C-39); 130.48 (C-49); 130.37 (C-40); 130.29 (C-50); 129.75 (C-38); 129.71 (C-48); 125.41 (C-37); 125.38 (C-47); 124.94 (C-12); 123.46 (C-33); 122.43 (C-43); 79.71 (C-3); 75.67 (C-23); 64.75 (C-41); 57.46 (C-31); 50.88 (C-34); 50.80 (C-44); 49.75 (C-9); 47.50 (C-17); 46.70 (C-5); 45.80 (C-19); 41.83 (C-4); 41.68 (C-14); 41.27 (C-18); 39.23 (C-8); 38.01 (C-1); 36.80 (C-10); 33.81 (C-21); 33.05 (C-29); 32.36 (C-7); 32.32 (C-22); 30.64 (C-20); 27.60 (C-15); 25.96 (C-27); 25.77 (C-2); 23.59 (C-30); 23.30 (C-11); 22.94 (C-16); 18.57 (C-6); 16.77 (C-26); 15.58 (C-25); 12.15 (C-24); EIMS *m/z* 905.4 $[M+H]^+$; anal. C 66.14, H 12.60, N 12.11%, calcd for $C_{50}H_{64}N_8O_8$, C 66.35, H 7.13, N 12.38%.

*4.5.9 (m-Chlorobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(m chlorobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**10**)*

White solid (34 mg; 48%): R_f = 0.48 (hexane/ethyl acetate 1:1 v/v); m.p: 122.4-123.9 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3401, 3155, 3150, 3088, 1633, 1520, 1048, 774, 733 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 7.51 (s, 1H, H-33); 7.47 (s, 1H, H-43); 7.30 (m, 6H, H-36, H-38, H-39, H-46, H-48, H-49); 7.11 (brd, 2H, *J* = 6.7 Hz, H-40, H-50); 5.50 (brs, 2H, H-34); 5.47 (d, 2H, *J* = 5.6 Hz, H-44); 5.22 (brs, 1H, H-12); 5.15 (s, 2H, H-31); 4.63 (brs, 2H, H-41); 3.59 (d, 1H, *J* = 8.6 Hz, H-23_a); 3.52 (m, 1H, H-3); 3.27 (d, 1H, *J* = 8.7 Hz, H-23_b); 2.81 (brdd, 1H, *J* = 2.9, 13.6 Hz, H-18); 1.06 (s, 3H, CH_3); 0.88 (s, 9H, 3xCH_3); 0.84 (s, 3H, CH_3); 0.51 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.80 (C-28); 145.98 (C-13); 143.98 (C-32); 143.65 (C-42); 136.68 (C-35); 136.53 (C-45); 135.20 (C-37); 135.19 (C-47); 130.56 (C-39); 130.53 (C-49); 129.18 (C-36); 129.14 (C-46); 128.22 (C-40); 128.12 (C-50); 126.18 (C-38); 126.12 (C-48); 124.05 (C-12); 122.51 (C-33); 122.31 (C-43); 80.28 (C-3); 76.12 (C-23); 65.04 (C-41); 57.61 (C-31); 53.59 (C-34); 53.54 (C-44); 50.06 (C-9); 47.63 (C-17); 46.84 (C-5); 45.97 (C-19); 41.92 (C-4); 41.83 (C-14); 41.44 (C-18); 39.37 (C-8); 38.14 (C-1); 36.94 (C-10); 33.94 (C-21); 33.16 (C-29); 32.52 (C-7); 32.43 (C-22); 30.76 (C-20); 27.73 (C-15); 26.05 (C-27); 25.88 (C-2); 23.70 (C-30); 23.41 (C-11); 23.09 (C-16); 18.74 (C-6); 16.84 (C-26); 15.71 (C-25); 12.22 (C-24); EIMS *m/z* 905.3 $[M+Na]^+$; anal. C 67.75, H 9.74, N 9.36%, calcd for $C_{50}H_{64}Cl_2N_6O_4$, C 67.93, H 7.30, N 9.51%.

*4.5.10 (m-Nitrobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(m nitrobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**11**)*

White solid (46 mg; 63%): R_f = 0.37 (hexane/ethyl acetate 1:1 v/v); m.p: 120.9-122.3 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3404, 3156, 3153, 3092, 1719, 1534, 1174, 1158, 1048, 729 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 8.20 (d, 2H, *J* = 7.4 Hz, H-38, H-48); 8.14 (d,

2H, *J* = 4.8 Hz, H-36, H-46); 7.58 (m, 6H, H-33, H-39, H-40, H-42, H-49, H-50); 5.62 (m, 4H, H-34, H-44); 5.21 (brs, 1H, H-12); 5.16 (s, 2H, H-31); 4.64 (brs, 2H, H-41); 3.58 (d, 1H, *J* = 8.6 Hz, H-23_a); 3.52 (m, 1H, H-3); 3.26 (d, 1H, *J* = 8.7 Hz, H-23_b); 2.80 (brdd, 1H, *J* = 3.0, 13.7 Hz, H-18); 1.04 (s, 3H, CH_3); 0.87 (s, 3H, CH_3); 0.86 (s, 3H, CH_3); 0.84 (s, 3H, CH_3); 0.81 (s, 3H, CH_3); 0.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.76 (C-28); 148.65 (C-37, C47); 146.17 (C-13); 144.14 (C-32); 143.53 (C-42); 136.87 (C-35); 136.73 (C-45); 133.94 (C-40); 133.90 (C-50); 130.38 (C-49); 130.35 (C-49); 124.25 (C-12); 123.84 (C-36); 123.81 (C-46); 122.91 (C-38); 122.83 (C-48); 122.48 (C-33, C-43); 79.89 (C-3); 75.84 (C-23); 65.91 (C-41); 57.47 (C-31); 53.17 (C-34); 53.11 (C-44); 49.83 (C-9); 47.53 (C-17); 46.78 (C-5); 45.88 (C-19); 41.89 (C-4); 41.75 (C-14); 41.34 (C-18); 39.29 (C-8); 38.07 (C-1); 36.85 (C-10); 33.85 (C-21); 33.07 (C-29); 32.45 (C-7); 32.38 (C-22); 30.63 (C-20); 27.66 (C-15); 26.02 (C-27); 25.79 (C-2); 23.61 (C-30); 23.34 (C-11); 23.01 (C-16); 18.61 (C-6); 16.78 (C-26); 15.64 (C-25); 12.15 (C-24); EIMS *m/z* 905.6 $[M+H]^+$; anal. C 66.11, H 7.36, N 12.18%, calcd for $C_{50}H_{64}N_8O_8$, C 66.35, H 7.13, N 12.38%.

4.5.11 (m-Bromobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(m bromobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate ***(12)***

White solid (37 mg; 48%): R_f = 0.52 (hexane/ethyl acetate 1:1 v/v); m.p: 166.1-167.3 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3394, 3151, 3148, 3061, 1730, 1639, 1519, 1241, 1048, 772, 732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.50 (s, 1H, H-33); 7.46 (brs, 2H, H-38, H-48); 7.44 (brs, 1H, H-43); 7.38 (brs, 2H, H-36, H-46); 7.21 (brt, 2H, *J* = 7.8 Hz, H-40, H-50); 7.15 (brd, 2H, *J* = 7.6 Hz, H-39, H-49); 5.47 (brs, 2H, H-44); 5.42 (m, 2H, *J* = 5.6 Hz, H-34); 5.19 (brs, 1H, H-12); 5.12 (s, 2H, H-31); 4.60 (brs, 2H, H-41); 3.56 (d, 1H, *J* = 8.6 Hz, H-23_a); 3.50 (m, 1H, H-3); 3.24 (d, 1H, *J* = 8.6 Hz, H-23_b); 2.78 (brdd, 1H, *J* = 2.2, 13.6 Hz, H-18); 1.03 (s, 3H, CH_3); 0.85 (s, 9H, 3xCH_3); 0.81 (s, 3H, CH_3);

0.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.72 (C-28); 145.92 (C-13); 143.91 (C-32); 143.58 (C-42); 136.90 (C-35); 136.74 (C-45); 132.05 (C-38); 132.00 (C-48); 131.06 (C-36); 130.96 (C-46); 130.75 (C-39); 130.73 (C-49); 126.61 (C-40); 126.55 (C-50); 124.00 (C-12); 123.19 (C-37, C-47); 122.45 (C-33); 122.30 (C-43); 80.00 (C-3); 75.92 (C-23); 64.96 (C-41); 57.55 (C-31); 53.44 (C-34); 53.39 (C-44); 49.91 (C-9); 47.57 (C-17); 46.77 (C-5); 45.91 (C-19); 41.88 (C-4); 41.77 (C-14); 41.35 (C-18); 39.31 (C-8); 38.09 (C-1); 36.87 (C-10); 33.88 (C-21); 33.11 (C-29); 32.44 (C-7); 32.37 (C-22); 30.70 (C-20); 27.68 (C-15); 26.02 (C-27); 25.83 (C-2); 23.67 (C-30); 23.36 (C-11); 23.04 (C-16); 18.68 (C-6); 16.78 (C-26); 15.68 (C-25); 12.20 (C-24); EIMS *m/z* 995.2 $[M+Na]^+$; anal. C 61.52, H 6.81, N 8.53%, calcd for $C_{50}H_{64}Br_2N_6O_4$, C 61.73, H 6.63, N 8.64%.

*4.5.12 (m-Methylbenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(m-methylbenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**13**)*

White solid (28 mg; 41%): R_f = 0.56 (hexane/ethyl acetate 1:1 v/v); m.p: 101.7-103.3 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3406, 3153, 3150, 3067, 1730, 1626, 1534, 1047, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 7.48 (s, 1H, H-33); 7.44 (brs, 1H, H-44); 7.26 (m, 2H, H-39, H-50); 7.17 (brs, 1H, H-36); 7.15 (brs, 1H, H-47); 7.06 (m, 4H, H-38, H-40, H-49, H-51); 5.49 (d, 1H, J = 14.7 Hz, H-34_a); 5.48 (brs, 2H, H-45); 5.43 (d, 1H, J = 14.7 Hz, H-34_b); 5.22 (t, 1H, J = 3.2 Hz, H-12); 5.15 (s, 2H, H-31); 4.62 (brs, 2H, H-42); 3.59 (d, 1H, J = 8.7 Hz, H-23_a); 3.52 (m, 1H, H-3); 3.28 (d, 1H, J = 8.6 Hz, H-23_b); 2.82 (brdd, 1H, J = 3.9, 13,9 Hz, H-18); 2.34 (s, 6H, 2xCH_3); 1.07 (s, 3H, CH_3); 0.89 (s, 9H, 3xCH_3); 0.85 (s, 3H, CH_3); 0.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.74 (C-28); 145.60 (C-13); 143.66 (C-32); 143.66 (C-43); 136.08 (C-37); 136.05 (C-48); 134.58 (C-35); 134.43 (C-46); 129.68 (C-36); 129.64 (C-47); 129.11 (C-39, C-50); 128.92 (C-38); 128.83 (C-49); 125.28 (C-40); 125.18 (C-51); 123.89 (C-12); 122.48 (C-

33); 122.23 (C-44); 80.40 (C-3); 76.16 (C-23); 65.04 (C-42); 57.67 (C-31); 54.31 (C-34); 54.26 (C-45); 50.11 (C-9); 47.63 (C-17); 46.79 (C-5); 45.96 (C-19); 41.88 (C-4); 41.81 (C-14); 41.39 (C-18); 39.35 (C-8); 38.12 (C-1); 36.92 (C-10); 33.93 (C-21); 33.15 (C-29); 32.48 (C-7); 32.39 (C-22); 30.74 (C-20); 27.71 (C-15); 25.99 (C-27); 25.88 (C-2); 23.69 (C-30); 23.39 (C-11); 23.06 (C-16); 21.43 (C-41, C-52); 18.73 (C-6); 16.82 (C-26); 15.67 (C-25); 12.20 (C-24); EIMS *m/z* 843.5 $[M+H]^+$; anal. C 73.83, H 8.43, N 9.75%, calcd for $C_{52}H_{70}N_6O_4$, C 74.07, H 8.37, N 9.97%.

*4.5.13 (Benzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(benzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**14**)*

White solid (46 mg; 70%): R_f = 0.61 (hexane/ethyl acetate 1:1 v/v); m.p: 104.9-106.1 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3404, 3150, 3147, 3062, 1725, 1636, 1523, 1048, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 7.47 (s, 1H, H-33); 7.44 (brs, 1H, H-44); 7.35 (brs, 6H); 7.26 (brs, 4H); 5.51 (brs, 2H, H-44); 5.48 (m, 2H, H-34); 5.21 (brs, 1H, H-12); 5.13 (brs, 2H, H-31); 4.61 (brs, 2H, H-41); 3.58 (d, 1H, J = 8.3 Hz, H-23_a); 3.52 (m, 1H, H-3); 3.26 (d, 1H, J = 8.3 Hz, H-23_b); 2.80 (dl, 1H, J = 3.9, 13.0 Hz, H-18); 1.06 (s, 3H, CH_3); 0.87 (s, 9H, 3xCH_3); 0.83 (s, 3H, CH_3); 0.50 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.73 (C-28); 145.65 (C-13); 143.70 (C-32); 143.63 (C-42); 134.68 (C-35); 134.52 (C-45); 122.29 (C-37, C-39, C-47, C-49); 128.92 (C-38); 128.89 (C-48); 128.18 (C-36, C-40); 128.10 (C-46, C-50); 123.89 (CH-12); 122.70 (C-33); 122.44 (C-43); 80.35 (C-3); 76.11 (C-23); 65.02 (C-41); 57.62 (C-31); 54.30 (C-34); 54.25 (C-44); 50.08 (C-9); 47.61 (C-17); 46.79 (C-5); 45.96 (C-19); 41.88 (C-4); 41.81 (C-14); 41.39 (C-18); 39.35 (C-8); 38.12 (C-1); 36.91 (C-10); 33.92 (C-21); 33.14 (C-29); 32.47 (C-7); 32.39 (C-22); 30.72 (C-20); 27.70 (C-15); 25.99 (C-27); 25.87 (C-2); 23.68 (C-30); 23.40 (C-11); 23.05 (C-16); 18.72 (C-6); 16.81 (C-26); 15.68 (C-25); 12.19 (C-24);

EIMS *m/z* 815.4 $[M+H]^+$; anal. C 73.55, H 10.58, N 10.01%, calcd for $C_{50}H_{66}N_6O_4$, C 73.68, H 8.16, N 10.31%.

*4.5.14 (2,4-Difluorobenzyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(2,4-difluorobenzyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**15**)*

White solid (32 mg; 45%): R_f = 0.47 (hexane/ethyl acetate 1:1 v/v); m.p: 100.9-102.1 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3404, 3151, 3148, 3083, 1639, 1508, 1273, 1048, 1004, 735 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.57 (s, 1H, H-33); 7.53 (s, 1H, H-41); 7.30 (m, 2H, H-40, H-50); 6.87 (m, 4H, H-37, H-39, H-47, H-49); 5.54 (brs, 2H, H-44); 5.54 (brs, 2H, H-34); 5.23 (brs, 1H, H-12); 5.14 (s, 2H, H-31); 4.61 (brs, 2H, H-39); 3.59 (d, 1H, *J* = 8.6 Hz, H-23_a); 3.53 (m, 1H, H-3); 3.28 (d, 1H, *J* = 8.6 Hz, H-23_b); 2.81 (dd, 1H, *J* = 3.3, 13.8 Hz, H-18); 1.07 (s, 3H, CH_3); 0.88 (s, 9H, 3xCH_3); 0.84 (s, 3H, CH_3); 0.50 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 177.74 (C-28); 163.50 (dd, *J* = 12.1, 249.1 Hz, C-38, C-48); 157.91 (dd, *J* = 13.2, 249.1 Hz, C-36, C-46); 145.76 (C-13); 143.83 (C-32); 143.61 (C-42); 131.73 (m, C-40, C-50); 123.96 (CH-12); 122.49 (C-33); 122.33 (C-43); 118.13 (dd, *J* = 3.8, 12.6 Hz, C-35); 117.90 (dd, *J* = 3.9, 10.9 Hz, C-45); 112.26 (dd, *J* = 3.7, 21.5 Hz, C-39, C-49); 104.40 (t, *J* = 23.2 Hz, C-37, C-47); 80.22 (C-3); 75.99 (C-23); 64.97 (C-41); 57.55 (C-31); 49.99 (C-9); 47.61 (C-17); 47.26 (d, *J* = 3.7 Hz, C-34); 47.17 (d, *J* = 3.7 Hz, C-44); 46.80 (C-5); 45.93 (C-19); 41.90 (C-4); 41.79 (C-14); 41.37 (C-18); 39.33 (C-8); 38.12 (C-1); 36.90 (C-10); 33.91 (C-21); 33.11 (C-29); 32.48 (C-7); 32.40 (C-22); 30.72 (C-20); 27.68 (C-15); 26.00 (C-27); 25.84 (C-2); 23.64 (C-30); 23.38 (C-11); 23.05 (C-16); 18.67 (C-6); 16.74 (C-26); 15.63 (C-25); 12.17 (C-24); EIMS *m/z* 887.3 $[M+H]^+$; anal. C 67.42, H 7.30, N 9.37%, calcd for $C_{50}H_{62}F_4N_6O_4$, C 67.70, H 7.04, N 9.47%.

*4.5.15 (Phenyl-oxoethyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(phenyl-oxoethyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**16**)*

White solid (50 mg; 72%): R_f = 0.46 (hexane/ethyl acetate 1:1 v/v); m.p: 90.8-92.1 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3404, 3156, 3150, 3062, 1702, 1699, 1694, 1630, 1518, 1048, 724 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 7.97 (d, 4H, *J* = 7.4 Hz H-37, H-41, H-48, H-52); 7.73 (s, 1H, H-33); 7.69 (brs, 1H, H-44); 7.65 (brt, 2H, *J* = 7,3 Hz, H-39, H-50); 7.52 (brt, 4H, *J* = 7.2 Hz H-38, H-40, H-49, H-51); 5.87 (dl, 2H, *J* = 18.4 Hz, H-34_a, H-45_a); 5.79 (brd, 2H, *J* = 18.4 Hz, H-34_b, H-45_b); 5.24 (brs, 1H, H-12); 5.19 (brs, 2H, H-31); 4.66 (brs, 2H, H-42); 3.59 (d, 1H, *J* = 8.5 Hz, H-23_a); 3.54 (brt, 1H, *J* = 6.0 Hz, H-3); 3.29 (d, 1H, *J* = 8.3 Hz, H-23_b); 2.82 (brd, 1H, *J* = 12.7 Hz, H-18); 1.08 (s, 3H, CH_3); 0.86 (s, 9H, 3xCH_3); 0.82 (s, 3H, CH_3); 0.57 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 190.34 (C-35); 190.08 (C-46); 177.69 (C-28); 145.54 (C-13); 143.63 (C-32); 143.58 (C-43); 134.70 (C-36, C-39, C-47); 133.97 (C-50); 129.25 (C-38, C-40, C-49, C-51); 128.21 (C-37, C-41, C-48, C-52); 125.81 (C-12); 124.37 (C-33); 122.49 (C-44); 79.93 (C-3); 75.86 (C-23); 64.88 (C-42); 57.58 (C-31); 55.54 (C-34); 55.38 (C-45); 49.29 (C-9); 47.60 (C-17); 46.76 (C-5); 45.92 (C-19); 41.89 (C-4); 41.78 (C-14); 41.35 (C-18); 39.33 (C-8); 38.06 (C-1); 36.89 (C-10); 33.88 (C-21); 33.14 (C-29); 32.44 (C-7); 32.35 (C-22); 30.71 (C-20); 27.69 (C-15); 26.00 (C-27); 25.90 (C-2); 23.69 (C-30); 23.38 (C-11); 23.04 (C-16); 18.68 (C-6); 16.87 (C-26); 15.64 (C-25); 12.22 (C-24); EIMS *m/z* 893.2 $[M+Na]^+$; anal. C 71.51, H 7.92, N 9.51%, calcd for $C_{52}H_{66}N_6O_6$, C 71.70, H 7.64, N 9.65%.

*4.5.16 (p-Methylphenyl-oxoethyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(p-methylphenyl-oxoethyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**17**)*

White solid (45 mg; 62%): R_f= 0.48 (hexane/ethyl acetate 1:1 v/v); m.p: 99.7-101.5 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3408, 3153, 3151, 3064, 1706, 1698, 1695, 1633, 1522,

1048, 725 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.85 (d, 4H, *J* = 7.9 Hz H-37, H-37', H-47, H-47'); 7.72 (s, 1H, H-33); 7.69 (brs, 1H, H-44); 7.29 (d, 4H, *J* = 7.9 Hz, H-38, H-38', H-48, H-48'); 5.78 (m, 4H, H-34, H-44); 5.23 (brs, 1H, H-12); 5.18 (brs, 2H, H-31); 4.64 (brs, 2H, H-41); 3.57 (d, 1H, *J* = 9.0 Hz, H-23_a); 3.54 (brt, 1H, *J* = 7.3 Hz, H-3); 3.27 (d, 1H, *J* = 8.7 Hz, H-23_b); 2.82 (brdd, 1H, *J* = 2.4, 13.6 Hz, H-18); 2.41 (s, 6H, 2xCH_3); 1.07 (s, 3H, CH_3); 0.87 (s, 3H, CH_3); 0.85 (s, 6H, 2xCH_3); 0.79 (s, 3H, CH_3); 0.57 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 189.90 (C-35); 189.63 (C-45); 177.54 (C-28); 145.71 (C-39, C-49); 145.38 (C-13); 143.55 (C-32); 143.40 (C-42); 131.49 (C-36, C-46); 129.81 (C-38, C-38', C-48, C-48'); 128.24 (C-37, C-37', C-47, C-47'); 125.69 (C-12); 124.33 (C-33); 122.42 (C-43); 79.37 (C-3); 75.43 (C-23); 64.77 (C-41); 57.52 (C-31); 55.36 (C-34); 55.21 (C-44); 49.66 (C-9); 47.53 (C-17); 46.68 (C-5); 45.86 (C-19); 41.86 (C-4); 41.71 (C-14); 41.30 (C-18); 39.27 (C-8); 38.03 (C-1); 36.80 (C-10); 33.81 (C-21); 33.05 (C-29); 32.38 (C-7); 32.29 (C-22); 30.62 (C-20); 27.61 (C-15); 25.98 (C-27); 25.82 (C-2); 23.61 (C-30); 23.31 (C-11); 22.98 (C-16); 21.79 (C-40, C-50); 18.56 (C-6); 16.81 (C-26); 15.56 (C-25); 12.15 (C-24); EIMS *m/z* 921.5 $[M+Na]^+$; anal. C 71.97, H 9.56, N 9.17%, calcd for $C_{54}H_{70}N_6O_6$, C 72.13, H 7.85, N 9.35%.

*4.5.17 (Naphthyl-oxoethyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(naphthyl-oxoethyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**18**)*

White solid (64 mg; 82%): R_f = 0.45 (hexane/ethyl acetate 1:1 v/v); m.p: 93.8-95.2 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3401, 3156, 3152, 3066, 1703, 1699, 1696, 1630, 1512, 1048, 731 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 8.47, (brd, 2H, *J* = 4.1 Hz H-37, H-52); 7.94 (m, 4H, H-42, H-44, H-57, H-59); 7.87 (m, 4H, H-39, H-45, H-54, H-60); 7.79 (brs, 1H, H-33); 7.75 (brs, 1H, H-48); 7.60 (m, 4H, H-40, H-41, H55, H-56); 5.95 (m, 4H, H-34, H-49); 5.24 (brs, 1H, H-12); 5.21 (brs, 2H, H-31); 4.65 (brs, 2H, H-46); 3.55 (m, 2H, H-

3, H-23$_a$); 3.27 (d, 1H, *J* = 8.6 Hz, H-23$_b$); 2.84 (brd, 1H, *J* = 12.9 Hz, H-18); 1.07 (s, 3H, CH_3); 0.87 (s, 3H, CH_3); 0.85 (s, 3H, CH_3); 0.83 (s, 3H, CH_3); 0.77 (s, 3H, CH_3); 0.58 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 190.05 (C-35); 190.32 (C-50); 177.54 (C-28); 145.45 (C-13); 143.53 (C-32); 143.48 (C-47); 136.06 (C-36, C-51); 132.31 (C-43, C-58); 131.23 (C-38); 131.19 (C-53); 130.22 (C-39, C-54); 129.71 (C-37, C-52); 129.34 (C-44, C-59); 129.11 (C-42); 129.08 (C-57); 127.89 (C-41, C-56); 127.31 (C-40); 127.29 (C-55); 125.75 (C-12); 124.40 (C-33); 123.19 (C-45, C-60); 122.41 (C-48); 79.32 (C-3); 75.40 (C-23); 64.77 (C-46); 57.52 (C-31); 55.51 (C-34); 55.36 (C-49); 49.62 (C-9); 47.51 (C-17); 46.67 (C-5); 45.85 (C-19); 41.83 (C-4); 41.69 (C-14); 41.29 (C-18); 39.25 (C-8); 37.99 (C-1); 36.77 (C-10); 33.78 (C-21); 33.03 (C-29); 32.35 (C-7); 32.28 (C-22); 30.59 (C-20); 27.59 (C-15); 25.98 (C-27); 25.80 (C-2); 23.59 (C-30); 23.29 (C-11); 22.98 (C-16); 18.53 (C-6); 16.81 (C-26); 15.53 (C-25); 12.14 (C-24); EIMS *m/z* 993.4 $[M+Na]^+$; anal. C 74.01, H 7.54, N 8.43%, calcd for $C_{60}H_{70}N_6O_6$, C 74.20, H 7.26, N 8.56%.

*4.5.18 (p-Cyanophenyl-oxoethyl)-1H-1,2,3-triazol-4-yl-methyl-(3β)3-hydroxyolean-12-en-23-(p-cyanophenyl-oxoethyl)-1H-1,2,3-triazol-4-yl-methyloxy-28-oate (**19**)*

White solid (64 mg; 87%): R_f = 0.41 (hexane/ethyl acetate 1:1 v/v); m.p: 113.6-115.1 °C; IR (KBr): $\bar{\upsilon}_{max}$ = 3401, 3155, 3151, 3061, 2221, 2218, 1707, 1701, 1699, 1630, 1512, 1049, 728 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 8.07 (d, 4H, *J* = 7.5 Hz, H-37, H-37', H-47, H-47'); 7.80 (d, 4H, *J* = 7.5 Hz, H-38, H-38', H-48, H-48'); 7.72 (s, 1H, H-33); 7.68 (brs, 1H, H-44); 5.87 (m, 4H, H-34, H-44); 5.20 (brs, 1H, H-12); 5.15 (m, 2H, H-31); 4.64 (d, *J* = 12.8 Hz, 1H, H-41$_a$); 4.59 (d, *J* = 12.8 Hz, 1H, H-41$_b$); 3.53 (m, 2H, *J* = 9.0 Hz, H-3, H-23$_a$); 3.26 (d, 1H, *J* = 8.3 Hz, H-23$_b$); 2.79 (brdd, 1H, *J* = 11.6 Hz, H-18); 1.05 (s, 3H, CH_3); 0.84 (s, 6H, 2xCH_3); 0.83 (s, 3H, CH_3); 0.76 (s, 3H, CH_3); 0.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 189.68 (C-35); 189.38 (C-

45); 177.74 (C-28); 145.76 (C-13); 143.69 (C-32); 143.52 (C-42); 136.87 (C-36, C-46); 132.97 (C-38, C-38', C-48, C-48'); 128.71 (C-37, C-37', C-47, C-47'); 125.79 (C-12); 124.29 (C-33); 122.55 (C-43); 117.79 (CH_3-40, CH_3-50); 117.49 (C-39, C-49); 79.77 (C-3); 75.69 (C-23); 64.75 (C-41); 57.40 (C-31); 55.72 (C-34); 55.55 (C-44); 49.80 (C-9); 47.54 (C-17); 46.72 (C-5); 45.86 (C-19); 41.88 (C-4); 41.73 (C-14); 41.31 (C-18); 39.28 (C-8); 38.03 (C-1); 36.84 (C-10); 33.81 (C-21); 33.04 (C-29); 32.39 (C-7); 32.32 (C-22); 30.64 (C-20); 27.64 (C-15); 25.99 (C-27); 25.82 (C-2); 23.60 (C-30); 23.32 (C-11); 23.01 (C-16); 18.62 (C-6); 16.81 (C-26); 15.59 (C-25); 12.18 (C-24); EIMS *m/z* 921.3 $[M+H]^+$; anal. C 70.16, H 7.18, N 12.02%, calcd for $C_{54}H_{64}N_8O_6$, C 70.41, H 7.00, N 12.16%.

4.6 Cell lines and culture conditions

The cell lines used are human cancer cell lines: pharynx carcinoma - FaDu; ovarian carcinoma - A2780; colon adenocarcinoma - HT29; malignant melanoma - A375; thyroid carcinoma - SW1736). Cultures were maintained as monolayers in RPMI 1640 medium with L-glutamine (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) supplemented with 10% heat inactivated fetal bovine serum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and penicillin/streptomycin (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) at 37 °C in a humidified atmosphere with 5% CO_2.

4.7 Bioassays

The hederagenine derivatives were subjected to the following bioassays: i) Cytotoxicity assay; ii) AO/PI dye exclusion assay; iii) *In vitro* growth of *Leishmania infantum* intracellular amastigotes; iv) Metabolic viability assay. The details for all these assays can be found in the Supplementary Material.

4.8 *Molecular Docking*

Molecular docking studies for hederagenin and compounds **2**, **5** and **17** with active site of *Leishmania infantum* $CYP51_{Li}$ (PDB code: 3L4D; resolution: 2.75 Å) were performed by using AutoDock 4.2 program suite [37-38]. Each compound was generated using Chemdraw 14.0 followed by MM2 energy minimization. The enzyme targets were prepared for molecular docking simulation by removing water, and all hydrogens were added, Gasteiger charges were calculated and non-polar hydrogens were merged to carbon atoms. A grid box size of 70x70x70 point (x, y, z) with a spacing of 0.486 Å was centered on the X, Y, and Z at 34.321, -25.721, and 3.139, respectively. The genetic algorithm with local search (GALS) was chosen to search the best conformers. The Lamarckian was opted to search for the best conformers. Each docking experiment was performed 50 times, yielding 50 docked conformations. The docking procedure of ligands (**He**, **2**, **5** and **17**) with the enzyme ($CYP51_{Li}$) was performed as described.

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Appendix A. Supplementary material

Supplementary material related to this article can be found at…

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Table 1. Cytotoxicity for hederagenin (**He**) and derivatives **2-19** (cut-off in all experiments was 30 μM except for parent **He** where the cut-off was 60 μM); employing five human tumor cell lines; betulinic acid (**BA**) was used as a standard.

Compound	EC_{50} (μM)*				
	FaDu	**A2780**	**HT29**	**A375**	**SW1736**
He	>60	>60	>60	>60	>60
2-4	>30	>30	>30	>30	>30
5	>30	21.6± 0.1	16.3±1.9	28.5±0.5	>30
6	>30	>30	27.0±0.1	>30	>30
7	>30	29.1± 0.2	21.7±0.1	26.1±0.1	>30
8-18	>30	>30	>30	>30	>30
19	12.1±2.2	11.2±1.4	7.4±0.3	10.6±1.1	11.9±2.7
BA	13.4±1.3	12.7±0.9	18.4±1.4	12.0±0.4	16.4±2.1

*EC_{50} values in μM from SRB assays after 96 h of treatment; the values are averaged from at least three independent experiments performed each in four replicate; confidence interval CI = 95%; with errors. Compounds with EC_{50} > 30 μM are considered inactive.

Table 2. Antileishmanial activity *in vitro* of hederagenin (**He**) and its derivatives (**1**, **2**, **5**, **15** and **17**) against the intracellular amastigotes of *L. infantum* parasite of macrophages.

Compound	Amastigotes IC_{50}[a] (µM)	Toxicity DH82 Canine macrophages CC_{50}[b] (µM)	SI[c] Intracellular amastigote forms
He	61.6 ± 0.25	>1000	>10
1	28.8 ± 0.12	259 ± 0.17	9
2	25.9 ± 0.12	>1000	>38
5	5.6 ± 0.14	>1000	>178
15	154.8 ± 0.15	140 ± 0.11	0.9
17	7.4 ± 0.12	>1000	>135
Control[d]	80.0 ± 0.13	4.7 ± 0.07	0.1

[a] IC_{50} = half maximal concentration represents the concentration of drug able to inhibit by 50% the *in vitro* growth.

[b] CC_{50} = cytotoxic concentration for 50% of canine macrophages DH82.

[c] SI = selective index corresponding to the ratio between CC_{50} and IC_{50}.

[d] Potassium antimonyl tartrate trihydrate used as positive control.

Table 3. Cytotoxic concentration and selectivity index for hederagenin (**He**) and its derivatives (**1**, **2**, **5**, **15** and **17**) and positive drug control against BGM and HepG2 cells.

Compound	Monkey African green kidney (BGM) CC_{50}[a] (µM)	SI[b]	Human hepatocytes (HepG2) CC_{50}[a] (µM)	SI[b]
He	57 ± 0.33	0.9	>1000	>16.2
1	99 ± 0.18	3.5	104 ± 0.86	3.7
2	>1000	>38	>1000	>38
5	>1000	>178	>1000	>178
15	99.7 ± 0.10	0.6	105 ± 0.11	0.7
17	>1000	>135	>1000	>135
Control[c]	18 ± 0.09	1.6	0.5 ± 0.07	0.3

[a] CC_{50} = cytotoxic concentration for 50% of BGM and HepG2 cells at concentrations that weakly inhibit intracellular amastigote growth.

[b] SI = selective index corresponding to the ratio between CC_{50} and IC_{50} values presented in Table 2.

[c] Potassium antimonyl tartrate trihydrate used as positive control.

Table 4. Docking statistics of hederagenin (**He**) and derivatives **2**, **5** and **17** with $CYP51_{Li}$ and their interactions with the enzyme.

Compound	Binding Energy	H-Bond	Possible hydrophobic interactions
He	-7.42	Lys-406:OH; Glu-425:OH	Gly-416; Phe-412; Met-405; Arg-346; Phe-415, Hem-481
2	-7.65	Glu-204:OH; Ser-205:O; Arg-227:O;	Met-459; Thr-458; His-293; Phe-213; Ile-208; Pro-209; Ala-210; Leu:-207; Cys-206, Hem-481
5	-8.42	Val-460:NH; Glu-204:OH; Met-459:NH	Pro-463; Gli-462; Trh-458; His-457; His-293; Phe-289; Ser-205; Gln-224; Pro-209: Leu-207; Leu-167; Hem-481
17	-8.38	Met-459:NH	Ala-465; Pro-463; Gly-462; His-457; Asn-455; Trh-458; Ile-297; His-293; Pro-209; Glu-204; Ser-205; Hem-481

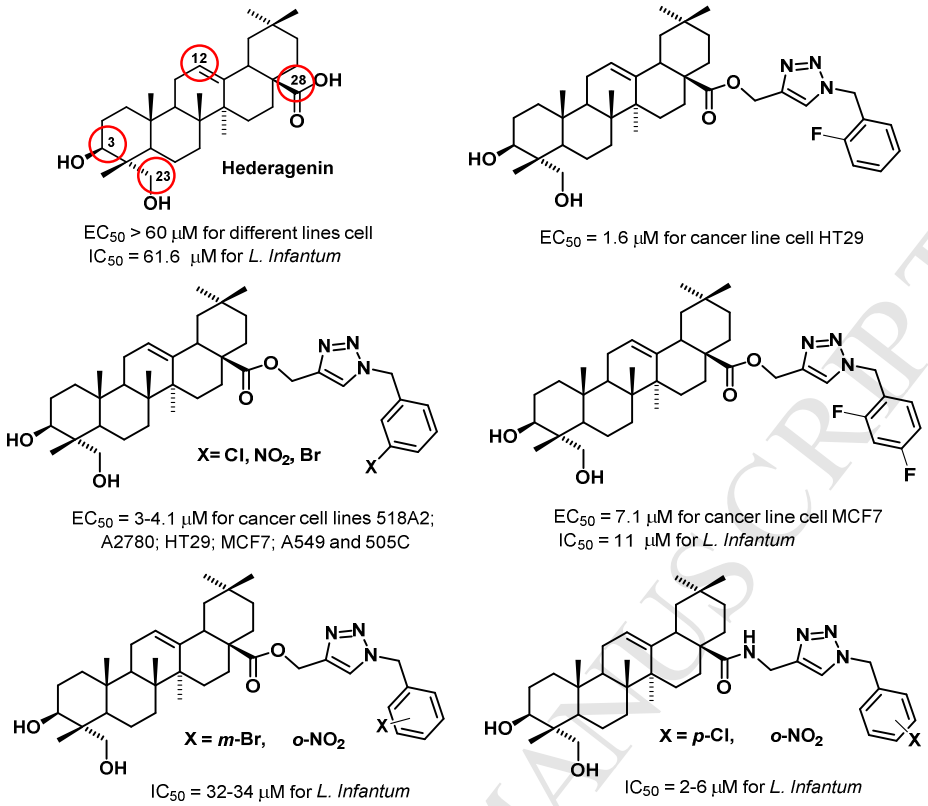


Figure 1. Chemical structure, cytotoxic and leishmania activity of hederagenin and some triazolyl derivatives [19, 26].

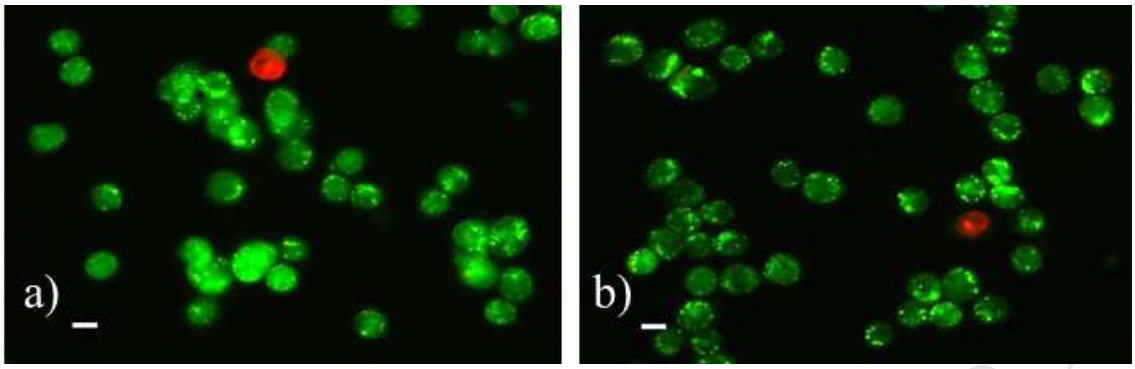


Figure 2. Treatment of HT29 cells with **19** after 24h. Fluorescence microscopic images (scale bar = 5 μm), AO and PI were used a) control, b) **19** (15 μM).

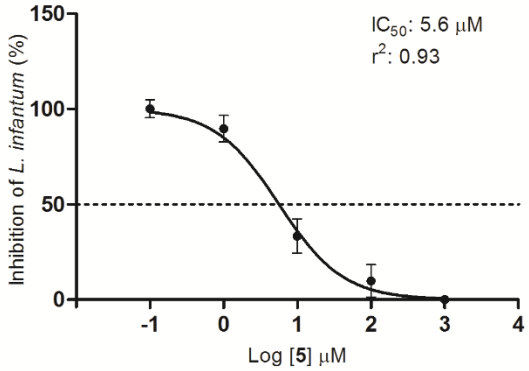


Figure 3 Inhibition of intracellular amastigote forms of *L. infantum* (BH46) by **5**. The dose-response curve [log (concentration of **5**) versus inhibition] was performed in four replicates.

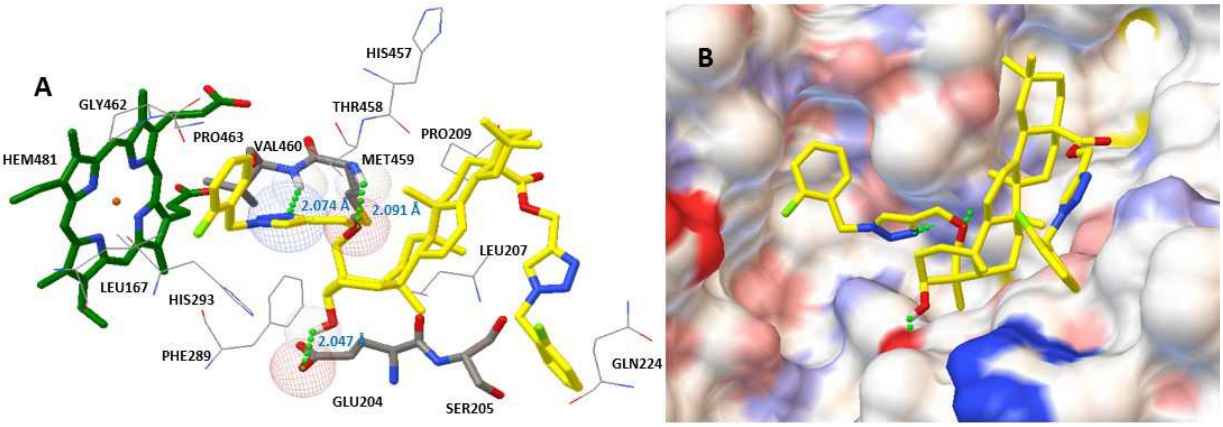
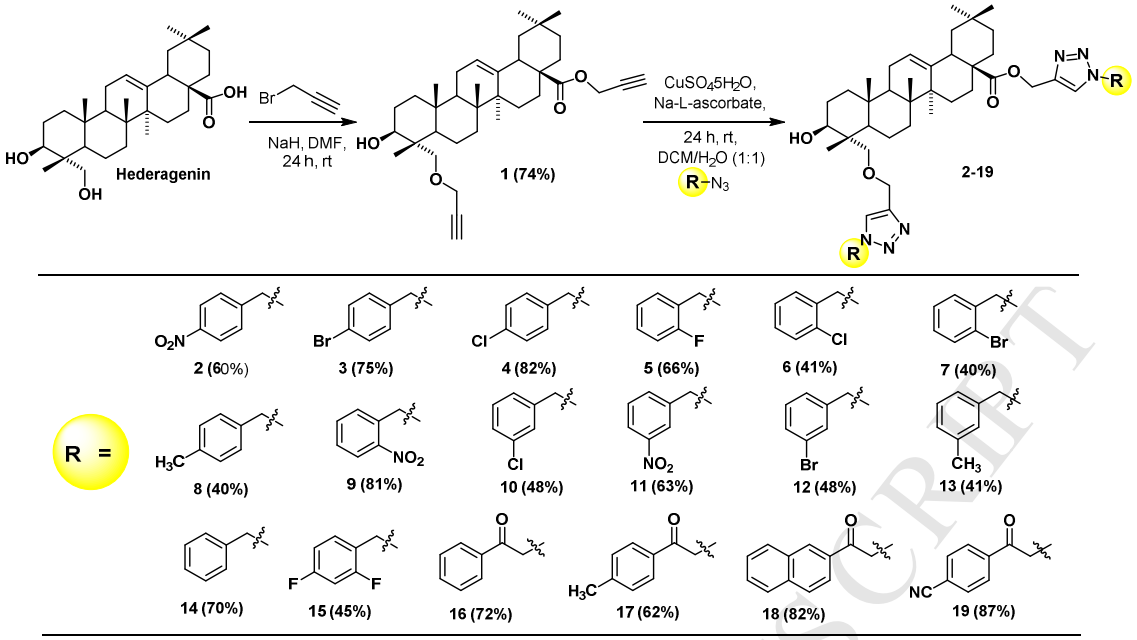


Figure 4. (**A**) Docking binding pose of the hederagenin derivative (**5**) (yellow stick) with the enzyme $CYP51_{Li}$ (PDB ID: 3L4D). (**B**) A vacuum electrostatics depiction of $CYP51_{Li}$ bound to hederagenin derivative (**5**) (yellow stick), showing protein contact potential.



Scheme 1. Synthesis of the dipropargylated (**1**) and the bis-triazolyl-hederagenin derivatives **2-19**.

Highlights

- A series of 18 novel bis-triazolylhederagenin derivatives has been synthesized
- Compound **5** is at least 1780 times more selective than commercial antimony drug
- Derivatives **2**, **5** and **17** showed IC_{50} around of 5-29 μM against *L. infantum*
- Derivative **19** was the most cytotoxic with EC_{50} around of 7.4-12.1 μM.
- Hederagenin and **2**, **5**, **17** interact in the binding site of the enzyme $CYP51_{Li}$