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Title:
Derivatives of grindelic acid: from a non-active natural diterpene to synthetic antitumor derivatives

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1. Introduction

The plant kingdom comprise a relatively small group within biodiversity, with about 250,000 species, of which near 6%, has been studied for their biological activity, and only about 15% has been studied in a phytochemical way. Arguably, the plant kingdom is the largest source of structural diversity in organic compounds, forming a reservoir of chemical structures which is not possible to find in another environment. In this sense, a large number of natural products isolated from plants (or their transformed products) are known, to have relevant biological activities showing an important applicative potential [1].

The quest for new chemical structures with antiproliferative properties, which can be used in the future as chemotherapeutic agents, is an active area of research stimulated by the discovery of new biological targets and the possibility of arising new drugs without undesirable side effects. In the past years, there has been an increasing interest in the development of new pharmaceuticals based on a nucleus present in natural products along with the increase of life-threatening diseases such as AIDS, cancer, hepatitis, etc. [2]. It is noteworthy that some semi synthetic compounds derived from a natural framework sometimes show higher bioactivity than the original natural product. Taking into account their broad range of biological activities, such as enzyme inducing, modulation of immune cell function, and cytotoxic against human tumor cell lines, [3-6] labdane diterpenes have attracted scientific interest.

Analyzing the structure of molecules used in cancer therapy, most of them show nitrogenated functional groups in their framework like amide and carbamate moieties [7]. Recently, it has been reported a series of disubstituted 1,2,3-triazole exhibiting potent cytotoxicity in the nanomolar range and tubulin inhibitory activity in the low micromolar range [8]. This kind of compounds is considered an interesting unit in the design of anticancer drugs. Such heterocycle may act due to their dipolar character, rigidity, ability to form hydrogen bridge bonds or as simple connectors [9].

Synthetic organic chemists have gained great interest for 1,2,3-triazoles in the development of new biologically active molecules [10]. The triazole moiety does not occur in nature, but 1,2,3-triazole cores may form the basis of small-molecule pharmaceutical leads. Molecules containing this heterocyclic nucleus have being reported having anti-HIV, antimicrobial, anti-allergic, antifungal and antitumor activities [10].

Grindelic acid (1) is a labdane-type diterpene that was reported as the main secondary metabolite from Grindelia chiloensis Cabr and G. pulchella Dunal var. pulchella (Asteraceae). This compound possesses a suitable C-14-side-chain for inserting functionalities carrying nitrogen and oxygen [11]. In a previous work, we have reported about the enhancement of the cytotoxicity of some sesquiterpenes and iridoids when their lipophilicity was increased by the introduction of alkyl and/or aryl-silyl functionalities on the natural framework [12].

In consideration of the above mentioned factors, herein we describe our findings aimed to the synthesis and cytotoxic evaluation of oxygenated and oxy-nitrogenated derivatives from grindelic acid (1). It should be emphasized that this diterpene can be isolated in significant quantities from the aforementioned natural sources. Furthermore,
these plants have a wide distribution in the Patagonian steppe zones of Argentina and Chile [13].

2. Result and Discussion
2.1. Chemistry

From *Grindelia pulchella* Dunal var. *pulchella*, we obtained the metabolites grindelic acid (1) and 1α-hydroxygrindelic acid (2), as reported earlier (Figure 1) [13].

Insert Figure 1

In preliminary tests, compound 1 (main secondary metabolite) exhibited greater bioactivity than 2. Therefore, it was chosen as a starting material for the preparation of a series of thirty-six derivatives. Firstly, we prepared several oxygenated compounds 3-10 taking advantage of the presence of the carboxylic acid group (Scheme 1). Methyl ester derivative 3 was obtained from 1 by direct esterification under standard conditions. Subsequent reduction of the ester group at the side chain, with LiAlH4 gave alcohol 4 in high yields. The oxidation of 4 under Parikh-Doering conditions and subsequent Wittig reaction using ethyl 2-(triphenylphosphoranylidene)-propanoate, led to the α,β-unsaturated ester 5 that after reduction with DIBAL-H afforded in good yields alcohol 6. Following the same sequence with ethyl(triphenylphosphoranylidene)acetate, compounds 7 and 8, were obtained from 4. Epoxides 9 and 10 were achieved by Katsuki-Sharpless asymmetric epoxidation of the allylic alcohol 8 using *R,R-*(+)-DET and *S,S-*(-)-DET, respectively.

Insert Scheme 1
Insert Legend Scheme 1

Cycloaddition and multicomponent reactions are convergent procedures of high synthetic utility combining chemical and atomic efficiency. Within the first type, azide-alkyne Huisgen cycloaddition is a powerful tool to prepare 1,2,3-triazoles in a straightforward manner [14]. Multicomponent Reactions (MCRs) are convergent reactions in which three or more starting materials react to form a product where, basically, all or most of the atoms contribute to the newly formed product. In MCRs a product is assembled according to a cascade of elementary chemical reactions. Thus, there is a network of reaction equilibrium which all finally flow into an irreversible step yielding the product. In this work, it was prepared a series of oxy-nitrogenated derivatives, compounds 11-22 by Ugi reaction, 27-32 and 37 by Huisgen cycloadditions, and 23-26 and 33-36 were prepared by classical chemistry. Noteworthy, in all proposals the natural product stereochemistry remained intact.

Derivatives 11-22 were obtained by the Ugi reaction using 1 as carboxylic acid and acetone as a source of carbonyl group. Benzylamine (compounds 11-17), and aniline (compounds 18-22) were used as amines varying the corresponding isocyanides (Table 1).

Insert Table 1
One of the most popular reactions within the click chemistry paradigm is the Cu(I)-
catalyzed 1,3-dipolar Huisgen cycloaddition of alkynes and azides. This reaction
proceeds with great efficiency and selectivity in aqueous media and yields a triazole
moiety [15]. Our first objective was to obtain azide 23 and alkyne 26, using as starting
material alcohol 4 (see Scheme 2).

Insert Scheme 2
Insert Legend Scheme 2

The coupling of azide 23 and various commercial alkynes, under Huisgen conditions,
provided the 1,2,3-triazoles 27-28 in good yields. In a similar manner, the quasi
symmetrical hybrid 29 was prepared from azide 23 and alkyne 26 (Table 2).

Insert Table 2

Using Huisgen conditions and adjusting 1 equivalent of the suitable di-alkyne per 2
equivalents of the azide 23, the dimers 30-32 were achieved (see Table 3). Alkynes
used for the preparations of compounds 30 and 31 are commercially available, and the
alkyne used to arrive to compound 32 was synthesized according to the procedure
described in the literature [16].

Insert Table 3

The final set of derivatives was prepared with the aim to introduce the maximum of
structural diversity at the final hybrid compounds. Thus, carbonylimidazole 33 was
prepared by the reaction of alcohol 4 with carbonyldiimidazole (CDI) in CH₂Cl₂.
Derivatives 34-35 were obtained from compound 33 by treatment with diverse amines.
The use of ethylenediamine provided dimeric carbamate 36. Finally, Huisgen coupling
of 35 with azide 23 yielded the hybrid triazol 37.

Insert Scheme 3
Insert Legend Scheme 3

2.2. Antiproliferative activity

The in vitro antiproliferative activity was evaluated using the National Cancer Institute
(NCI) protocol after 48 h of drug exposure using the sulforhodamine B (SRB) assay.
The results, expressed as GI₅₀ values, are shown in Table 4. Derivatives 20-22, 31,
and 36 were not evaluated due to their low solubility in DMSO under the testing
conditions.

From the growth inhibition results we can depict some preliminary structure-activity
relationships. The group of oxygenated derivatives (3-10) is more active than the
natural products (1-2) where they derive from, having GI₅₀ values in all cell lines in the
range 16-37 µM. In this series, compounds 5 and 7, both bearing an ethyl ester in the
side chain, gave higher GI₅₀ values (>30 µM) in the most resistant cell lines T-47D and
When compared to the methyl ester analog 3, one could speculate with the bulkiness of the ester group on the side chain and its reverse effect on the activity. However, when the side chain was elongated through an Ugi reaction (compounds 11-22) a clear enhancement in the antiproliferative activity was observed. In fact, compound 17 resulted the most active compound of the whole study with GI\textsubscript{50} values in the range 0.95-1.9 µM. In this second group of compounds, a relationship between substituents and biological activity could not be established. When considering the derivatives of the Huisgen reaction 27-29, no improvement was observed in the activity when compared to the most active derivative 17. Finally, for the analogs bearing two grindelic scaffolds (29-32 and 36-37), the activity profile was variable. Thus, derivatives 32 and 37 were inactive (GI\textsubscript{50} >100 µM), whilst 30 and 34 showed GI\textsubscript{50} values <10 µM against all cell lines tested.

3. Conclusion

Given these results, the use of a natural product can be a viable strategy to prepare new active molecules. With adequate quantities of a natural product, and using accessible synthetic routes, it has been possible to obtain new structures exhibiting interesting bioactivities as antitumor agents. Grindelic acid (1) was the starting material for the transformation into several oxygenated, and oxy-nitrogenated moieties at the C-14 side chain by means of diverse reactions. Our strategy allowed to reach thirty-five new compounds including functionalities such as epoxides, diamides, carbamates and 1,2,3-triazoles, among others. A significant number of these synthetic derivatives showed higher activity than the natural product. It is noteworthy that the oxy-nitrogenated and nitrogenated systems were more active than oxygenated ones, being the diamides the most active compounds. The N-benzyl-N-(1-(benzylamino)-2-methyl-1-oxopropan-2-yl) amide of grindelic acid (17) proved to be the most active product in all cell lines tested.

Finally, this work aims to demonstrate the possibility of achieving new antitumor leaders from molecular frameworks provided by nature, getting sometimes a dramatic enhance of the bioactivity through usual chemical transformations.

4. Experimental

4.1. Chemistry

Unless stated otherwise, all solvents were purified by standard techniques. Reactions requiring anhydrous conditions were performed under argon. Anhydrous magnesium sulfate was used for drying solutions. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), and were visualized by use of UV light, 2.5% phosphomolybdic acid in ethanol or vanillin with acetic and sulfuric acid in ethanol with heating [17]. Purification was performed by column
chromatography on silica gel (230-400 mesh) using n-hexane and ethyl acetate gradient as solvent. $^1$H NMR spectra were recorded on a Bruker 500 or 400 MHz, $^{13}$C NMR spectra were recorded at 100 MHz, and chemical shifts are reported relative to internal Me$_4$Si ($\delta$ = 0). Melting points were determined by using an Electrothermal IA9000 melting point apparatus, are reported in degrees Celsius and are uncorrected. Optical rotations were recorded in a Perkin Elmer polarimeter 343. High resolution ESI mass spectra were obtained from a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, an RF-only hexapole ion guide, and an external electrospray ion source. HRMS spectra were obtained on a mass spectrometer Micromass AutoSpec.

4.1.1. Preparation of grindelic acid methyl ester (3).

Grindelic acid (1) (10 g, 31.2 mmol) was dissolved in 124 ml of dry Et$_2$O (0.25 M) and was cooled at 0 ºC. A solution of CH$_2$N$_2$ in Et$_2$O (1M) was added dropwise until the formation of N$_2$ disappear. Reaction was checked by TLC. A few drops of acetic acid were added to quench the excess of diazomethane. Solvent was evaporated and the crude material was purified by silica gel chromatography to obtain 9.85 g, as yellow oil of 3, (29.46 mmol, 94% yield). $\alpha$$_D$$^{20} = -117.2 (c 8.1, MeOH); IR $\nu_{max}$ cm$^{-1}$ (KBr): 3030, 2922, 1749 (C=O), 1672 (C=C), 991 (=C-H); $^1$H NMR (500MHz, CDCl$_3$): $\delta$ 5.52 (s, 1H, H-7), 3.67 (s, 3H, OCH$_3$), 2.75 (d, $J = 14$, 1H, H-14 proR), 2.64 (d, $J = 14$, 1H, H-14 proS), 2.22 (m, 1H, H-12A), 2.07 (m, 2H, H-6A and H-11A), 1.87 (m, 3H, H-1 and H-12B), 1.84 (m, 2H, H-6B and H-11B), 1.79 (s, 3H, H-17), 1.66 (m, 3H, H-3 and H-5), 1.39 (m, 2H, H-2), 1.36 (s, 3H, H-16), 0.92 (s, 3H, H-19), 0.90 (s, 3H, H-18), 0.84 (s, 3H, H-20); $^{13}$C NMR (100MHz, CDCl$_3$): $\delta$ 171.9 (C-15), 134.9 (C-8), 126.5 (C-7), 90.6 (C-9), 81.6 (C-13), 51.4 (OMe), 47.9 (C-14), 42.7 (C-5), 40.7 (C-3), 38.2 (C-12), 33.2 (C-4), 32.9 (C-18), 32.8 (C-1), 28.5 (C-11), 27.3 (C-16), 24.2 (C-6), 22.4 (C-19), 21.3 (C-17), 18.7 (C-2), 16.6 (C-20); HRMS-ES (m/z): calcd for C$_{21}$H$_{34}$O$_3$ [M]$^+$: 334.2508, found 334.2560. All data are consistent with literature [13].

4.1.2. Preparation of grindelic alcohol (4).

A solution of 5 gr of 3 (14.94 mmol) and 2.83 gr (74.74 mmol) of LiAlH$_4$ in dry THF (150 mL, 0.1 M) was refluxed during 2 h under Ar pressure. When reaction was completed the mixture was cooled and ethyl acetate was added until complete elimination of LiAlH$_4$ residues. The crude was filtered and solids washed with 100 mL Et$_2$O. Water (100 mL) was added and extracted with Et$_2$O (3 x 50mL). Organic layers were dried with MgSO$_4$ and resulting crude was purified by silica gel chromatography. 4.18 gr (13.64 mmol, 91% yield) of 4 was recovered as a white solid, mp 64-66 ºC. $[\alpha]$$_D$$^{20} = -131.7 (c 1.25, CHCl$_3$); $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 5.51 (s, 1H), 3.97 (t, $J = 11$ Hz, 1H), 3.69 (dd, $J = 8.8$ and 3 Hz, 1H), 2.10-1.95 (m, 4H), 1.91-1.72 (m, 3H), 1.82 (s, 3H), 1.65-1.50 (m, 6H), 1.48-1.36 (m, 2H), 1.31 (s, 3H), 1.25 (m, 1H), 0.91 (s, 3H), 0.87 (s, 3H), 0.82 (s, 3H); $^{13}$C NMR (100MHz, CDCl$_3$): $\delta$ 134.5, 126.9, 91.1, 84.4, 60.0, 43.7, 42.9, 41.9, 40.7, 40.5, 33.2, 32.9, 32.5, 27.5, 26.6, 24.1, 22.2, 21.1, 18.7, 16.6; HRMS-ES (m/z): calcd for C$_{20}$H$_{34}$O$_2$ [M]$^+$: 306.2559, found 306.2548. All data are consistent with literature [13].

4.1.3. Preparation of (E)-ethyl 2-methyl-4-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8,8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-enoate (5).
2.32 g of 4 (7.58 mmol), 6.31 ml of Et₃N (4.6 g, 45.51 mmol) and 5.28 g of ethyl 2-(triphenyl-phosphoranylidene)propanoate (15.17 mmol) were stirred with 30 ml of dry CH₂Cl₂ (0.25 M) and 6.1 ml of DMSO (0.8 ml/mmol of alcohol). When phosphorane was completely dissolved 3.62 g of SO₃.Py complex (22.75 mmol) was added. Reaction was monitored by TLC. When reaction was completed, 20 ml of HCl (5%) was added. The layers were partitioned, and the aqueous phase was extracted with Et₂O (3 x 20 ml). Organic layers were washed with water (3 x 20 ml) and aqueous NaCl saturated solution (1 x 20 ml), dried with MgSO₄, filtered and concentrated. The crude was purified by silica gel column chromatography to give 5 (2.18 g, 5.6 mmol, 74% yield). 

\[\alpha\]D20 = -36.1 (c 2.81, MeOH); ¹H NMR (400MHz, CDCl₃): δ 6.77 (t, J = 7 Hz, 1H), 5.52 (s, 1H), 4.21 (q, J = 7 Hz, 2H), 2.61(dd, J = 14 and 8 Hz, 1H), 2.46 (dd, J = 14 and 8 Hz, 1H), 2.15-1.95 (m 4H), 1.90-1.65 (m, 6H), 1.86 (s, 3H), 1.78 (s, 3H), 1.60-1.35 (m, 4H), 1.31 (t, J = 7 Hz, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H); ¹³C NMR (100MHz, CDCl₃): δ 168.2, 138.8, 135.0, 129.3, 126.5, 90.5, 83.4, 60.4, 43.0, 42.6, 42.1, 40.7, 38.2, 33.2, 32.9, 28.4, 28.3, 27.5, 24.0, 22.4, 21.3, 18.8, 16.7, 14.3, 12.6; HRMS-ES (m/z): calcd for C₂₅H₄₀O₃[M]+: 388.2977, found 388.2992.

4.1.4. Preparation of (E)-2-methyl-4-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-en-1-ol (6).

896 mg of 5 (2.39 mmol) was dissolved in 24 ml of dry Et₂O at 0 ºC. 5.26 ml of DIBAL-H solution (0.1 M in cyclohexane, 5.26 mmol) was added dropwise. When reaction was completed 5 ml of water was added and reaction was stirred for 15 minutes at room temperature. Reaction mixture was dried with MgSO₄, filtered through celite and purified by silica gel column chromatography to give 6 (2.12 mmol, 89% yield). 

\[\alpha\]D20 = -28.2 (c 1.06, MeOH); ¹H NMR (400MHz, CDCl₃): δ 5.51 (s, 1H), 5.45 (td, J = 7 and 2 Hz, 1H), 4.04 (s, 2H), 2.48 (dd, J = 14 and 8 Hz, 1H), 2.34 (dd, J = 14 and 8 Hz, 1H), 2.13-1.92 (m, 4H), 1.88-1.80 (m, 2H), 1.79 (s, 3H), 1.71-1.68 (m, 2H), 1.69 (s, 3H), 1.60-1.50 (m, 2H), 1.28-1.22 (m, 2H), 1.20 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H); ¹³C NMR (100MHz, CDCl₃): δ 136.4, 135.3, 126.3, 122.9, 90.3, 83.9, 69.0, 42.6, 42.0, 41.9, 40.8, 38.0, 33.2, 32.9, 32.8, 28.4, 28.3, 27.5, 24.2, 22.3, 21.2, 18.8, 16.7, 14.0; HRMS-ES (m/z): calcd for C₂₃H₃₈O₂ [M]+: 346.2872, found 346.2872.

4.1.5. Preparation of (E)-ethyl 4-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-enoate (7).

995 mg of 4 (3.25 mmol), 2.72 ml of Et₃N (1.97 g, 19.5 mmol) and 2.26 g of ethyl(triphenyl-phosphoranylidene)acetate (6.5 mmol) were stirred with 13 ml of dry CH₂Cl₂ (0.25 M) and 2.6 ml of DMSO (0.8 ml/mmol of alcohol). When phosphorane was completely dissolved, 1.55 g of SO₃.Py complex (9.74 mmol) was added. Reaction was monitored by TLC. When reaction was completed, 20 ml of HCl (5%) was added. The layers were partitioned, and the aqueous phase was extracted with Et₂O (3 x 20 ml). Organic layers were washed with water (3 x 20 ml) and aqueous NaCl saturated solution (1 x 20 ml), dried with MgSO₄, filtered and concentrated. Crude material was purified by silica gel column chromatography yielding 1.14 g of 7 (3.05 mmol, 94% yield). 

\[\alpha\]D20 = -121.76 (c 1.71, MeOH); ¹H NMR (500MHz, CDCl₃): δ 6.95 (m, 1H), 5.87 (d, J = 12 Hz, 1H), 5.52 (s, 1H), 4.21 (q, J = 7 Hz, 2H), 2.60 (m, 1H), 2.47 (m, 1H),
2.21-1.98 (m, 4H), 1.88-1.79 (m, 3H), 1.72-1.38 (m, 6H) 1.31 (t, J = 7 Hz, 3H), 1.22 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H); $^{13}$C NMR (100MHz, CDCl$_3$): δ 166.6, 146.1, 134.9, 126.6, 123.6, 90.6, 82.6, 60.2, 46.8, 42.6, 42.0, 40.7, 38.2, 33.2, 32.9, 32.8, 28.4, 27.5, 24.2, 22.4, 21.3, 18.8, 16.7, 14.3; HRMS-ES (m/z): calcd for C$_{24}$H$_{38}$O$_3$ [M]$^+$: 374.2821, found 374.2838.

4.1.6. Preparation of (E)-2-methyl-4-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-en-1-ol (8).

426.2 mg of 7 (1.13 mmol) was dissolved in 12 ml of dry Et$_2$O at 0 ºC. 2.5 ml of DIBAL-H (0.1 M in cyclohexane, 2.5 mmol) was added dropwise. When reaction was completed 3 ml of water was added and reaction was stirred for 15 minutes at room temperature. Reaction mixture was dried with MgSO$_4$, filtered through celite and purified by silica gel column chromatography to yield 8 (308.8 mg, 0.93 mmol, 82% yield). $[^{[\alpha]}_D^{20}$ = -75.3 (c 6.22, MeOH); $^1$H NMR (500MHz, CDCl$_3$): δ 5.66 (m, 2H), 5.49 (bs, 1H), 4.09 (bs, 2H), 2.44 (m, 1H), 2.32 (m, 1H), 2.11-1.92 (m, 4H), 1.88-1.70 (m, 2H), 1.76 (s, 3H), 1.70-1.60 (m, 4H), 1.58-1.45 (m, 2H), 1.44-1.20 (m, 2H), 1.18 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.80 (s, 3H); $^{13}$C NMR (100MHz, CDCl$_3$): δ 135.0, 131.6, 129.4, 126.2, 90.3, 83.1, 62.4, 46.6, 42.5, 41.9, 40.6, 37.9, 33.1, 32.8, 32.7, 28.3, 27.0, 24.1, 22.3, 21.2, 18.7, 16.6; HRMS-ES (m/z): calcd for C$_{22}$H$_{36}$O$_2$ [M]$^+$: 332.2715, found 332.2718.

4.1.7. Preparation of ((2S,3S)-3-(((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)methyl)oxiran-2-yl)methanol (9).

In a reaction flask containing powered activated 4 Å molecular sieves (30 mg), 130 mg of 8 (0.39 mmol) was dissolved in 4 ml of dry CH$_2$Cl$_2$ (0.1 M) and cooled at -20 ºC under argon atmosphere. 138.5 µl of Ti(OPr-i)$_4$ (133.46 mg, 0.47 mmol) and 93.8 µl of ((R,R)-(+)-DET (112.98 mg, 0.55 mmol) were added sequentially. The mixture was stirred at the same temperature for 20 min, and TBHP (0.14 ml, 5.5 M in isooctane, 0.78 mmol) was added slowly. After the addition, the reaction was maintained with stirring for 12 h at low temperature. Tartaric acid aqueous solution (15%w/v) was added and the stirring was continued at room temperature for 1 h. The phases were separated and the aqueous phase was extracted with CH$_2$Cl$_2$ (3 x 3 ml). The combined organic phase were washed with brine, concentrated, diluted with Et$_2$O, and treated with precooled, at 0 ºC, 15%(w/v) NaOH aqueous solution. The two-phase mixture was stirred vigorously for 15 minutes at 0 ºC. The organic phase was separated, and the aqueous phase was extracted with CH$_2$Cl$_2$ (3 x 3 ml). The combined organic phase were washed with brine, dried, filtered, concentrated, and purified by silica gel column chromatography to yield 9 (96.8 mg, 0.28 mmol, 71% yield). $[^{[\alpha]}_D^{20}$ = -97.3 (c 1.1, MeOH); $^1$H NMR (500MHz, CDCl$_3$): δ 5.51 (s, 1H), 3.92 (dd, J = 12 and 2 Hz, 1H), 3.66 (dd, J = 12 and 4 Hz, 1H), 3.06 (m, 1H), 2.92 (m, 1H), 2.15-2.00 (m, 2H), 1.98-1.80 (m, 2H), 1.88-1.77 (m, 2H) 1.78 (s, 3H), 1.71-1.65 (m, 2H), 1.62-1.51 (m, 4H), 1.48-1.35 (m, 1H), 1.30 (s, 3H), 1.28-1.18 (m, 1H) 0.90 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H); $^{13}$C NMR (100MHz, CDCl$_3$): δ 135.0, 126.5, 90.6, 82.3, 61.7, 58.4, 53.4, 45.4, 42.6, 42.0, 40.7, 38.5, 33.2, 32.9, 32.8, 28.3, 28.0, 24.2, 22.4, 21.2, 18.8, 16.7; HRMS-ES (m/z): calcd for C$_{22}$H$_{36}$O$_3$ [M]$^+$: 348.2664, found 348.2672.
4.1.8. Preparation of ***((2R,3R)-3-(((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)methyl)oxiran-2-yl)methanol*** (10).

The procedure used above to obtain 9 was applied again to 8 (64 mg, 0.19 mmol), using the (S,S)-(−)-DET instead of (R,R)-(−)-DET, to yield 10 (57.0 mg, 0.16 mmol, 85% yield). \([\alpha]_D^{20} = -137.6 (c 2.82, \text{MeOH})\); \(^1\text{H NMR (500MHz, CDCl}_3): \delta 5.49 (s, 1H), 3.89 (dd, \(J = 12\) and 2 Hz, 1H), 3.63 (dd, \(J = 12\) and 4 Hz, 1H), 3.10 (m, 1H), 2.90 (m, 1H), 2.19-1.95 (m, 5H), 1.86-1.73 (m, 2H), 1.74 (s, 3H), 1.72-1.60 (m, 2H), 1.58-1.49 (m, 2H), 1.48-1.35 (m, 2H), 1.32 (s, 3H), 1.28-1.17 (m, 2H), 0.91 (s, 3H), 0.88 (s, 3H), 0.82 (s, 3H); \(^{13}\text{C NMR (100MHz, CDCl}_3): \delta 135.1, 126.3, 90.4, 82.2, 61.8, 58.1, 53.8, 45.8, 42.8, 42.0, 40.7, 39.2, 33.2, 33.0, 32.7, 18.8, 16.7; HRMS-ES (m/z): calcd for C\(_{22}\)H\(_{36}\)O\(_3\)[M]+: 348.2664, found 348.2672.

4.1.9. General procedure for Ugi reaction. Preparation of 2-(N-benzyl-2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-N-tert-butyl-2-methylpropanamide (11).

A solution of acetone (0.030 mL, 0.41 mmol), and benzylamine (0.038 mL, 0.35 mmol) in methanol (0.7 mL, 0.5 M) were stirred for 3 hours. After that, grindelic acid (107.1 mg, 0.35 mmol) and tert-butyl-isocyanide (0.044 mL, 0.38 mmol) were added. Reaction mixture was stirred for 24 h. The solvent was removed under reduced pressure and the crude reaction mixture was purified by flash chromatography on silica gel to afford the desired product 11 (111 mg, 59% yield) as a white solid: mp 72-74 °C. \([\alpha]_D^{20} = -137.6 (c 2.82, \text{MeOH})\); \(^1\text{H NMR (500MHz, CDCl}_3): \delta 7.37 (m, 2H, H-3' and H-5'), 7.29 (m, 3H, H-2', H-4' and H-6'), 5.59 (s, 1H, N-H), 5.46 (s, 1H, H-7), 5.07 (d, \(J = 14.4\) Hz, 1H, H-7'A), 4.45 (d, \(J = 14.4\) Hz, 1H, H-7'B), 2.89 (d, \(J = 11.6\) Hz, 1H, H-14A), 2.56 (d, \(J = 11.6\) Hz, 1H, H-14B), 2.07 (m, 1H, H-11A), 2.04 (m, 3H, H-6A and H-12), 1.86 (m, 1H, H-6B), 1.73 (m, 1H, H-11B), 1.62 (m, 1H, H-5), 1.56 (s, 3H, H-17), 1.55 (m, 2H, H-1), 1.52 (m, 2H, H-2), 1.40 (s, 3H, H-16), 1.38 (s, 6H, H-3'' and H-4''), 1.36 (s, 9H, H-2'', H-3''' and H-4''), 1.18 (m, 1H, H-3A), 1.13 (m, 1H, H-3B), 0.92 (s, 3H, H-19), 0.88 (s, 3H, H-18), 0.81 (s, 3H, H-20); \(^{13}\text{C NMR (100MHz, CDCl}_3): \delta 174.0 (C-1''), 171.9 (C-15), 139.0 (C-1'), 135.0 (C-8), 128.8 (2C, C-3 and C-5'), 127.1 (C-7), 126.4 (C-4'), 125.7 (2C, C-2 and C-6'), 90.4 (C-9), 83.1 (C-13), 63.1 (C-2''), 50.7 (C-1'''), 48.0 (C-14), 47.6 (C-7'), 43.0 (C-5'), 42.0 (C-3), 40.6 (C-10), 39.7 (C-12), 33.2 (C-4), 33.1 (C-18), 32.6 (C-1), 28.6 (3C, C-2'', C-3''' and C-4''), 28.3 (C-11), 27.7 (C-16), 24.4 (C-6), 24.3 (2C, C-3'' and C-4''), 22.3 (C-19), 21.2 (C-17), 18.7 (C-2), 16.6 (C-20); HRMS-ES (m/z): calcd for C\(_{35}\)H\(_{54}\)N\(_2\)O\(_3\)[M]+: 550.3734, found 550.3722.

4.1.10. Preparation of 2-(N-benzyl-2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-2-methyl-N-(2,3,3-trimethylbutan-2-yl)propanamide (12).

This compound was prepared by General procedure for Ugi reaction using benzylamine and 1,1,3,3-tetramethylbutylisocyanide to obtain 444 mg of 12 (75% yield) as a clear oil color less oil. \([\alpha]_D^{20} = -66.73 (c 1.2, \text{CHCl}_3); \(^1\text{H NMR (500MHz, CDCl}_3): \delta 7.38 (m, 2H), 7.30 (m, 3H), 5.63 (s, 1H), 5.45 (s, 1H), 5.00 (d, J = 18 Hz, 1H), 4.48 (d, J = 18 Hz, 1H), 2.89 (d, J = 15 Hz, 1H), 2.57 (d, J = 15 Hz, 1H), 2.15-2.00 (m, 5H), 1.91-1.82 (m, 1H), 1.77-1.65, (m, 2H), 1.61 (s, 3H), 1.57 (m, 1H), 1.54-1.49 (m, 2H), 1.45 (s, 6H), 1.40 (s, 3H), 1.38 (s, 3H), 1.37(s, 3H), 1.30-1.25 (m, 1H), 1.22-1.14 (m, 1H) 1.01 (s, 9H), 0.91 (s, 3H), 0.88 (s, 3H), 0.81 (s, 3H); \(^{13}\text{C NMR (100MHz, CDCl}_3): \delta 173.4,
171.9, 138.9, 135.1, 128.8 (2C), 127.1, 126.4, 125.8 (2C), 90.3, 83.2, 63.1, 54.9, 53.0, 48.0, 47.6, 43.0, 42.0, 40.6, 39.5, 33.2, 33.1, 32.6, 31.6 (3C), 31.5, 28.5, 28.4 (2C), 27.7, 24.4, 24.2, 22.3, 21.2, 18.7, 16.6; HRMS-ES (m/z): calcd for C_{38}H_{60}N_{2}O_{3} [M]^{+}: 606.4760, found 606.4756.

4.1.11. Preparation of 2-(N-benzyl-2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6,7',8',8'a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-N-cyclohexyl-2-methylpropanamide (13).

This compound was prepared by General procedure for Ugi reaction using benzylamine and cyclohexyl isocyanide to obtain 378 mg of 13 (71% yield) as a white solid: mp 81-83 °C. [α]_{D}^{20} = -91.08 (c 1.0, CHCl_{3}); ^1H NMR (500MHz, CDCl_{3}): δ 7.36 (m, 4H), 7.26 (m, 1H), 5.55 (d, J = 8 Hz, 1H), 5.48 (s, 1H), 5.10 (d, J = 18 Hz, 1H), 4.43 (d, J = 18 Hz, 1H), 3.76 (m, 1H), 2.84 (d, J = 14 Hz, 1H), 2.51 (d, J = 14 Hz, 1H), 2.09-1.81 (m, 7H), 2.09-1.81 (m, 7H), 1.75-1.60 (m, 10H), 1.54 (bs, 3H), 1.45-1.30 (m, 3H), 1.40 (s, 6H), 1.40 (s, 6H), 1.37 (s, 3H), 1.28-1.05 (m, 5H), 0.91 (s, 3H), 0.88 (s, 3H), 0.80 (s, 3H); ^13C NMR (100MHz, CDCl_{3}): δ 174.0, 172.0, 139.1, 135.0, 128.7 (2C), 127.0, 126.4, 125.7 (2C), 90.4, 82.9, 62.5, 48.2, 47.8, 47.4, 43.1, 41.2, 40.6, 39.7, 33.2 (2C), 33.0, 32.6, 28.2, 27.8, 25.7, 24.9 (2C), 24.5 (2C), 24.3, 24.2, 22.3, 21.2, 18.7, 16.6; HRMS-ES (m/z): calcd for C_{37}H_{56}N_{2}O_{3} [M]^{+}: 576.4291, found 576.4288.

4.1.12. Preparation of 2-(N-benzyl-2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6,7',8',8'a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-N-(2,6-dimethylphenyl)-2-methylpropanamide (14).

This compound was prepared by General procedure for Ugi reaction using benzylamine and 2,6-dimethylphenyl isocyanide to obtain 350 mg of 14 (63% yield) as a white solid: mp 181-183 °C. [α]_{D}^{20} = -74.63 (c 1.023, CHCl_{3}); ^1H NMR (500MHz, CDCl_{3}): δ 7.29 (m, 5H), 7.00 (m, 3H), 5.41 (bs, 1H), 5.37 (s, 1H), 5.07 (d, J = 19 Hz, 1H), 4.54 (d, J = 19 Hz, 1H), 2.89 (d, J = 15 Hz, 1H), 2.78 (d, J = 15 Hz, 1H), 2.56 (d, J = 15 Hz, 1H), 2.45 (d, J = 15 Hz, 1H), 2.45 (d, J = 15 Hz, 1H), 2.45 (d, J = 15 Hz, 1H), 2.28 (bs, 6H), 2.15-1.90 (m, 4H), 1.59 (s, 3H), 1.54 (s, 3H), 1.49-1.18 (10H), 1.37 (s, 3H), 0.82 (s, 3H), 0.81 (s, 3H), 0.78 (s, 3H); ^13C NMR (100MHz, CDCl_{3}): δ 175.1, 172.1, 138.8, 134.9, 128.7 (2C), 127.0, 126.4, 125.7 (2C), 90.4, 82.9, 62.5, 48.2, 47.8, 47.4, 43.1, 42.1, 40.6, 39.7, 33.2 (2C), 33.0, 32.6, 28.2, 27.8, 25.7, 24.9 (2C), 24.5 (2C), 24.3, 24.2, 22.3, 21.2, 18.7, 16.6; HRMS-ES (m/z): calcd for C_{39}H_{54}N_{2}O_{3} [M]^{+}: 598.4134, found 598.4149.

4.1.13. Preparation of 2-(N-benzyl-2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6,7',8',8'a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-N-butyl-2-methylpropanamide (15).

This compound was prepared by General procedure for Ugi reaction using benzylamine and butyl isocyanide to obtain 438 mg of 15 (79% yield) as a white solid: mp 70-72 °C. [α]_{D}^{20} = -78.53 (c 1.34, CHCl_{3}); ^1H NMR (500MHz, CDCl_{3}): δ 7.35 (m, 5H), 5.77 (bs, 1H), 5.51 (s, 1H), 5.16 (d, J = 19 Hz, 1H), 4.41 (d, J = 19 Hz, 1H), 3.32 (m, 1H), 3.23 (m, 1H), 2.83 (d, J = 14 Hz, 1H), 2.49 (d, J = 14 Hz, 1H), 2.23-2.00 (m, 5H), 2.00-1.97 (m, 2H), 1.92-1.80 (m, 4H), 1.80-1.60 (m, 2H), 1.60-1.30 (m 4H), 1.56 (bs, 3H), 1.42 (bs, 3H), 1.41 (bs, 3H), 1.36 (s, 3H), 0.93 (t, J = 6 Hz 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.81 (s, 3H); ^13C NMR (100MHz, CDCl_{3}): δ 175.1, 172.1, 138.9, 134.9, 128.7 (2C), 127.0, 126.6, 125.7 (2C), 90.5, 82.9, 62.4, 47.7, 47.6, 43.3, 42.1, 40.6,


This compound was prepared by General procedure for Ugi reaction using benzylamine and 2-morpholinoethyl isocyanide to obtain 106.8 mg of 16 (79% yield) as a white solid: mp 164-166 °C. [α]_D^{20} = -87.98 (c 1.32, CHCl_3); ^1H NMR (500MHz, CDCl_3): δ 7.27 (m, 4H), 7.18 (m, 1H), 6.20 (bs, 1H), 5.41 (s, 1H), 5.04 (d, J = 19 Hz, 1H), 4.29 (d, J = 19 Hz, 1H), 3.65 (m, 4H), 3.30 (m, 2H), 2.73 (d, J = 14 Hz, 1H), 2.50-2.41 (m, 6H), 2.38 (d, J = 14 Hz, 1H), 2.10-1.90 (m, 4H), 1.85-1.50 (m, 4H), 1.47 (bs, 3H), 1.45-1.09 (m, 4H), 1.35 (bs, 3H), 1.28 (bs, 3H), 1.25 (s, 3H), 1.22-1.03 (m, 2H), 0.83 (s, 3H), 0.80 (s, 3H), 0.73 (s, 3H); ^13C NMR (100MHz, CDCl_3): δ 175.4, 172.1, 139.1, 134.9, 128.7 (2C), 126.9, 126.6, 125.7 (2C), 90.4, 82.9, 67.1, 62.3, 56.9, 53.3 (3C), 47.5, 47.2, 43.1, 42.2, 41.9, 40.5, 39.7, 36.1, 33.2 (2C), 28.1, 27.8, 24.8, 24.3, 23.4, 22.2, 21.3, 18.7, 16.6; HRMS-ES (m/z): calcd for C_{37}H_{58}N_{3}O_{4} [M+H]^+: 608.4427, found 608.4443.

4.1.15. Preparation of N-benzyl-2-methyl-2-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)-N-phenylacetamido)propanamide (17).

This compound was prepared by General procedure for Ugi reaction using benzylamine and benzylisocyanide to obtain 528 mg of 17 (97% yield) as a white solid: mp 76-78 °C. [α]_D^{20} = -72.91 (c 1.18, CHCl_3); ^1H NMR (500MHz, CDCl_3): δ 7.25 (m, 10H), 6.94 (m, 1H), 5.41 (s, 1H), 5.04 (d, J = 19 Hz, 1H), 4.50 (dd, J = 19 and 6 Hz, 1H), 4.34 (m, 2H), 2.76 (d, J = 14 Hz, 1H), 2.41 (d, J = 19 Hz, 1H), 2.15-1.59 (m, 6H), 1.58-1.10 (m, 6H), 1.47 (bs, 3H), 1.37 (bs, 3H), 1.36 (bs, 3H), 1.22 (s, 3H), 1.00 (m, 1H), 0.81 (s, 3H), 0.75 (s, 3H), 0.73 (s, 3H); ^13C NMR (100MHz, CDCl_3): δ 175.1, 172.1, 138.8 (2C), 134.9, 128.7 (2C), 128.5 (2C), 127.8 (2C), 127.2, 127.0, 126.6, 125.7 (2C), 90.4, 82.9, 62.5, 47.6, 47.5, 43.8, 43.2, 42.1, 40.5, 39.7, 36.1, 33.2 (2C), 28.1, 27.8, 24.8, 24.3, 23.4, 22.2, 21.3, 18.7, 16.6; HRMS-ES (m/z): calcd for C_{38}H_{52}N_{2}O_{3} [M]^+: 584.3978, found 584.436.
4.1.17. Preparation of \( \text{N-}(2,6\text{-dimethylphenyl})\text{-2-methyl-2-} \text{-}(2-((1'R,5S,8a'S)-2',5,5',8a'\text{-pentamethyl-}4,4a',5,5',6',7',8',8a'\text{-octahydro}-3H,4'H-spiro[furan-2,1'\text{-naphthalen}]\text{-5-yl})\text{-N-phenylacetamido})\text{propanamide (19)}.\)

This compound was prepared by General procedure for Ugi reaction using aniline and 2,6-dimethylphenyl isocyanide to obtain 140 mg of \( \textbf{19} \) (26% yield) as a white solid: mp 90-92 °C. \([\alpha]_D^{20} = -62.58 \) (c 10.47, CHCl\(_3\)); \(^1\)H NMR (500MHz, CDCl\(_3\)): \( \delta \) 7.41 (m, 3H), 7.30 (m, 3H), 7.20 (s, 1H), 7.10 (m, 2H), 5.40 (s, 1H), 2.49 (d, \( J = 18 \) Hz, 1H), 2.32 (bs, 6H), 2.30-2.15 (m, 4H), 2.12-1.89 (m, 4H), 1.80-1.58 (m, 6H) 1.53 (bs, 6H), 1.48 (bs, 3H), 0.86 (s, 3H), 0.79 (s, 3H), 0.76 (s, 3H); \(^13\)C NMR (100MHz, CDCl\(_3\)): \( \delta \) 172.9, 171.0, 139.9, 139.7, 135.3, 130.7, 130.4, 129.5 (2C), 128.6, 128.1 (2C), 126.9, 126.0, 124.3, 118.0, 90.0, 83.5, 63.0, 47.9, 42.8, 41.9, 40.7, 38.7, 33.2, 33.0, 32.9, 28.7, 27.6, 26.1, 25.5, 24.1, 22.4, 21.3, 18.7, 18.5 (2C), 16.7; HRMS-ES (m/z): calcd for \( \text{C}_{38}\text{H}_{52}\text{N}_2\text{O}_3 \) [M]+: 584.3978, found 584.3971.

4.1.18 Preparation of \( \text{N-butyl-2-methyl-2-} \text{-}(2-((1'R,5S,8a'S)-2',5,5',5',8a'\text{-pentamethyl-}4,4a',5,5',6',7',8',8a'\text{-octahydro}-3H,4'H-spiro[furan-2,1'\text{-naphthalen}]\text{-5-yl})\text{-N-phenylacetamido})\text{propanamide (20)}.\)

This compound was prepared by General procedure for Ugi reaction using aniline and butyl isocyanide to obtain 115 mg of \( \textbf{20} \) (23% yield) as a white solid: mp 187-189 °C. \([\alpha]_D^{20} = -52.86 \) (c 1.17, CHCl\(_3\)); \(^1\)H NMR (500MHz, CDCl\(_3\)): \( \delta \) 7.38 (m, 3H), 7.27 (m, 2H), 5.87 (t, \( J = 6 \) Hz, 1H), 5.41 (s, 1H), 3.32 (m, 2H), 2.47 (d, \( J = 15 \) Hz, 1H), 2.26 (d, \( J = 15 \) Hz, 1H), 2.15 (m, 1H), 2.05-1.90 (m, 3H), 1.85-1.72 (m, 1H), 1.70-1.60 (m, 4H) 1.60-1.45 (m, 4H), 1.52 (bs, 3H), 1.45-1.25 (m, 3H), 1.35 (s, 3H), 1.32 (s, 3H), 1.31 (s, 3H), 1.15 (m, 1H) 0.95 (t, \( J = 7 \) Hz, 3H), 0.89 (s, 3H), 0.84 (s, 3H), 0.79 (s, 3H); \(^13\)C NMR (100MHz, CDCl\(_3\)): \( \delta \) 174.9, 171.0, 140.0, 135.2, 130.7, 130.3, 129.4 (2C), 128.4, 128.0, 90.1, 83.5, 62.5, 47.9, 42.9, 42.0, 40.7, 39.7, 38.9, 33.1, 33.0, 32.9, 31.7, 28.6, 27.6, 25.9, 25.1, 24.1, 22.4, 21.3, 20.2, 18.7, 16.7, 13.8; HRMS-ES (m/z): calcd for \( \text{C}_{34}\text{H}_{52}\text{N}_2\text{O}_3 \) [M]+: 536.3978, found 536.3962.

4.1.19. Preparation of \( \text{N-benzyl-2-methyl-2-} \text{-}(2-((1'R,5S,8a'S)-2',5,5',5',8a'\text{-pentamethyl-}4,4a',5,5',6',7',8',8a'\text{-octahydro}-3H,4'H-spiro[furan-2,1'\text{-naphthalen}]\text{-5-yl})\text{-N-phenylacetamido})\text{propanamide (21)}.\)

This compound was prepared by General procedure for Ugi reaction using aniline and benzyl isocyanide to obtain 229 mg of \( \textbf{21} \) (43% yield) as a white solid: mp 194-196 °C. \([\alpha]_D^{20} = -57.56 \) (c 1.16, CHCl\(_3\)); \(^1\)H NMR (500MHz, CDCl\(_3\)): \( \delta \) 7.37 (t, \( J = 6 \) Hz, 1H), 5.41 (s, 1H), 4.60 (dd, \( J = 15 \) and 6 Hz, 1H), 4.49 (dd, \( J = 15 \) and 6 Hz, 1H), 2.47 (d, \( J = 15 \) Hz, 1H), 2.26 (d, \( J = 15 \) Hz, 1H), 2.07-1.81 (m, 3H), 1.75-1.55 (m, 5H) 1.53 (bs, 3H), 1.51-1.42 (m, 4H) 1.37 (s, 6H), 1.30 (s, 3H), 1.15 (m, 1H) 0.89 (s, 3H), 0.82 (s, 3H), 0.79 (s, 3H); \(^13\)C NMR (100MHz, CDCl\(_3\)): \( \delta \) 174.9, 171.0, 139.8, 138.7, 135.3, 130.7, 130.4, 129.5 (2C), 128.6 (2C), 128.5, 127.8 (2C), 127.3, 126.0, 90.1, 83.5, 62.6, 47.9, 43.9, 42.9, 42.0, 40.6, 38.8, 33.2, 33.0, 32.9, 28.7, 27.6, 25.7, 25.2, 24.1, 22.5, 21.3, 18.7, 16.7; HRMS-ES (m/z): calcd for \( \text{C}_{37}\text{H}_{52}\text{N}_2\text{O}_3 \) [M]+: 571.3900, found 571.3872.

4.1.20. Preparation of \( \text{N-cyclohexyl-2-methyl-2-} \text{-}(2-((1'R,5S,8a'S)-2',5,5',5',8a'\text{-pentamethyl-}4,4a',5,5',6',7',8',8a'\text{-octahydro}-3H,4'H-spiro[furan-2,1'\text{-naphthalen}]\text{-5-yl})\text{-N-phenylacetamido})\text{propanamide (22)}.\)
This compound was prepared by General procedure for Ugi reaction using aniline and cyclohexyl isocyanide to obtain 481 mg of **22** (92% yield) as a white solid: mp 222-224 ºC. [α]_D^20 = -60.91 (c 1.33, CHCl₃); **1H NMR** (500MHz, CDCl₃): δ 7.38 (m, 3H), 7.23 (m, 2H), 5.68 (d, J = 8 Hz, 1H), 5.40 (s, 1H), 3.79 (m, 1H), 2.45 (d, J = 15 Hz, 1H), 2.25 (d, J = 15 Hz, 1H), 2.12-1.98 (m, 1H), 1.95-1.78 (m, 5H), 1.75-1.50 (m, 9H), 1.53 (bs, 3H), 1.42-1.30 (m, 3H), 1.33 (s, 3H), 1.28 (s, 3H), 1.27-1.02 (m, 5H), 0.87 (s, 3H), 0.82 (s, 3H), 0.77 (s, 3H); **13C NMR** (100MHz, CDCl₃): δ 173.9, 170.9, 140.0, 135.2, 130.6, 130.3, 129.4 (2C), 128.4, 125.9, 90.0, 83.5, 62.6, 48.4, 48.0, 42.8, 42.0, 40.6, 38.7, 33.2, 33.0 (2C), 32.9, 29.7, 28.7, 27.6, 25.8, 25.7, 25.1, 24.9 (2C), 24.1, 22.47, 21.2, 18.7, 16.7; HRMS-ES (m/z): calcd for C₃₆H₅₄N₂O₃ [M]+: 562.4134; found 562.4127.

4.1.21. Preparation of (1'R,4a'S,5S,8a'S)-5-(2-azidoethyl)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene] (**23**).

Alcohol **4** (2 g, 6.53 mmol) was dissolved in dry CH₂Cl₂ (65 mL) and the solution was cooled to 0 ºC. Triethylamine (1.8 mL, 13.06 mmol) was added via syringe followed by methanesulfonyl chloride (758 µL, 9.78 mmol) in one portion. After 4.5 h, the reaction mixture was diluted with CH₂Cl₂ (20 mL), washed with H₂O (30 mL) and brine (30 mL), dried with MgSO₄, filtered, and evaporated under reduced pressure to leave a yellow residue. The crude residue was purified by flash-chromatography column on silica gel, using n-hexane/ethyl acetate as the eluent. The mesylate obtained (2.26 g, 5.8 mmol, 90% yield) was dissolved in dry DMF (37 mL, 0.16 M). Sodium azide (764 mg, 11.7 mmol) was added and the mixture was stirred at 40 ºC. After 24 h, the reaction mixture was diluted with Et₂O (40 mL), washed with H₂O (3 x 20 mL) and brine (30 mL), dried with MgSO₄, filtered, and concentrated under reduced pressure. The crude oil was purified by column chromatography on silica gel, using n-hexane/ethyl acetate as the eluent, to afford **23** as an oil (1.55 g, 80% yield). [α]_D^20 = -140.8 (c 1.15, CHCl₃); IR ν max, cm⁻¹ (KBr): 2968, 2924, 2870, 2098 (azide), 1460, 1377, 1315, 1267, 1003, 874, 677.; **1H NMR** (500MHz, CDCl₃): δ 5.50 (s, 1H, H-7), 3.42 (m, 1H, H-15A), 3.32 (m, 1H, H-15B), 2.07 (m, 1H, H-11A), 2.05 (m, 1H, H-6A), 1.98 (m, 1H, H-12A), 1.97 (m, 1H, H-14A), 1.86 (m, 1H, H-14B), 1.83 (m, 1H, H-6B), 1.81 (m, 1H, H-11B), 1.77 (m, 1H, H-12B), 1.76 (s, 3H, H-17), 1.69 (m, 1H, H-5), 1.65 (m, 1H, H-1A), 1.57 (m, 2H, H-2), 1.41 (m, 1H, H-1B), 1.39 (m, 1H, H-3A), 1.23 (s, 3H, H-16), 1.22 (m, 1H, H-3B), 0.91 (s, 3H, H-19), 0.89 (s, 3H, H-18), 0.82 (s, 3H, H-20); **13C NMR** (100MHz, CDCl₃): δ 135.0 (C-8), 126.4 (C-7), 90.4 (C-9), 81.5 (C-13), 48.2 (C-15), 42.6 (C-5), 42.1 (C-14), 42.0 (C-3), 40.7 (C-10), 39.5 (C-12), 33.2 (C-4), 33.0 (C-18), 32.8 (C-1), 28.2 (C-11), 26.9 (C-16), 24.2 (C-6), 22.3 (C-19), 21.2 (C-17), 18.8 (C-2), 16.8 (C-20); HRMS-ES (m/z): calcd for C₂₀H₃₄N₃O [M+H]+: 332.2702, found 332.2709.

4.1.22. Preparation of 3-((1'R,4a'S,5S,8a'S)-5-(2-azidoethyl)-2',5,5',5',8a'-octamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)propanal (**24**).

To an oxalyl chloride solution (60 µL, 0.622 mmol) in CH₂Cl₂ (8 mL) at -78 ºC was added dropwise DMSO (90 µL, 1.24 mmol), and the resulting mixture was stirred for 15 min. A solution of **4** (150.5 mg, 0.49 mmol) in CH₂Cl₂ (2 mL) was added dropwise at -78 ºC. The mixture was stirred for 30 min. Triethylamine (0.7 ml, 4.9 mmol) was added dropwise and the resultant mixture was gradually warmed to room temperature and stirred for 7h. The mixture was quenched with water (10 mL) and CH₂Cl₂ (10 mL). The organic layer was washed with brine, dried over MgSO₄, filtered and concentrated in
vacuum. Purification of the residue by silica gel column chromatography (n-hexane/ethyl acetate, 85:15) gave the aldehyde 24 (148 mg, 99%). \([\alpha]_D^{25} = -72.14 \ (c 0.14, \text{CHCl}_3)\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta 9.82 \ (s, 1H), 5.52 \ (s, 1H), 2.80 \ (dd, J = 13 \text{ and } 2 \text{ Hz}, 1H), 2.60 \ (dd, J = 13 \text{ and } 2 \text{ Hz}, 1H), 2.08 \ (m, 4H), 1.87 \ (m, 3H), 1.76 \ (bs, 3H), 1.57 \ (m, 5H), 1.33 \ (m, 3H), 1.22 \ (m, 1H), 0.91 \ (s, 3H), 0.88 \ (s, 3H), 0.83 \ (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 202.8, 134.6, 126.8, 80.9, 57.1, 42.7, 42.0, 40.7, 39.4, 33.2, 33.0, 32.8, 28.3, 28.1, 24.2, 22.3, 21.3, 18.8, 18.7, 16.7; HRMS-ES \((m/z)\): calcd for C\(_{20}\)H\(_{32}\)O\(_2\)[M]\(^+\): 304.24022, found 304.2412.

4.1.23. Preparation of \((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-5-(prop-2-ynyl)-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene] (26).\)

Aldehyde 24 (148 mg, 0.48 mmol) was dissolved in dry CH\(_2\)Cl\(_2\) (5 mL, 0.1 M) at 0 ºC under argon atmosphere. Triphenylphosphine (514 mg, 1.96 mmol), triethylamine (0.3 mL, 1.96 mmol) and CBr\(_4\) (325 mg, 0.98 mmol) were added. The mixture was stirred for 15 min. and the work-up was carried out by addition of NaHCO\(_3\) saturated solution. The product was extracted with Et\(_2\)O (3 x 25 mL), washed with brine, dried over MgSO\(_4\), filtered and concentrated in vacuum. The dibromoolefin 25 obtained was used without purification in the next reaction. The crude material was dissolved in THF (5 mL, 0.1 M) and cooled at -78 ºC under argon with stirring. A solution of n-BuLi (0.36 mL, 2.7 M in heptane) was added using triphenylmethane as indicator. After 30 min. a NH\(_4\)Cl saturated solution was added and the product was extracted with Et\(_2\)O (3 x 25 mL). The organic layer was washed with brine, dried over MgSO\(_4\), filtered and concentrated in vacuum. The residue was purified by column chromatography on silica gel, using a mixture of n-hexane-ethyl acetate as eluent, to afford 26 as a clear oil (78 mg, 53% total yield). \([\alpha]_D^{25} = -77.5 \ (c 0.48, \text{CHCl}_3)\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta 5.53 \ (s, 1H), 2.57 \ (dd, J = 8 \text{ and } 2 \text{ Hz}, 1H), 2.71 \ (t, J = 8 \text{ and } 2 \text{ Hz}, 1H), 2.19 \ (m, 3H), 2.00 \ (s, 1H), 1.91-1.78 \ (m, 2H), 1.79 \ (bs, 3H), 1.75-1.62 \ (m, 1H), 1.58-1.45 \ (m, 2H), 1.41 \ (s, 3H), 1.32-1.23 \ (m, 2H), 0.92 \ (s, 3H), 0.91 \ (s, 3H), 0.89 \ (s, 3H), 0.84 \ (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 134.9, 126.6, 91.2, 82.9, 82.5, 69.3, 42.6, 42.0, 40.9, 38.1, 33.4, 33.2, 32.9, 32.8, 28.3, 26.8, 24.2, 22.4, 21.3, 18.8, 16.7; HRMS-ES \((m/z)\): calcd for C\(_{21}\)H\(_{32}\)O \([M]^+\): 300.2453, found 300.2453.

4.1.24. General procedure for Huisgen reaction. Preparation of \((4\text{-decyl-1-(2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazole (27).\)

The azide 23 (53.7 mg, 0.16 mmol) and 1-dodecyne (35 µL, 0.16 mmol) were suspended in a 1:1 mixture of water and ethanol (0.1 M). Sodium ascorbate (9.6 mg, 0.05 mmol) and copper(II) sulfate pentahydrate (4 mg, 0.02 mmol) were added. The resulting mixture was stirred at room temperature for 24 h. Then, the reaction was diluted with water, extracted with ethyl acetate (10 mL × 3), dried over MgSO\(_4\), filtered, and concentrated in vacuum. The residue was purified by column chromatography on silica gel, using a mixture of n-hexane-ethyl acetate as eluent, to afford 27 as a clear oil (78 mg, 53% total yield). \([\alpha]_D^{25} = -64.35 \ (c 0.20, \text{CHCl}_3)\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta 7.32 \ (s, 1H), 5.51 \ (s, 1H), 4.46 \ (t, J = 8 \text{ Hz}, 2H), 2.71 \ (t, J = 8 \text{ Hz}, 2H), 2.19 \ (m, 3H), 2.15-1.78 \ (m, 5H), 1.74 \ (bs, 3H), 1.71-1.49 \ (m, 5H), 1.45-1.08 \ (m, 18H) 1.28 \ (s, 3H), 0.92 \ (t, J = 7 Hz, 3H), 0.91 \ (s, 3H), 0.90 \ (s, 3H), 0.82 \ (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 148.3, 134.9, 126.6, 120.6, 90.6, 81.3, 68.0, 47.0, 43.7, 42.7, 42.1, 40.7, 39.7, 33.2, 33.0, 32.8, 31.9, 29.6, 29.5 (2C), 29.3, 28.1, 26.9, 25.7, 24.3,
22.7, 22.2, 21.3, 18.8, 18.4, 16.7, 14.1; HRMS-ES (m/z): calcd for C_{32}H_{55}N_{3}O [M]^+ : 497.4345, found 497.4329.

4.1.25. Preparation of 4-(3-ethynylphenyl)-1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8'a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazole (28).

This compound was prepared by General procedure for Huisgen reaction using 50 mg of azide 23 (0.15 mmol) and 9.5 mg of 1,3-diethynilbenzene (0.075 mmol) to obtain 54 mg of 28 (78% yield) as a white solid: mp 129-131 ºC. [α]D_{20} = -175.6 (c 1.57, CHCl3); IR ν_{max}, cm⁻¹ (KBr): 3311 (Csp-H), 3130 (=C-H), 2966, 2916, 2866, 2114 (Csp-Csp), 1479, 1373, 1232, 1001, 804, 619; 1H NMR (500MHz, CDCl3): δ 7.92 (s, 1H, H-5'), 7.86 (m, 2H, H-9' and H-11'), 7.46 (d, J = 6.3 Hz, 1H, H-7'), 7.39 (t, J = 7.6 Hz, 1H, H-10'), 5.50 (s, 1H, H-7), 4.57 (t, J = 7.6 Hz, 2H, H-15), 3.12 (s, 1H, H-13'), 2.25 (t, J = 7.6 Hz, 2H, H-14), 2.01 (m, 1H, H-6A) 1.99 (m, 1H, H-11A), 1.98 (m, 1H, H-12A), 1.83 (m, 2H, H-11B and H-12B), 1.78 (m, 1H, H-16B), 1.69 (s, 1H, H-5), 1.63 (m, 1H, H-1A), 1.56 (m, 2H, H-2), 1.44 (m, 1H, H-1B). 1.39 (m, 3H, H-3A), 1.30 (s, 3H, H-16), 1.22 (m, 1H, H-3B), 0.91 (s, 3H, H-19), 0.87 (s, 3H, H-18), 0.82 (s, 3H, H-20); 13C NMR (100MHz, CDCl3): δ 146.6 (C-4'), 134.8 (C-8), 131.5 (C-5'), 131.2 (C-6'), 129.2 (C-9'), 128.8 (C-7'), 126.7 (C-7), 126.0 (C-10'), 122.6 (C-8'), 120.2 (C-11'), 90.8 (C-9), 83.3 (C-12'), 81.4 (C-13' and C-13), 47.2 (C-15), 43.5 (C-14), 42.8 (C-5), 42.1 (C-3), 40.1 (C-12), 33.2 (C-4), 33.0 (C-18), 32.8 (C-1), 28.1 (C-6), 27.0 (C-16), 24.2 (C-11), 22.3 (C-19), 21.2 (C-17), 18.8 (C-2), 16.7 (C-20); HRMS-ES (m/z): calcd for C_{30}H_{39}N_{3}O [M]^+ : 457.3093, found 457.3088.

4.1.26. Preparation of 1-(2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-4-(((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8'a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)methyl)-1H-1,2,3-triazole (29).

This compound was prepared by General procedure for Huisgen reaction using 48.0 mg of azide 23 (0.14 mmol) and 44.3 mg of alquine 26 (0.14 mmol) to obtain 58.5 mg of 29 (64% yield) as a clear oil. [α]D_{25} = -103.33 (c 0.12, CHCl3); 1H NMR (500MHz, CDCl3): δ 7.39 (s, 1H), 5.51 (s, 2H), 4.44 (t, J = 8 Hz, 4H, H-15), 3.00 (s, 2H), 2.18 (m, 4H), 2.06 (m, 6H), 1.98-1.73 (m, 6H), 1.74 (bs, 3H), 1.69 (s, 3H), 1.68-1.47 (m, 6H), 1.41 (m, 5H), 1.25 (m, 3H); 13C NMR (100MHz, CDCl3): δ 144.9, 135.5, 134.8, 126.6, 126.2, 122.7, 90.6, 90.5, 82.7, 81.3, 60.4, 47.0, 43.6, 42.7, 42.6, 42.1 (2C), 40.8, 40.7, 39.6, 39.5, 38.4, 33.3, 33.2, 33.1, 33.0, 32.8, 29.7, 28.3, 28.1, 27.2, 26.9, 24.3 (2C), 22.3 (2C), 21.3, 21.0, 18.8, 16.7, 14.2; HRMS-ES (m/z): calcd for C_{41}H_{65}N_{3}O_{2} [M]^+ : 631.5077, found 631.5073.

4.1.27. Preparation of 1,4-bis(1-(2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8'a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)butane (30).

This compound was prepared by General procedure for Huisgen reaction using 27.0 mg of azide 23 (0.08 mmol) and 5 µL of 1,7-octadiyne (0.04 mmol) to obtain 22.4 mg of 30 (64% yield) as a clear oil, yield 64%. [α]D_{25} = -84.57 (c 0.31, CHCl3); 1H NMR (500MHz, CDCl3): δ 7.34 (s, 2H, H-5'), 5.50 (s, 2H, H-7), 4.46 (t, J = 7.6 Hz, 4H, H-15), 3.96 (m, 4H, H-13'), 3.24 (m, 4H, H-12'), 2.94 (m, 4H, H-11'), 2.17 (m, 4H, H-10'), 2.07 (m, 4H, H-16), 1.94 (m, 4H, H-9), 1.65 (s, 3H, H-19), 1.58 (m, 3H, H-18), 1.44 (m, 3H, H-17), 1.39 (m, 3H, H-16), 1.22 (m, 1H, H-3B), 0.91 (s, 3H, H-19), 0.87 (s, 3H, H-18), 0.82 (s, 3H, H-17), 0.81 (s, 3H, H-16), 0.91 (s, 3H, H-20); HRMS-ES (m/z): calcd for C_{41}H_{65}N_{3}O_{2} [M]^+ : 631.5077, found 631.5073.
2.74 (t, \(J = 7.14\) Hz, H-6'), 2.18 (m, 4H, H-14), 2.03 (m, 2H, H-11A), 1.95 (m, 2H, H-12A), 1.85 (m, 2H, H-11B), 1.80 (m, 8H and H-7'), 1.79 (m, 2H, H-12B), 1.73 (s, 6H, H-17), 1.70 (m, 2H, H-5), 1.63 (m, 2H, H-1A), 1.61 (m, 4H, H-2), 1.43 (m, 2H, H-1B), 1.41 (m, 2H, H-3A), 1.26 (s, 6H, H-16), 1.21 (m, 2H, H-3B), 0.91 (s, 6H, H-19), 0.89 (s, 6H, H-18), 0.81 (s, 6H, H-20); \(^{13}\)C NMR (100MHz, CDCl\(_3\)): \(\delta 147.7\) (2C, C-4'), 134.8 (2C, C-8), 126.3 (2C, C-7), 120.6 (2C, C-5'), 90.8 (2C, C-9), 81.3 (2C, C-13), 47.1(2C, C-15), 43.7 (2C, C-14), 42.7 (2C, C-5), 42.1 (2C, C-3), 40.7 (2C, C-10), 39.8 (2C, C-12), 33.2 (2C, C-4), 33.0 (2C, C-18), 32.8 (2C, C-1), 28.5 (2C, C-6), 28.2 (2C, C-7'), 26.9 (2C, C-16), 25.2 (2C, C-6'), 24.3 (2C, C-11), 23.3 (2C, C-19), 21.3 (2C, C-17), 18.8 (2C, C-2), 16.7 (2C, C-20).

4.1.28. Preparation of 1,3-bis(1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)benzene (31).

This compound was prepared by General procedure for Huisgen reaction using 87.0 mg of azide 23 (0.26 mmol) and 17 \(\mu\)L of 1,3-diethynylbenzene (0.13 mmol) to obtain 51.0 mg of 31 (51% yield) as a white solid: mp 101-103 °C. \([\alpha]_D^{20} = -144.5\) (c 8.8, CHCl\(_3\)); IR \(\nu_{\text{max}},\) cm\(^{-1}\) (KBr): 3136 (=C-H), 2962, 2926, 2870, 1699, 1460, 1379, 1261, 1101, 1020, 800; \(^{1}H\) NMR (500MHz, CDCl\(_3\)): \(\delta 8.28\) (s, 1H), 7.91 (s, 2H), 7.82 (dd, \(J = 8\) and 2Hz, 2H), 7.49 (t, \(J = 8\) Hz, 1H), 5.52 (s, 2H), 4.58 (m, 4H), 2.28 (m, 4H), 2.17-1.92 (m, 8H), 1.89-1.78 (m, 6H), 1.77 (s, 6H), 1.74-1.49 (m, 6H), 1.46-1.38 (m, 4H), 1.32 (s, 6H), 1.29-1.10 (m, 2H), 0.92 (s, 6H), 0.88 (s, 6H), 0.83 (s, 6H); \(^{13}\)C NMR (100MHz, CDCl\(_3\)): \(\delta 134.7\) (2C), 131.4 (2C), 126.7 (2C), 125.2 (2C), 122.8 (2C), 120.0 (2C), 90.7 (2C), 81.3 (2C), 77.2 (2C), 47.3 (2C), 43.7 (2C), 42.7 (2C), 42.1 (2C), 40.7 (2C), 39.9 (2C), 33.2 (2C), 33.0 (2C), 32.8 (2C), 28.2 (2C), 24.2 (2C), 22.3 (2C), 21.3 (2C), 18.8 (2C), 16.7 (2C); HRMS-ES (m/z): calcd for C\(_{50}\)H\(_{72}\)N\(_6\)O\(_2\) [M+H]+: 788.5717, found 788.5724. HRMS-ESI (m/z): calcd for C\(_{50}\)H\(_{72}\)N\(_6\)O\(_2\)Na [M+Na]+: 811.5614, found 811.5606.

4.1.29. Preparation of 1,3-bis((1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzene (32).

This compound was prepared by General procedure for Huisgen reaction using 100.0 mg of azide 23 (0.30 mmol) and 28.0 mg of 1,3-bis(prop-2-yn-1-yloxy)benzene (0.15 mmol) to obtain 59.0 mg of 32 (46% yield) as a white solid: mp 75-77 °C. \([\alpha]_D^{20} = -123.4\) (c 1.08, CHCl\(_3\)); 1H NMR (500MHz, CDCl\(_3\)): \(\delta 7.67\) (bs, 2H), 7.20 (t, \(J = 8\) Hz, 1H), 6.63 (s, 1H), (6.62 (d, \(J = 8\) Hz, 2H), 5.49 (bs, 2H), 5.19 (s, 4H), 4.51 (m, 4H), 2.21 (t, \(J = 8\), 4H), 2.15-1.90 (m, 8H), 1.90-1.75 (m, 6H), 1.72 (s, 6H), 1.71-1.46 (m, 8H), 1.45-1.30 (m, 2H), 1.28 (s, 6H), 1.28-1.15 (m, 2H), 0.91 (s, 6H), 0.89 (s, 6H), 0.81 (s, 6H); \(^{13}\)C NMR (100MHz, CDCl\(_3\)): \(\delta 159.5\) (2C), 144.0 (2C), 134.7, 130.0, 126.6 (2C), 122.9 (2C), 107.5 (2C), 102.2 (2C), 90.7 (2C), 81.2 (2C), 62.1 (2C), 47.4 (2C), 43.6 (2C), 42.7 (2C), 42.1 (2C), 40.7 (2C), 39.9 (2C), 33.2 (2C), 33.0 (2C), 32.7 (2C), 28.0 (2C), 26.9 (2C), 24.3 (2C), 22.3 (2C), 21.3 (2C), 18.8 (2C), 16.7 (2C); HRMS-ESI (m/z): calcd for C\(_{52}\)H\(_{76}\)N\(_6\)O\(_4\)Na [M+Na]+: 871.5825, found 871.5832.

4.1.30. Preparation of 2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl-1H-imidazole-1-carboxylate (33).
To a solution of alcohol 4 (1 g, 3.26 mmol) in CH₂Cl₂ (65 mL, 0.05 M) was added carbonyl diimidazole (2.64 g, 16.4 mmol). The reaction mixture was heated to 40 °C and stirred for 4.5 h. Solvent was removed and the residue subjected to a flash chromatography to provide carbonyl imidazole 33 (927 mg, yield 71%) as clear oil. [α]₀²⁰ = -117.90 (c 15.54, CHCl₃); IR νmax, cm⁻¹ (KBr): 3124, 2970, 1747 (C=O), 1672 (C=C), 1466, 1379, 1271, 1005, 872, 758; ¹H NMR (500MHz, CDCl₃): δ 8.11 (s, 1H, H-2'), 7.41 (s, 1H, H-4'), 7.05 (s, 1H, H-5'), 5.46 (bs, 1H, H-7), 4.56 (m, 2H, H-15), 2.16 (dt, J = 14 and 7 Hz, 1H, H-14A), 2.07 (m, 1H, H-11A), 2.03 (m, 1H, H-14B), 2.00 (m, 1H, H-12A), 1.96 (m, 1H, H-6A), 1.79 (m, 2H, H-11B and H-12B), 1.75 (m, 1H, H-6B), 1.67 (m, 1H, H-5), 1.61 (m, 2H, H-3), 1.51 (m, 2H, H-2), 1.40 (m, 2H, H-1), 1.28 (s, 3H, H-16), 0.89 (s, 3H, H-19), 0.85 (s, 3H, H-18), 0.80 (s, 3H, H-20); ¹³C NMR (100MHz, CDCl₃): δ 137.1 (C-2'), 134.8 (C-8), 130.5 (C-4'), 126.6 (C-7), 117.1 (C-5'), 90.6 (C-9), 81.3 (C-13), 65.9 (C-15), 42.6 (C-5), 42.0 (C-3), 41.8 (C-14), 40.7 (C-10), 40.0 (C-12), 33.2 (C-4), 33.0 (C-18), 32.7 (C-1), 27.1 (C-16), 24.2 (C-11 and C-6), 22.3 (C-19), 21.3 (C-17), 18.7 (C-2), 16.7 (C-20); HRMS-ES (m/z): calcd for C₂₄H₃₇N₂O₃ [M+H]+: 401.2799, found 401.2791.

4.1.31. General procedure for synthesis of carbamates derivatives. Preparation of 2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl-3-(dimethylamino)propylcarbamate (34).

A solution of the carbonyl imidazole 33 (24.2 mg, 0.06 mmol) was dissolved in acetonitrile (0.12 mL, 0.5 M). Methyl iodide (0.75 mL, 12 mmol) was added and the solution was heated to 60 °C for 2 h. The volatiles were removed by evaporation and the residue was redissolved in methylene chloride (1.5 mL, 0.04 M), 3-(dimethylamino)-1-propylamine (0.15 mL, 1.2 mmol) was added, and the resulting reaction mixture was stirred 4 h. The volatiles were removed by evaporation and the crude residue was purified by flash chromatography to give 20.7 mg of 34 (79% yield) as a clear oil. [α]₀²⁵ = -38 (c 0.1, CHCl₃); ¹H NMR (500MHz, CDCl₃): δ 5.53 (bs, 1H), 5.49 (bs, 1H), 4.16 (bs, 2H), 3.26 (m, 2H), 2.58 (m, 2H), 2.40 (bs, 6H), 2.06 (m, 6H), 1.78 (m, 1H), 1.76 (s, 3H), 1.69 (m, 4H), 1.51 (m, 2H), 1.40 (m, 2H), 1.23 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.81 (s, 3H); ¹³C NMR (100MHz, CDCl₃): δ 156.9, 135.2, 126.2, 90.4, 81.8, 62.6, 56.8, 44.4, 42.6, 42.4, 42.0, 40.7, 39.2 (2C), 33.2, 32.9, 32.8, 29.7, 28.4, 27.0, 26.3, 24.2, 22.4, 21.2, 18.8, 16.7; HRMS-ESI (m/z): calcd for C₂₆H₄₆N₂O₃Na [M+Na]+: 457.3406, found 457.3407.

4.1.32. Preparation of 2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl prop-2-ynylcarbamate (35).

This compound was prepared by General procedure for synthesis of carbamates derivatives using 97.0 mg carbonyl imidazole 33 (0.24 mmol) and 0.35 mL of propargylamine (5.52 mmol) to obtain 78 mg of 35 (83% yield) as a white solid: mp 116-118 °C. [α]₀²⁵ = -113.7 (c 0.918, CHCl₃); ¹H NMR (500MHz, CDCl₃): δ 5.49 (bs, 1H), 4.83 (bs, 1H), 4.20 (m, 2H), 3.98 (bs, 2H), 2.25 (m, 1H), 2.10-1.88 (m, 4H), 1.78-1.60 (m, 4H), 1.76 (s, 3H), 1.59-1.45 (m, 3H), 1.40-1.35 (m, 2H), 1.28-1.20 (m, 2H), 1.23 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.82 (s, 3H); ¹³C NMR (100MHz, CDCl₃): δ 135.2, 134.2, 126.3, 90.4, 81.8, 80.8, 71.5, 63.2, 42.6, 42.3, 42.0, 40.7, 39.3, 33.2, 32.9, 32.8, 29.7, 28.4, 27.0, 24.2, 22.4, 21.2, 18.8, 16.7; HRMS-ESI (m/z): calcd for C₂₄H₃₇NO₃ [M]+: 387.2773, found 387.2761.
4.1.33. Preparation of bis(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl) ethane-1,2-diyldicarbamate (36).

This compound was prepared by General procedure for synthesis of carbamates derivatives using 100.0 mg carbonyl imidazole 33 (0.25 mmol) and 0.38 mL of ethylenediamine (5.75 mmol) to obtain 46 mg of 36 (51% yield) as a white solid: mp 170-172 °C. [α]D 20 = -126.7 (c 1.12, CHCl3); 1H NMR (500MHz, CDCl3): δ 5.50 (bs, 2H), 4.99 (bs, 2H), 4.17 (m, 4H), 3.30 (bs, 4H), 2.09-1.80 (m, 8H), 1.79-1.62 (m, 2H), 1.77 (s, 6H), 1.60-1.52 (m, 4H), 1.51-1.38 (m, 10H), 1.35-1.22 (m, 4H), 1.20-1.09 (m, 2H), 0.91 (s, 3H), 0.90 (s, 6H), 0.89 (s, 3H), 0.82 (s, 6H); 13C NMR (100MHz, CDCl3): δ 178.8 (2C), 135.2 (2C), 126.2 (2C), 126.2 (2C), 115.0 (2C), 90.6 (2C), 81.8 (2C), 62.9 (2C), 56.5 (2C), 42.6 (2C), 42.3 (2C), 42.0 (2C), 40.7 (2C), 33.2 (2C), 33.0 (2C), 32.8 (2C), 28.4 (2C), 27.0 (2C), 24.2 (2C), 22.4 (2C), 21.3 (2C), 18.8 (2C), 16.7 (2C); HRMS-ESI (m/z): calcd for C44H72N2O6Na [M+Na]+: 747.5294, found 747.5262.

4.1.34. Preparation of 2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl(1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)methylcarbamate (37).

This compound was prepared by General procedure for Huisgen reaction using 81.0 mg azide 23 (0.24 mmol) and 43.0 mg of propynylcarbamate 35 (0.11 mmol) to obtain 51 mg of 37 (64% yield) as a white solid, mp 69-71 °C. [α]D 20 = -137.1 (c 1.022, CHCl3); 1H NMR (500MHz, CDCl3): δ 7.58 (s, 1H), 5.49 (bs, 2H), 5.27 (bs, 1H), 4.46 (m, 4H), 4.16 (m, 2H), 2.19 (m, 2H), 2.12-1.86 (m, 7H), 1.85-1.70 (m, 8H), 1.75 (s, 3H), 1.73 (s, 3H), 1.71-1.45 (m, 6H), 1.42-1.32 (m, 4H), 1.28-1.20 (m, 3H), 1.27 (s, 3H), 1.21 (s, 3H), 0.91 (s, 3H), 0.90 (s, 6H), 0.89 (s, 3H), 0.81 (s, 6H); 13C NMR (100MHz, CDCl3): δ 176.2, 156.6, 135.2, 134.7, 126.7 (2C), 126.2 (2C), 122.1, 90.7, 90.2, 81.8, 81.2, 62.9, 47.3, 43.6, 42.6 (2C), 42.3, 42.1 (2C), 40.7 (2C), 39.9, 39.2, 36.3, 33.2, 33.0, 32.9, 32.8, 32.7, 28.4, 28.0, 27.0, 26.9, 24.3, 24.2, 22.4, 21.3 (2C), 18.8 (2C), 16.7 (2C); HRMS-ESI (m/z): calcd for C44H70N4O4Na [M+Na]+: 741.5292, found 741.5292.

4.2. Cells, culture and plating

The human solid tumor cell lines HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast) and WiDr (colon) were used in this study. Cells were maintained in 25 cm² culture flasks in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum and 2 mM L-glutamine in a 37 °C, 5% CO₂, 95% humidified air incubator. Exponentially growing cells were trypsinized and resuspended in antibiotic containing medium (100 units penicillin G and 0.1 mg of streptomycin per mL). Single cell suspensions displaying >97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto 96-well microtiter plates. Cells were inoculated in a volume of 100 µL per well at densities of 20,000 (WiDr), 15,000 (T-47D) and 10,000 (HeLa, SW1573 and HBL-100) cells per well, based on their doubling times.

4.3. Antiproliferative tests
Chemosensitivity tests were performed using the SRB assay of the NCI with slight modifications. Briefly, pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, negative control). Each agent was tested in triplicates at different dilutions in the range 1–100 µM. The drug treatment was started on day 1 after plating. Drug incubation times were 48 h, after which time cells were precipitated with 25 µL ice-cold 50% (w/v) trichloroacetic acid and fixed for 60 min at 4 ºC. Then the SRB assay was performed. The optical density (OD) of each well was measured at 492 nm, using BioTek's PowerWave XS Absorbance Microplate Reader. Values were corrected for background OD from wells only containing medium. The percentage growth (PG) was calculated with respect to untreated control cells (C) at each of the drug concentration levels based on the difference in OD at the start (T0) and end of drug exposure (T), according to NCI formulas. Therefore, if T is greater than or equal to T0 the calculation is 100 × [(T–T0)/(C–T0)]. If T is less than T0 denoting cell killing the calculation is 100 × [(T–T0)/(T0)]. The effect is defined as percentage of growth, where 50% growth inhibition (GI50) represents the concentration at which PG is +50. With these calculations a PG value of 0 corresponds to the amount of cells present at the start of drug exposure, while negative PG values denote net cell kill.

Acknowledgments
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References and notes


Figure 1: Grindelic acid and 1-(α)-hydroxygrindelic acid
**Scheme 1**: Reagents and conditions: a) CH\(_2\)N\(_2\), Et\(_2\)O, rt. 94%; b) LiAlH\(_4\), THF, 70 °C, 91%; c) SO\(_3\).Py, Et\(_3\)N, DMSO, Ph\(_3\)P=CH(CH\(_3\))CO\(_2\)Et, CH\(_2\)Cl\(_2\), rt, 74%; d) DIBAL-H, Et\(_2\)O, 0 °C, 82-89%; e) SO\(_3\).Py, Et\(_3\)N, DMSO, Ph\(_3\)P=CHCO\(_2\)Et, CH\(_2\)Cl\(_2\), rt, 94%; f) Ti(OPr-i)\(_4\), R,R-(+)-DET, TBHP, CH\(_2\)Cl\(_2\), -20 °C, 71%; g) Ti(OPr-i)\(_4\), S,S-(-)-DET, TBHP, CH\(_2\)Cl\(_2\), -20 °C, 85%.

**Scheme 2**: Reagents and conditions: a) MsCl, TEA, CH\(_2\)Cl\(_2\), 0 °C, 12h, 90%; b) NaN\(_3\), DMF, 40 °C, 48h, 80%; c) (COCl)\(_2\), Et\(_3\)N, DMSO, Cl\(_2\)CH\(_2\), -78 °C, 30 min, 99%; d) PPh\(_3\), CBr\(_4\), Et\(_3\)N, Cl\(_2\)CH\(_2\), 0 °C, 15 min; e) n-BuLi, THF, -78 °C, 30 min, 53%.

**Scheme 3**: Reagents and conditions: a) CDI, Cl\(_2\)CH\(_2\), 40 °C, 16 h, 71%; b) i: ICH\(_3\), acetonitrile, 60 °C, 2h, ii: RNH\(_2\), Cl\(_2\)CH\(_2\), 4h, rt, 79-83%; c) i: ICH\(_3\), acetonitrile, 60 °C, 2h, ii: ethylenediamine, Cl\(_2\)CH\(_2\), 4h, rt, 51%; d) 23, sodium ascorbate, CuSO\(_4\).5H\(_2\)O, H\(_2\)O-EtOH, rt, 64%.
Table 1: Diamides obtained from the multicomponent Ugi reaction of grindelic acid (1).\(^a\)

\[ 
\begin{align*}
\text{Table 1: Diamides obtained from the multicomponent Ugi reaction of grindelic acid (1).} & \quad ^a
\end{align*}
\]

<table>
<thead>
<tr>
<th>Compound</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>Yield (^b)</th>
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<tr>
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\(^a\) All the reactions were carried out using 1 eq of compound 1, 1 eq of the corresponding amine, 1.2 eq of propanone and 1.1 eq of the corresponding isocyanide.

\(^b\) Isolated yield.
Table 2: 1,2,3-triazole obtained by Huisgen reaction using mono-alkynes.\(^a\)

\[
\begin{align*}
\text{Compound} & & R & & \text{yield} \\
27 & & \text{(CH)}_2 & & 48 \\
28 & & & & 78 \\
29 & & & & 64
\end{align*}
\]

\(^a\) All the reactions were carried out using the azide 23 (1 equiv), alkyne (1 equiv), sodium ascorbate (0.3 equiv) and copper(II) sulfate pentahydrate (0.12 equiv) in a 1:1 mixture of water and ethanol (0.1 M).

\(^b\) Isolated yield.
Table 3: 1,2,3-triazole obtained by Huisgen reaction using di-alkynes.\textsuperscript{a}

![Reaction Diagram]

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<tr>
<th>Compound</th>
<th>R</th>
<th>yield</th>
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<tr>
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</tr>
<tr>
<td>32</td>
<td></td>
<td>46</td>
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</table>

\textsuperscript{a} All the reactions were carried out using the azide 23 (1 equiv), di-alkyne (0.5 equiv), sodium ascorbate (0.3 equiv) and copper(II) sulfate pentahydrate (0.12 equiv) in a 1:1 mixture of water and ethanol (0.1 M).

\textsuperscript{b} Isolated yield.
### Table 4: *In vitro* antiproliferative activity against human solid tumor cell lines.\(^a\)

<table>
<thead>
<tr>
<th>Comp.</th>
<th>HBL-100 (breast)</th>
<th>HeLa (cervix)</th>
<th>SW1573 (NSCLC)</th>
<th>T-47D (breast)</th>
<th>WiDr (colon)</th>
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<tbody>
<tr>
<td>1</td>
<td>46 (±10)</td>
<td>60 (±31)</td>
<td>49 (±28)</td>
<td>69 (±5.5)</td>
<td>57 (±13)</td>
</tr>
<tr>
<td>2</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;72 (±39)</td>
<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
<td>3</td>
<td>17 (±2.4)</td>
<td>16 (±2.9)</td>
<td>18 (±1.9)</td>
<td>19 (±5.4)</td>
<td>23 (±3.9)</td>
</tr>
<tr>
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<td>17 (±2.6)</td>
<td>17 (±2.5)</td>
<td>19 (±1.1)</td>
<td>16 (±1.8)</td>
<td>21 (±0.65)</td>
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<td>5</td>
<td>17 (±4.4)</td>
<td>18 (±5.8)</td>
<td>20 (±4.5)</td>
<td>32 (±9.1)</td>
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</tr>
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<td>6</td>
<td>16 (±2.6)</td>
<td>16 (±2.3)</td>
<td>18 (±1.3)</td>
<td>15 (±1.7)</td>
<td>19 (±0.23)</td>
</tr>
<tr>
<td>7</td>
<td>16 (±3.8)</td>
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<td>8</td>
<td>17 (±1.7)</td>
<td>18 (±2.5)</td>
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<td>16 (±2.3)</td>
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<td>18 (±0.64)</td>
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<td>10</td>
<td>16 (±1.9)</td>
<td>17 (±1.4)</td>
<td>18 (±1.0)</td>
<td>15 (±2.5)</td>
<td>17 (±0.14)</td>
</tr>
<tr>
<td>11</td>
<td>4.6 (±3.9)</td>
<td>5.3 (±5.6)</td>
<td>11 (±1.4)</td>
<td>3.9 (±0.87)</td>
<td>12 (±0.84)</td>
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<tr>
<td>12</td>
<td>10 (±3.5)</td>
<td>30 (±6.9)</td>
<td>14 (±2.6)</td>
<td>40 (±4.5)</td>
<td>49 (±7.9)</td>
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<tr>
<td>13</td>
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<td>4.7 (±0.84)</td>
<td>3.6 (±0.71)</td>
<td>4.4 (±1.0)</td>
<td>5.1 (±0.82)</td>
</tr>
<tr>
<td>14</td>
<td>8.9 (±2.2)</td>
<td>11 (±1.6)</td>
<td>5.1 (±1.9)</td>
<td>31 (±5.8)</td>
<td>38 (±7.8)</td>
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<td>15</td>
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<td>2.9 (±0.89)</td>
<td>1.8 (±0.51)</td>
<td>3.0 (±0.78)</td>
<td>3.1 (±1.3)</td>
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<td>16</td>
<td>16 (±0.88)</td>
<td>18 (±2.0)</td>
<td>16 (±3.3)</td>
<td>19 (±2.3)</td>
<td>12 (±2.4)</td>
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<td>17</td>
<td>0.95 (±0.38)</td>
<td>1.6 (±0.46)</td>
<td>1.2 (±0.54)</td>
<td>1.9 (±0.62)</td>
<td>1.8 (±0.81)</td>
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<td>18</td>
<td>5.4 (±2.1)</td>
<td>5.7 (±2.6)</td>
<td>5.8 (±2.6)</td>
<td>5.3 (±2.5)</td>
<td>6.8 (±0.28)</td>
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<td>8.7 (±0.63)</td>
<td>22 (±2.3)</td>
<td>5.9 (±1.2)</td>
<td>21 (±3.5)</td>
<td>37 (±8.2)</td>
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<tr>
<td>24</td>
<td>14 (±3.5)</td>
<td>17 (±2.6)</td>
<td>17 (±3.3)</td>
<td>26 (±8.1)</td>
<td>15</td>
</tr>
<tr>
<td>26</td>
<td>17 (±1.9)</td>
<td>16 (±1.2)</td>
<td>15 (±2.4)</td>
<td>28 (±10)</td>
<td>4.8</td>
</tr>
<tr>
<td>27</td>
<td>16 (±5.0)</td>
<td>25 (±6.8)</td>
<td>15 (±2.3)</td>
<td>44 (±13)</td>
<td>15</td>
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<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
<td>29</td>
<td>9.3 (±1.4)</td>
<td>17 (±0.82)</td>
<td>11 (±0.5)</td>
<td>17 (±1.0)</td>
<td>n.a.</td>
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<td>3.0 (±0.39)</td>
<td>4.3 (±0.63)</td>
<td>1.3</td>
</tr>
<tr>
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<td>&gt;100</td>
<td>&gt;100</td>
</tr>
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<td>33</td>
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<td>18 (±1.1)</td>
<td>17 (±0.85)</td>
<td>17 (±0.88)</td>
<td>13</td>
</tr>
<tr>
<td>34</td>
<td>6.1 (±0.57)</td>
<td>7.8 (±2.9)</td>
<td>8.0 (±6.9)</td>
<td>4.3 (±0.98)</td>
<td>1.4</td>
</tr>
<tr>
<td>35</td>
<td>14 (±0.35)</td>
<td>14 (±1.6)</td>
<td>13 (±2.3)</td>
<td>16 (±2.4)</td>
<td>12 (±1.9)</td>
</tr>
<tr>
<td>37</td>
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<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
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</table>

**Cisplatin**  
1.9 (±0.2)  
2.0 (±0.3)  
3.0 (±0.4)  
15 (±2.3)  
26 (±5.3)  

**Camptothecin**  
0.23 (±0.05)  
0.6 (±0.4)  
0.25 (±0.12)  
2.0 (±0.5)  
1.8 (±0.7)  

\(^a\) Values expressed as GI\(_{50}\) (50% growth inhibition) are given in µM and are means of two to four experiments, standard deviation is given in parentheses.
Figure 1: Grindelic acid and 1-(α)-hydroxygrindelic acid
Highlights

- Syntheses of grindelic acid derivatives.
- Oxy-nitrogenated and nitrogenated groups are key for the cytotoxic activity.
- The more active derivative showed a GI50 values in the range 0.95-1.8 µM.
- Labdanes-type diterpenes represent a potential source for new anticancer drug candidates.
European Journal of Medicinal Chemistry

Supplementary data

Derivatives of grindelic acid: from a non-active natural diterpene to synthetic antitumor derivatives

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*Corresponding author: e-mail:odonadel@gmail.com, Tel/Fax: +54 266 4439909
Figure S1. Spectra of Grindelic acid methyl ester (3)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S2. Spectra of Grindelic alcohol, (4)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S3. Spectra of (E)-ethyl 2-methyl-4-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-enolate, (5).

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S4. Spectra of (E)-2-methyl-4-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-en-1-ol, (6).

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S5. Spectra of (E)-ethyl 4-((1'R,4a'S,5S,8a'S)-2',5,5',5', 8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-enoate, (7).

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S6. Spectra of (E)-2-methyl-4-((1'R,4a'S,5S,8a'S)-2',5, 5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H, 4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-en-1-ol, (8).

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S7. Spectra of ((2S,3S)-3-(((1'R,4a'S,5S,8a'S)-2',5,5',5', 8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)methyl)oxiran-2-yl)methanol, (9)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S8. Spectra of ((2R,3R)-3-(((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro [furan-2,1'-naphthalene]-5-yl)methyl)oxiran-2-yl)methanol, (10)

\(^1\)H-NMR (CDCl\(_3\))

\(^{13}\)C-NMR (CDCl\(_3\))
Figure S9. Spectra of 2-(N-benzyl-2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H, 4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-N-tert-butyl-2-methylpropanamide, \(11\)

\(^1\)H-NMR (CDCl\(_3\))

\(^{13}\)C-NMR (CDCl\(_3\))
Figure S10. Spectra of 2-(N-benzyl-2-((1'R,5S,8α'S)-2',5,5',5',8α'-pentamethyl-4,4α',5,5',6',7',8',8α'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-2-methyl-N-(2,3,3-trimethylbutan-2-yl)propanamide, (12)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
**Figure S11.** Spectra of 2-(N-benzyl-2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-N-cyclohexyl-2-methylpropanamide, (13)

\[ \text{\(^1H\)-NMR (CDCl}_3\)]

\[ \text{\(^{13}C\)-NMR (CDCl}_3\)]
Figure S12. Spectra of 2-(N-benzyl-2-((1'R,5S,8S')-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-N-(2,6-dimethylphenyl)-2-methylpropanamide, (14)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S13. Spectra of 2-(N-benzyl-2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-N-buty1-2-methylpropanamide, (15)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S14. Spectra of 2-(N-benzyl-2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-2-methyl-N-(2-morpholinoethyl)propanamide, (16) 

$^1$H-NMR (CDCl$_3$)

13C-NMR (CDCl$_3$)
**Figure S15.** Spectra of N-benzyl-2-methyl-2-(2-(1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7', 8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)-N-phenylacetamido)propanamide, (17)

\[ ^1H\text{-NMR (CDCl}_3\text{)} \]

\[ ^{13}\text{C-NMR (CDCl}_3\text{)} \]

- **\( ^1H\)-NMR (CDCl\(_3\))**
- **\( ^{13}\text{C-NMR (CDCl}_3\text{)} \)**

GFR-046-C13_400MHz
Figure S16. Spectra of Preparation of N-tert-butyl-2-methyl-2-(2-((1'R,4a'S,5S, 8a'S)-2',5',5',8a'-pentamethyl-4,4a',5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)-N-phenylacetamido)propanamide. (18)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S17. Spectra of N-(2,6-dimethylphenyl)-2-methyl-2-(2-(((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)-N-phenylacetamido)propanamide, (19)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
**Figure S18.** Spectra of N-butyl-2-methyl-2-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8', 8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)-N-phenylacetamido)propaneamide, (20)

$^1\text{H-NMR (CDCl}_3\text{)}$

$^{13}\text{C-NMR (CDCl}_3\text{)}$
Figure S19. Spectra of N-benzyl-2-methyl-2-(2-((1'R,5S,8a'S)-2',5,5',5',8a'pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)-N-phenylacetamido)propanamide, (21)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S20. Spectra of N-cyclohexyl-2-methyl-2-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)-N-phenylacetamido)propanamide, (22)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S21. Spectra of Preparation of (1'R,4a'S,5S,8a'S)-5-(2-azidoethyl)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene], (23)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S22. Spectra of 3-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)propanal, (24)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S23. Spectra of (1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-5-(prop-2-ynyl)-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene], (26)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S24. Spectra of (4-decyl-1-t-2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazole, (27)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S25. Spectra of 4-(3-ethynylphenyl)-1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazole, (28)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S26. Spectra of 1-(2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-4-(((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)methyl)-1H-1,2,3-triazole, (29)

$^1$H-NMR (CDCl$_3$)

[Image of $^1$H-NMR spectrum]

$^{13}$C-NMR (CDCl$_3$)

[Image of $^{13}$C-NMR spectrum]
Figure S27. Spectra of 1,4-bis(1-(2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)butane, (30)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S28. Spectra of 1,3-bis(1-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)benzene, (31)

\[ ^1H-NMR \text{ (CDCl}_3\text{)} \]

\[ ^{13}C-NMR \text{ (CDCl}_3\text{)} \]
Figure S29. Spectra of 1,3-bis((1'-(2-(((1'R,5S,8a'S)-2',5,5',8a'-pentamethyl-4,4a',5,5',6',7',8a'-octahydro-3H,4'H-spiro[furan-2,1'-'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzene, (32)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
**Figure S30.** Spectra of 2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl-1H-imidazole-1-carboxylate, (33)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S31. Spectra of 2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl-3-(dimethylamino)propylcarbamate, (34)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S32. Spectra of 2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl prop-2-ynylcarbamate, (35)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
**Figure S33.** Spectra of bis(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl) ethane-1,2-diylidicarbamate, (36)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S34. Spectra of 2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl (1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)methylcarbamate, (37)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Natural products. Starting material:

Figure S35. Spectra of grindelic acid (1)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CDCl$_3$)
Figure S36. Spectra of 1α-hydroxygrindelic acid (2)

$^1$H-NMR (CDCl$_3$)

$^{13}$C-NMR (CCl$_3$)

Note: Additional spectroscopic and spectrometric data available for request to the corresponding author.