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(54) Title: ERINACINE A AND ANALOGS THEREOF FOR THERAPEUTIC USE

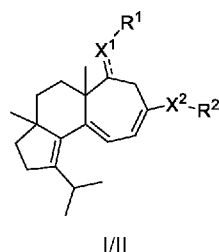
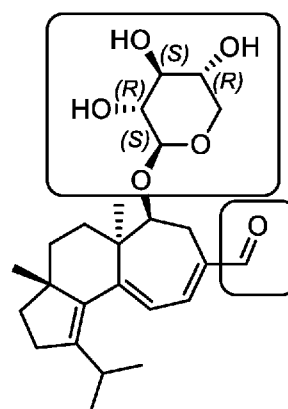


FIGURE 1



Erinacine A (ErA)

(57) Abstract: The present application includes compounds of Formula I and II, compositions comprising these compounds and uses thereof, in particular for treatment of central nervous system (CNS) or peripheral nervous system (PNS) diseases, disorders or conditions, and for promoting or improving wound healing. (Formula I/II)

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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TITLE: ERINACINE A AND ANALOGS THEREOF FOR THERAPEUTIC USE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority from U.S. provisional patent application no. 63/613,778 filed on December 22, 2023, the contents of which are incorporated by reference in their entirety.

FIELD

[0002] The present application relates to Erinacine A and analogues and compositions thereof and their use, for example, for the treatment of central or peripheral nervous system disorders. Further, the present application relates to the use of Erinacine A and/or said analogues in wound healing.

BACKGROUND

[0003] Neurological disorders such as Alzheimer's disease, Parkinson's disease, spinal cord injury and others have a profound impact on millions of individuals worldwide and pose a significant global burden [M. O. Owolabi, 2023]. These conditions are characterized by neurodegeneration and dysregulation of neurotrophic factors, also referred as neurotrophins (NTs), including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which play significant roles in the central nervous system (CNS) and in peripheral nervous system (PNS) [J. Kim, 2023] [Huang EJ, 2001]. Since neurotrophins are unable to penetrate the blood brain barrier (BBB) due to their high molecular weights, discovery of NGF or BDNF inducing small molecules holds particular relevance, and they represent promising drug candidates against neurodegenerative diseases [J. Xu, 2014].

[0004] Several NGF inducing cyathane diterpenoids such as Cyathin Q, Erinacine A (ErA) and Sarcodonin G have been isolated from various higher Basidiomycetes of the genera *Cyathus*, *Hericium*, and *Sarcodon* respectively. ErA derived from the mycelium of *Hericium erinaceus*, is one of the representatives of the erinacine group of metabolites shown to have neuroprotective and neuritogenic properties [F. L. Zhang, 2022 , [B. J. Ma, 2010]. The structure of ErA is shown in Figure 1.

[0005] ErA has been shown to possess potent stimulatory effects on the biosynthesis of NGF and BDNF *in vitro* as well as the ability to enhance catecholamine levels *in vivo* in rats [H. Kawagishi, 1994], [M. Shimbo, 2005], [Chiu CH, 2018].

[0006] In addition to the role of NTs in the context of neuronal development, recent research has unveiled their involvement in diverse biological processes, extending beyond the

nervous system. For instance, NTs have been implicated in the maintenance of skin homeostasis, tissue repair and regeneration, regulation of stem cell differentiation, immune modulation, angiogenesis, and muscle repair (Xiao N, 2016).

[0007] Chronic wounds pose a substantial medical and financial challenge to the healthcare system globally. In the United States, wounds make up 1% of all skin disease diagnoses and contribute to 6.07% of annual cutaneous-related deaths (Darwin E, 2018). Notably, the 5-year mortality rate for conditions like diabetic foot ulcers and ischemic ulcers surpasses that of certain cancers, such as breast and prostate cancer [Darwin E, 2018, Sen CK., 2021]. Besides the associated morbidity and mortality, wound care stands as the costliest skin ailment, with over \$14 billion dedicated annually solely to managing venous leg ulcers [Darwin E, 2018]. It is of utmost importance to develop new and effective therapies, especially for chronic wounds to improve patient outcomes and reduce healthcare costs. Cutaneous wound healing is a complex and dynamic biological process involving the coordinated efforts of various cell types (immune cells, keratinocytes, fibroblasts, and vascular endothelial cells), cytokines, signaling pathways, and extracellular matrix components [Wilkinson HN, 2023].

[0008] The wound healing process is divided into four overlapping phases of haemostasis, inflammation, proliferation, and tissue remodelling. Any disturbances in these processes can lead to complications, such as chronic nonhealing ulcers or keloids [Eming SA, 2014]. NTs like NGF have been established to play critical roles in the regulation of wound healing, by promoting epithelial cell and fibroblast migration, angiogenesis, inflammatory response regulation and tissue remodelling [Liu Z, 2021, Chen JC, 2014, Gostynska N, 2020]. The topical application of exogenous NGF has been explored as a potential therapy to accelerate wound repair and proven effective against various wound types, including diabetic foot ulcers, pressure ulcers, and corneal wounds [Liu Z, 2021]. Interestingly, the FDA has recently approved the use of NGF ocular drops for treatment of rare neurotrophic keratitis, which is not only the first-ever topical biologic pharmaceutical approved in ophthalmology, but also the first-ever use of human NGF as drug or treatment [Kanu LN, 2021]. However, the therapeutic potential of small molecules with neurotrophic properties in the specific domain of wound healing remains largely unexplored.

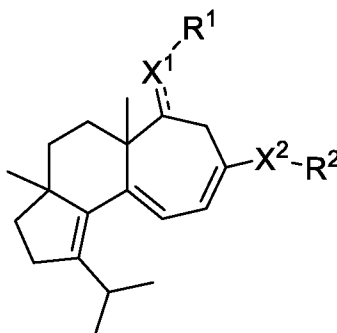
[0009] There remains a need to further develop treatments for central or peripheral nervous system disorders and wound healing.

SUMMARY

[0010] The present application discloses ErA analogues with, for example, enhanced efficacy, reduced toxicity, and improved pharmacokinetic properties for the treatment of CNS or

PNS related disorders in comparison to ErA. ErA and ErA analogues of the present application are also useful to improve or promote wound healing.

[0011] Therefore, the present application includes a compound of Formula I, or a pharmaceutically acceptable salt and/or solvate thereof:



I

wherein:

---- is a single or double bond;

when ---- is a double bond, X^1 is selected from O and NR^3 and R^1 is absent;

when ---- is a single bond, X^1 is selected from O, $OC(O)$ and NR^3 and R^1 is selected from H, $SO_2NR^4R^5$, $C_{1-10}alkyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{6-10}aryl$, $C_{5-10}heteroaryl$, $C_{1-10}alkylenearyl$, $C_{1-10}alkyleneheteroaryl$, $C_{1-10}alkyleneC_{3-8}cycloalkyl$ and $C_{1-10}alkyleneC_{3-8}heterocycloalkyl$, the latter nine groups being optionally substituted with one or more of OH, halo, $OC_{1-6}alkyl$, $OC(O)C_{1-6}alkyl$, $C(O)OC_{1-6}alkyl$, $OSi(CH_3)_2(C_{1-6}alkyl)$ and $C_{1-6}alkyl$;

X^2 is selected from a direct bond, $C(O)$, $C(O)Y^1$, $C(NR^{11})$, Y^1SO_2 , $C_{1-6}alkyleneY^1$, $C_{1-6}alkyleneY^1C(O)$, $C_{1-6}alkyleneY^1SO_2$, $C_{1-6}alkyleneY^1SO_2Y^2$ and $C_{1-6}alkyleneY^1C(O)Y^2$;

R^2 is selected from H, $C_{1-10}alkyl$, $C_{2-10}alkenyl$, $C_{2-10}alkynyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{6-10}aryl$, $C_{6-10}heteroaryl$, $C_{1-10}alkylenearyl$, $C_{1-10}alkyleneheteroaryl$, $C_{1-10}alkyleneC_{3-8}cycloalkyl$, $C_{1-10}alkyleneC_{3-8}heterocycloalkyl$ and $phenylC(O)phenylY^3C(O)R^6$, the latter twelve groups being optionally substituted with one or more of OH, $=O$, $C_{1-6}alkyl$, $OC_{1-6}alkyl$, NR^7R^8 , $NR^9C(O)OC_{1-6}alkyl$, and halo;

Y^1 , Y^2 and Y^3 are independently selected from NR^{10} and O;

R^3 , R^4 , R^5 , R^7 , R^8 , and R^9 are independently selected from H and $C_{1-6}alkyl$;

R^6 is selected from $C_{1-10}alkyl$, $C_{2-10}alkenyl$ and $C_{2-10}alkynyl$;

R^{10} is selected from H and C_{1-6} alkyl;

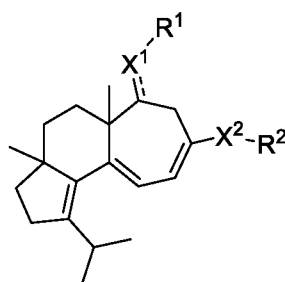
R^{11} is selected from H, C_{1-6} alkyl, C_{6-10} aryl and SO_2C_{6-10} aryl; and

wherein all available hydrogen atoms are optionally and independently replaced with a fluorine or chlorine atom,

provided that when X^1-R^1 is OH, then X^2-R^2 is not CH_2OH , $C(O)OH$ or $C(O)H$.

[0012] The present application also includes a pharmaceutical composition comprising one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, and a pharmaceutically acceptable carrier.

[0013] The present application includes a method of treating a disease, disorder or condition that benefits from induction of biosynthesis of nerve growth factor (NGF) and/or brain-derived neurotrophic factor (BDNF), the method comprising administering a therapeutically effective amount of one or more compounds of Formula II to a subject in need thereof:



II

wherein:

---- is a single or double bond;

when ---- is a double bond, X^1 is selected from O and NR^3 and R^1 is absent;

when ---- is a single bond, X^1 is selected from O, $OC(O)$ and NR^3 and R^1 is selected from H, $SO_2NR^4R^5$, C_{1-10} alkyl, C_{3-10} cycloalkyl, C_{3-10} heterocycloalkyl, C_{6-10} aryl, C_{5-10} heteroaryl, C_{1-10} alkylenearyl, C_{1-10} alkyleneheteroaryl, C_{1-10} alkylene C_{3-8} cycloalkyl and C_{1-10} alkylene C_{3-8} heterocycloalkyl, the latter nine groups being optionally substituted with one or more of OH, halo, OC_{1-6} alkyl, $OC(O)C_{1-6}$ alkyl, $C(O)OC_{1-6}$ alkyl, $OSi(CH_3)_2C_{1-6}$ alkyl and C_{1-6} alkyl;

X^2 is selected from a direct bond, $C(O)$, $C(O)Y^1$, $C(NR^{11})$, Y^1SO_2 , C_{1-6} alkylene Y^1 , C_{1-6} alkylene $Y^1C(O)$, C_{1-6} alkylene Y^1-SO_2 , C_{1-6} alkylene $Y^1-SO_2Y^2$ and C_{1-6} alkylene $Y^1C(O)Y^2$;

R² is selected from H, C₁₋₁₀alkyl, C₂₋₁₀alkenyl, C₂₋₁₀alkynyl, C₃₋₁₀cycloalkyl, C₃₋₁₀heterocycloalkyl, C₆₋₁₀aryl, C₆₋₁₀heteroaryl, C₁₋₁₀alkylenearyl, C₁₋₁₀alkyleneheteroaryl, C₁₋₁₀alkyleneC₃₋₈cycloalkyl, C₁₋₁₀alkyleneC₃₋₈heterocycloalkyl and phenylC(O)phenylY³C(O)R⁶, the latter twelve groups being optionally substituted with one or more of OH, =O, C₁₋₆alkyl, OC₁₋₆alkyl, NR⁷R⁸, NR⁹C(O)OC₁₋₆alkyl, and halo;

Y¹, Y² and Y³ are independently selected from NR¹⁰ and O;

R³, R⁴, R⁵, R⁷, R⁸, and R⁹ are independently selected from H and C₁₋₆alkyl;

R⁶ is selected from C₁₋₁₀alkyl, C₂₋₁₀alkenyl and C₂₋₁₀alkynyl;

R¹⁰ is selected from H and C₁₋₆alkyl;

R¹¹ is selected from H, C₁₋₆alkyl, C₆₋₁₀aryl and SO₂C₆₋₁₀aryl; and

wherein all available hydrogen atoms are optionally and independently replaced with a fluorine or chlorine atom.

[0014] In some embodiments, the disease, disorder or condition that benefits from induction of biosynthesis of nerve growth factor (NGF) and/or brain-derived neurotrophic factor (BDNF) is a central nervous system (CNS) or a peripheral nervous system (PNS) disease, disorder or condition.

[0015] The present application also includes a method of promoting or improving wound healing, the method comprising administering to a subject in need thereof, a therapeutically effective amount of ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of Formula II, or a pharmaceutically acceptable salt and/or solvate thereof.

[0016] Other features and advantages of the present application will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating embodiments of the application, are given by way of illustration only and the scope of the claims should not be limited by these embodiments but should be given the broadest interpretation consistent with the description as a whole.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Certain embodiments of the application will now be described in greater detail with reference to the attached drawings in which:

[0018] Figure 1 shows two potential sites for chemical modifications of ErA in exemplary embodiments of the application.

[0019] Figure 2 shows the results of the ErA identification study. A) total ion chromatogram (TIC) obtained by LC-MS analysis of *H. erinaceus* mycelial extract; (B) ErA peak detected at RT 8.305 minutes from HPLC-DAD (UV spectrum at $\lambda = 340$ nm); and (C) fragmentation spectrum of the detected ErA peak at 340 nm. The LC-MS-ESI detected the ErA aglycon m/z 301.2177, $[M+Na^+]$ m/z 455.2424 and $[2M+Na^+]$ m/z 887.4951 adducts.

[0020] Figure 3 shows the results of the ErA neurotrophic potential study using an *in vitro* neurite outgrowth assay in rat pheochromocytoma (PC12) cells: morphological differentiation of PC12 cells incubated with ErA (0.1 μ M – bottom left, and 1 μ M – bottom right), DMSO (0.5% - top right), nerve growth factor (NGF) (75 ng/mL – middle left), J147 (0.3 μ M – middle right) and PC12 cells treated with unconditioned medium (PC12-NOT CM – top left).

[0021] Figure 4 shows the results of the *in vitro* toxicity testing of ErA. Cell viability of cultured human peripheral blood mononuclear cells (PBMCs) exposed to ErA (0-60 μ M) was assessed after 24 hours of exposure.

[0022] Figure 5 shows the results of the *in vitro* blood-brain barrier (BBB) permeability testing of ErA. The graph shows the effective permeability (Pe) of ErA and respective kit controls, High Control (HC) and Low Control (LC).

[0023] Figure 6 shows the results of the biological activity analysis of ErA aglycon: (A) cell viability of PBMCs exposed to ErA aglycon (0-30 μ M) assessed after 24 hours after exposure; (B) neurite outgrowth activity of ErA aglycon on PC12 cells; (C) *in vitro* BBB permeability assessed by BBB-PAMPA assay: Comparison of the effective permeability (Pe) of ErA, ErA aglycon and respective kit controls, High Control (HC) and Low Control (LC).

[0024] Figure 7 shows the results of the *in vitro* toxicity studies for exemplary compounds of the application: I-32, I-33, I-107, I-34, I-36, I-37, I-38, I-39 and I-40, as well as the compounds II-1, II-2 and II-3. Cell viability of cultured human PBMCs in response to the analogs at (1-30 μ M) was assessed after 24 hours of exposure.

[0025] Figure 8 shows the results of the *in vitro* toxicity studies for exemplary compounds of the application: I-1 to I-8, I-52 and I-53. Cell viability of cultured human PBMCs in response to the compounds at (1-30 μ M) was assessed after 24 hours of exposure.

[0026] Figure 9 shows the results of the *in vitro* BBB permeability analysis assessed by BBB-PAMPA assay: Comparison of the effective permeability (Pe) of ErA, with exemplary compounds ErA aglycon, I-108, I-34, I-39, II-2, I-3, I-4, I-6 and respective kit controls; High Control (HC) and Low Control (LC).

[0027] Figure 10 shows the results of the neurite outgrowth assay of Il-2 at concentration 0.3 μ M on PC12 cells.

[0028] Figure 11 shows the results of the neurite outgrowth assay of ErA at indicated concentrations on HT-22 cells assessed after 48 hours of incubation in serum-free media. Cells were fixed and processed for immunofluorescence against β -tubulin and imaged under the microscope at 20X magnification. NGF at 100 ng/ml is used as the positive control.

[0029] Figure 12 shows the results of A) the neurite outgrowth assay on rat DRG neurons treated with ErA (0.003 μ M – 10 μ M) for four days. Cells were fixed and stained with antibody against beta-tubulin and visualised under the microscope. Representative images at 20X magnification are shown. B) the neurite length of rat sensory neurons treated with ErA (0.003 μ M – 10 μ M), calculated from the same experiment. Values are expressed in percentage of control (0.1% DMSO). NGF50: 50 ng/ml, positive control.

[0030] Figure 13 shows the number of rat primary sensory neurons treated with ErA (0.003 μ M – 10 μ M), expressed as percentage of control (0.1% DMSO). NGF50: 50 ng/ml, positive control.

[0031] Figure 14 shows the results of the *in vitro* wound healing assay in primary human fibroblast cells, treated with ErA (10 nM – 1000 nM) at 0h, 16h and 24h, when compared to vehicle control (0.5% DMSO). Images were captured at 4X magnification.

[0032] Figure 15 shows the results of the *in vitro* proliferation assay in primary human fibroblast cells. Live cells were monitored over time with the Presto Blue Cell Viability Reagent at time intervals of t = 6, 12, 24 and 48 h (shown in sections from left to right) after treatment with ErA at indicated concentrations (0.01- 1 μ M).

[0033] Figure 16 show the results of the *in vitro* tube formation assay. HUVEC cells were plated on matrigel precoated 96-well plates and treated with different concentrations of ErA for 16 h. Fluorescent micrographs show the effects of ErA on differentiation of HUVECs into capillary tube-like structures.

[0034] Figure 17 shows the results of the *in vitro* proliferation assay in primary human endothelial cells. Live HUVEC cells were monitored over time with the PrestoBlue Cell Viability Reagent at time intervals of t = 6, 12, 24 and 48 h (shown in section from left to right) upon treatment with ErA (0.01- 1 μ M).

[0035] Figure 18 shows the results of the *in vitro* trans-well migration assay on endothelial cells. HUVEC cells were treated with ErA (0.01 -10 μ M) and placed on the of the trans-well filter membrane. The lower chambers contained FBS as the chemoattractant and migratory cells were observed using nuclear staining dye, Hoechst. (A) shows fluorescent images of Hoechst-stained migratory endothelial cells adhering underneath the membrane surface were captured at 4X magnification (B) shows the quantified number of migratory cells in comparison to solvent control DMSO and positive control, NGF at 50 ng/ml.

[0036] Figure 19 shows photographs of biopsy punch wounds in diabetic mice, treated with ErA at dose 0.5 mg and 1 mg. The photographs were captured at regular intervals (0 to 8 days) and are shown for comparison with solvent (80 % butylene glycol).

[0037] Figure 20 shows the results of the histological analysis of wound tissues of diabetic mice by hematoxylin and eosin staining, after eight days of treatment at doses, 0.5 mg and 1 mg.

[0038] Figure 21 shows the results of the wound healing analysis as observed by Masson's Trichome staining. Wound tissues were treated with ErA at doses 0.5 mg and 1 mg, in contrast to control untreated wounds.

[0039] Figure 22 shows the results of the immunohistochemistry analysis of wounds to assess the effect of ErA on the expression of markers of inflammation (IL-1b), proliferation (Ki-67), angiogenesis (CD31) and, healing and reepithelialisation (Fibronectin) in tissue sections obtained from wounds of diabetic mice after eight days of treatment with ErA at doses 0.5 mg and 1 mg.

[0040] Figure 23 shows the neuroprotective and neuritogenic effects of Erinacine A in primary rat hippocampal neurons. (A) Representative image of neuron and the phenotypes measured for image analysis are shown. The neuron is shown in light grey, and arrows indicate the neurites originating from the cell body. The opaque white circles indicate the roots where the outgrowth occurs and circles with white outline denote neurite extremities. (B) Effect of ErA on neuronal survival, neurite network, branching points and neurite extremities on rat hippocampal neurons (DIV 3). Compound added on days in vitro (DIV) 0 and left for 3 days. BDNF50: 50 ng/ml BDNF. Results are expressed as a percentage of DMSO control as mean $\pm$ SEM (n = 4-6). One-way ANOVA followed by Fisher's LSD test was used for comparison. *p < 0.05, **p < 0.01, ***p < 0.001; (C) Representative images are shown. Treated cells were fixed, stained with MAP2 antibody and Hoechst dye for DNA and quantified.

I. Definitions

[0041] Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the present application herein described for which they are suitable as would be understood by a person skilled in the art.

[0042] All features disclosed in the specification, including the claims, abstract, and drawings, and all the steps in any method or process disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. Each feature disclosed in the specification, including the claims, abstract, and drawings, can be replaced by alternative features serving the same, equivalent, or similar purpose, unless expressly stated otherwise.

[0043] As used in this application and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "include" and "includes") or "containing" (and any form of containing, such as "contain" and "contains"), are inclusive or open-ended and do not exclude additional, unrecited elements or process steps.

[0044] The term "consisting" and its derivatives as used herein are intended to be closed terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, and also exclude the presence of other unstated features, elements, components, groups, integers and/or steps.

[0045] The term "consisting essentially of", as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of these features, elements, components, groups, integers, and/or steps.

[0046] The terms "about", "substantially" and "approximately" as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation of at least $\pm 5\%$ of the modified term if this deviation would not negate the meaning of the word it modifies or unless the context suggests otherwise to a person skilled in the art.

[0047] As used in the present application, the singular forms "a", "an" and "the" include plural references unless the content clearly dictates otherwise. For example, an embodiment including "a compound" should be understood to present certain aspects with one compound, or two or more additional compounds.

[0048] In embodiments comprising an “additional” or “second” component or effect, such as an additional or second compound, the second compound as used herein is different from the other compounds or first compound. A “third” compound is different from the other, first, and second compounds, and further enumerated or “additional” compounds are similarly different.

[0049] When “one or more” molecules or materials are referenced (such as one or more dendrons), it is understood that this is in reference to the “type” or “identity of the molecule or material. Therefore, a second molecule or material is different from the one, or first, molecule or material. Similarly, a “third” molecule or material is different from the one, first, and second molecules or materials, and further enumerated or “additional” molecules or materials are similarly different.

[0050] The term “and/or” as used herein means that the listed items are present, or used, individually or in combination. In effect, this term means that “at least one of” or “one or more” of the listed items is used or present. The term “and/or” with respect to salts and/or solvates thereof means that the compounds of the application exist as individual salts and solvates, as well as a combination of, for example, a salt of a solvate of a compound of the application.

[0051] The term “compound of the application” or “compound of the present application” and the like as used herein refers to a compound of Formula I or salts and/or solvates thereof, or a compound of Formula II or salts and/solvates thereof. It should be noted that the compounds of Formula II, or salts and/or solvates thereof, are the same as the compounds of Formula I, or salts and/or solvates thereof, with the exception that compounds wherein X^1-R^1 is OH, and X^2-R^2 is CH_2OH , $C(O)OH$ or $C(O)H$ are included in the compounds of Formula II, or salts and/or solvates thereof.

[0052] The term “composition of the application” or “composition of the present application” and the like as used herein refers to a composition comprising one or more compounds of the application.

[0053] The term “suitable” as used herein means that the selection of the particular compound or conditions would depend on the specific synthetic manipulation to be performed, the identity of the molecule(s) to be transformed and/or the specific use for the compound, but the selection would be well within the skill of a person trained in the art.

[0054] The present description refers to a number of chemical terms and abbreviations used by those skilled in the art. Nevertheless, definitions of selected terms are provided for clarity and consistency.

[0055] The term “protecting group” or “PG” and the like as used herein refers to a chemical moiety which protects or masks a reactive portion of a molecule to prevent side reactions in those reactive portions of the molecule, while manipulating or reacting a different portion of the molecule. After the manipulation or reaction is complete, the protecting group is removed under conditions that do not degrade or decompose the remaining portions of the molecule. The selection of a suitable protecting group can be made by a person skilled in the art. Many conventional protecting groups are known in the art, for example as described in “Protective Groups in Organic Chemistry” McOmie, J.F.W. Ed., Plenum Press, 1973, in Greene, T.W. and Wuts, P.G.M., “Protective Groups in Organic Synthesis”, John Wiley & Sons, 3rd Edition, 1999 and in Kocienski, P. Protecting Groups, 3rd Edition, 2003, Georg Thieme Verlag (The Americas).

[0056] The term “inert organic solvent” as used herein refers to a solvent that is generally considered as non-reactive with the functional groups that are present in the compounds to be combined together in any given reaction so that it does not interfere with or inhibit the desired synthetic transformation. Organic solvents are typically non-polar and dissolve compounds that are non soluble in aqueous solutions.

[0057] The term “alkyl” as used herein, whether it is used alone or as part of another group, means straight or branched chain, saturated alkyl groups. The number of carbon atoms that are possible in the referenced alkyl group are indicated by the prefix “C_{n1-n2}”. For example, the term C₁₋₁₀alkyl means an alkyl group having 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms. All alkyl groups are optionally fluoro-substituted unless otherwise indicated.

[0058] The term “alkylene”, whether it is used alone or as part of another group, means straight or branched chain, saturated alkylene group, that is, a saturated carbon chain that contains substituents on two of its ends. The number of carbon atoms that are possible in the referenced alkylene group are indicated by the prefix “C_{n1-n2}”. For example, the term C₂₋₆alkylene means an alkylene group having 2, 3, 4, 5 or 6 carbon atoms. All alkylene groups are optionally fluoro-substituted unless otherwise indicated.

[0059] The term “alkenyl” as used herein, whether it is used alone or as part of another group, means straight or branched chain, unsaturated alkyl groups containing at least one double bond. The number of carbon atoms that are possible in the referenced alkylene group are indicated by the prefix “C_{n1-n2}”. For example, the term C₂₋₆alkenyl means an alkenyl group having 2, 3, 4, 5 or 6 carbon atoms and at least one double bond. All alkenyl groups are optionally fluoro-substituted unless otherwise indicated.

[0060] The term “alkynyl” as used herein, whether it is used alone or as part of another group, means straight or branched chain, unsaturated alkynyl groups containing at least one triple bond. The number of carbon atoms that are possible in the referenced alkyl group are indicated by the prefix “C_{n1-n2}”. For example, the term C₂₋₆alkynyl means an alkynyl group having 2, 3, 4, 5 or 6 carbon atoms.

[0061] The term “aryl” as used herein, whether it is used alone or as part of another group, refers to a monovalent unsaturated aromatic group with an aromatic carbon atom as the point of attachment, said carbon atom forming part of a one or more six-membered aromatic ring structures, wherein the ring atoms are all carbon. Aryl groups can comprise 6 or more carbon atoms. All aryl groups are optionally fluoro-substituted unless otherwise indicated.

[0062] The term “cycloalkyl,” as used herein, whether it is used alone or as part of another group, means a saturated carbocyclic group containing one or more rings. The number of carbon atoms that are possible in the referenced cycloalkyl group are indicated by the numerical prefix “C_{n1-n2}”. For example, the term C₃₋₁₀cycloalkyl means a cycloalkyl group having 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms. All cycloalkyl groups are optionally fluoro-substituted unless otherwise indicated.

[0063] The term “heteroaryl” as used herein, whether it is used alone or as part of another group, refers to cyclic groups containing at least one heteroaromatic ring in which one or more of the atoms are a heteroatom selected from O, S and N. When a heteroaryl group contains the prefix C_{n1-n2} this prefix indicates the number of carbon atoms in the corresponding carbocyclic group, in which one or more, suitably 1 to 5, of the ring atoms is replaced with a heteroatom as defined above. All heteroaryl groups are optionally fluoro-substituted unless otherwise indicated.

[0064] The term “heterocycloalkyl” as used herein, whether it is used alone or as part of another group, refers to cyclic groups containing at least one non-aromatic ring in which one or more of the atoms are a heteroatom selected from O, S and N. Heterocycloalkyl groups are either saturated or unsaturated (i.e. contain one or more double bonds). When a heterocycloalkyl group contains the prefix C_{n1-n2} this prefix indicates the number of carbon atoms in the corresponding carbocyclic group, in which one or more, suitably 1 to 5, of the ring atoms is replaced with a heteroatom as defined above. All heterocycloalkyl groups are optionally fluoro-substituted unless otherwise indicated.

[0065] All cyclic groups, including aryl, heteroaryl, heterocycloalkyl and cycloalkyl groups, contain one or more than one ring (i.e. are polycyclic). When a cyclic group contains more than one ring, the rings may be fused, bridged, spirofused or linked by a bond.

[0066] The term “benzofused” as used herein refers to a polycyclic group in which a benzene ring is fused with another ring.

[0067] A first ring being “fused” with a second ring means the first ring and the second ring share two adjacent atoms there between.

[0068] A first ring being “bridged” with a second ring means the first ring and the second ring share two non-adjacent atoms there between.

[0069] A first ring being “spirofused” with a second ring means the first ring and the second ring share one atom there between.

[0070] The terms “halo” or “halogen” as used herein, whether it is used alone or as part of another group, refers to a halogen atom and includes fluoro, chloro, bromo and iodo.

[0071] The term “Me” refers to CH₃.

[0072] The term “OAc” refers to OC(O)CH₃.

[0073] The term “TBS” refers to tert-butyldimethylsilyl.

[0074] The symbol “

[0075] The term “available”, as in “available hydrogen atoms” or “available atoms” refers to atoms that would be known to a person skilled in the art to be capable of replacement by a substituent, such as a fluorine atom.

[0076] The term “subject” as used herein includes all members of the animal kingdom including mammals, and suitably refers to humans. Thus the methods of the present application are applicable to both human therapy and veterinary applications.

[0077] The term “pharmaceutically acceptable” means compatible with the treatment of subjects, for example humans.

[0078] The term “pharmaceutically acceptable carrier” means a non-toxic solvent, dispersant, excipient, adjuvant or other material which is mixed with the active ingredient in order to permit the formation of a pharmaceutical composition, i.e., a dosage form capable of administration to a subject.

[0079] The term “pharmaceutically acceptable salt” means either an acid addition salt or a base addition salt which is suitable for, or compatible with the treatment of subjects.

[0080] The term “solvate” as used herein means a compound, or a salt of a compound, wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered.

[0081] The term “treating” or “treatment” as used herein and as is well understood in the art, means an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission (whether partial or total), whether detectable or undetectable. “Treating” and “treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. “Treating” and “treatment” as used herein also include prophylactic treatment. For example, a subject with early cancer can be treated to prevent progression, or alternatively a subject in remission can be treated with a compound or composition of the application to prevent recurrence. Treatment methods comprise administering to a subject a therapeutically effective amount of one or more of the compounds of the application and optionally consist of a single administration, or alternatively comprise a series of administrations.

[0082] “Palliating” a disease or disorder means that the extent and/or undesirable clinical manifestations of a disorder or a disease state are lessened and/or time course of the progression is slowed or lengthened, as compared to not treating the disorder.

[0083] The term “prevention” or “prophylaxis”, or synonym thereto, as used herein refers to a reduction in the risk or probability of a patient becoming afflicted with a disease, disorder or condition.

[0084] The term “disease, disorder or condition that benefits from induction of the biosynthesis of NGF and/or BDNF” as used herein means that the disease, disorder or condition is affected by, modulated by and/or has some biological basis, either direct or indirect, that includes increased biosynthesis of the NGF and/or BDNF.

[0085] The term “increased biosynthesis of the NGF and/or BDNF” as used herein refers to any detectable increase in the biosynthesis of NGF and/or BDNF in the presence of one or more compounds of the application compared to the biosynthesis of NGF and/or BDNF under

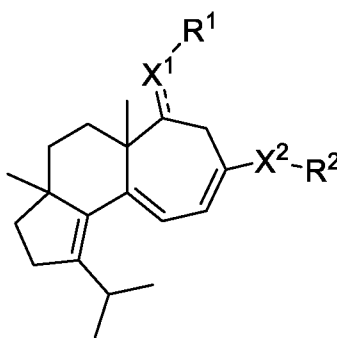
otherwise identical conditions except in the absence of the one or more compounds of the application.

[0086] As used herein, the term “effective amount” or “therapeutically effective amount” means an amount of a compound, or one or more compounds, of the application that is effective, at dosages and for periods of time necessary to achieve the desired result.

[0087] The term “administered” as used herein means administration of a therapeutically effective amount of a compound, or one or more compounds, or a composition of the application to a cell, a tissue, organ or a subject.

II. Compounds and Compositions of the Application

[0088] The present application includes a compound of Formula I, or a pharmaceutically acceptable salt and/or solvate thereof:



I

wherein:

---- is a single or double bond;

when ---- is a double bond, X^1 is selected from O and NR^3 and R^1 is absent;

when ---- is a single bond, X^1 is selected from O, $OC(O)$ and NR^3 and R^1 is selected from H, $SO_2NR^4R^5$, $C_{1-10}alkyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{6-10}aryl$, $C_{5-10}heteroaryl$, $C_{1-10}alkylenearyl$, $C_{1-10}alkyleneheteroaryl$, $C_{1-10}alkyleneC_{3-8}cycloalkyl$ and $C_{1-10}alkyleneC_{3-8}heterocycloalkyl$, the latter nine groups being optionally substituted with one or more of OH, halo, $OC_{1-6}alkyl$, $OC(O)C_{1-6}alkyl$, $C(O)OC_{1-6}alkyl$, $OSi(CH_3)_2(C_{1-6}alkyl)$ and $C_{1-6}alkyl$;

X^2 is selected from a direct bond, $C(O)$, $C(O)Y^1$, $C(NR^{11})$, Y^1SO_2 , $C_{1-6}alkyleneY^1$, $C_{1-6}alkyleneY^1C(O)$, $C_{1-6}alkyleneY^1SO_2$, $C_{1-6}alkyleneY^1SO_2Y^2$ and $C_{1-6}alkyleneY^1C(O)Y^2$;

R^2 is selected from H, C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-10} cycloalkyl, C_{3-10} heterocycloalkyl, C_{6-10} aryl, C_{6-10} heteroaryl, C_{1-10} alkylenearyl, C_{1-10} alkyleneheteroaryl, C_{1-10} alkylene C_{3-8} cycloalkyl, C_{1-10} alkylene C_{3-8} heterocycloalkyl and phenylC(O)phenylY³C(O)R⁶, the latter twelve groups being optionally substituted with one or more of OH, =O, C_{1-6} alkyl, OC₁₋₆alkyl, NR⁷R⁸, NR⁹C(O)OC₁₋₆alkyl, and halo;

Y¹, Y² and Y³ are independently selected from NR¹⁰ and O;

R³, R⁴, R⁵, R⁷, R⁸, and R⁹ are independently selected from H and C_{1-6} alkyl;

R⁶ is selected from C_{1-10} alkyl, C_{2-10} alkenyl and C_{2-10} alkynyl;

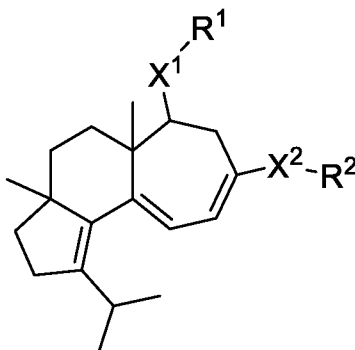
R¹⁰ is selected from H and C_{1-6} alkyl;

R¹¹ is selected from H, C_{1-6} alkyl, C_{6-10} aryl and SO₂C₆₋₁₀aryl; and

wherein all available hydrogen atoms are optionally and independently replaced with a fluorine or chlorine atom,

provided that when X¹-R¹ is OH, then X²-R² is not CH₂OH, C(O)OH or C(O)H.

[0089] The present application also includes a compound of Formula I, or a pharmaceutically acceptable salt and/or solvate thereof:



I

wherein X¹ is selected from O, OC(O) and NR³;

R¹ is selected from H, SO₂NR⁴R⁵, C_{1-10} alkyl, C_{3-10} cycloalkyl, C_{3-10} heterocycloalkyl, C_{6-10} aryl, C_{5-10} heteroaryl, C_{1-10} alkylenearyl, C_{1-10} alkyleneheteroaryl, C_{1-10} alkylene C_{3-8} cycloalkyl and C_{1-10} alkylene C_{3-8} heterocycloalkyl, the latter nine groups being optionally substituted with one or more of OH, halo, OC₁₋₆alkyl and C_{1-6} alkyl;

X^2 is selected from a direct bond, $C(O)$, $C(O)Y^1$, $C_{1-6}alkyleneY^1$, $C_{1-6}alkyleneY^1C(O)$, $C_{1-6}alkyleneY^1SO_2$, $C_{1-6}alkyleneY^1SO_2Y^2$ and $C_{1-6}alkyleneY^1C(O)Y^2$;

R^2 is selected from H, $C_{1-10}alkyl$, $C_{2-10}alkenyl$, $C_{2-10}alkynyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{6-10}aryl$, $C_{6-10}heteroaryl$, $C_{1-10}alkylenearyl$, $C_{1-10}alkyleneheteroaryl$, $C_{1-10}alkyleneC_{3-8}cycloalkyl$, $C_{1-10}alkyleneC_{3-8}heterocycloalkyl$ and $phenylC(O)phenylY^3C(O)R^6$, the latter twelve groups being optionally substituted with one or more of OH, $C_{1-6}alkyl$, $OC_{1-6}alkyl$, NR^7R^8 , $NR^9C(O)OC_{1-6}alkyl$, and halo;

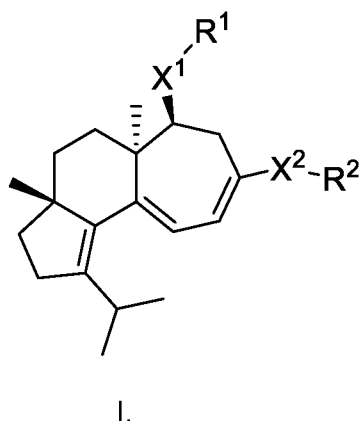
Y^1 , Y^2 and Y^3 are independently selected from NR^{10} and O;

R^3 , R^4 , R^5 , R^7 , R^8 , R^9 and R^{10} are independently selected from H and $C_{1-6}alkyl$;

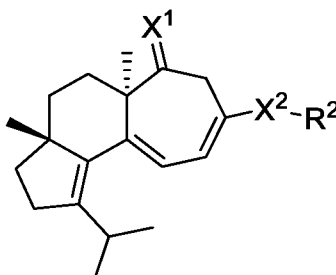
and

R^6 is selected from $C_{1-10}alkyl$, $C_{2-10}alkenyl$ and $C_{2-10}alkynyl$, provided that when X^1-R^1 is OH, then X^2-R^2 is not CH_2OH , $C(O)OH$ or $C(O)H$.

[0090] In some embodiments, the compounds of Formula I have the following relative stereochemistry:

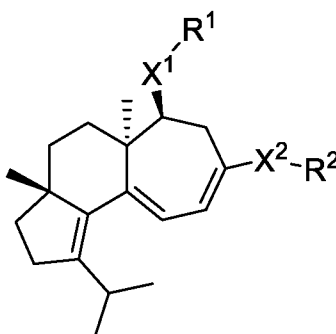


[0091] In some embodiments, when ---- is a double bond, the compounds of Formula I have the following relative stereochemistry:



I.

[0092] In some embodiments, when ---- is a single bond, the compounds of Formula I have the following relative stereochemistry:



I.

[0093] In some embodiments, when ---- is a double bond, X^1 is selected from O, NH and $NC_{1-6}alkyl$. In some embodiments, X^1 is O.

[0094] In some embodiments, when ---- is a single bond, X^1 is selected from O, $OC(O)$, NH and $NC_{1-6}alkyl$. In some embodiments, X^1 is selected from O, $OC(O)$, NH and $NC_{1-4}alkyl$. In some embodiments, X^1 is O. In some embodiments, X^1 is $OC(O)$. In some embodiments, X^1 is NH. In some embodiments, X^1 is NCH_3 .

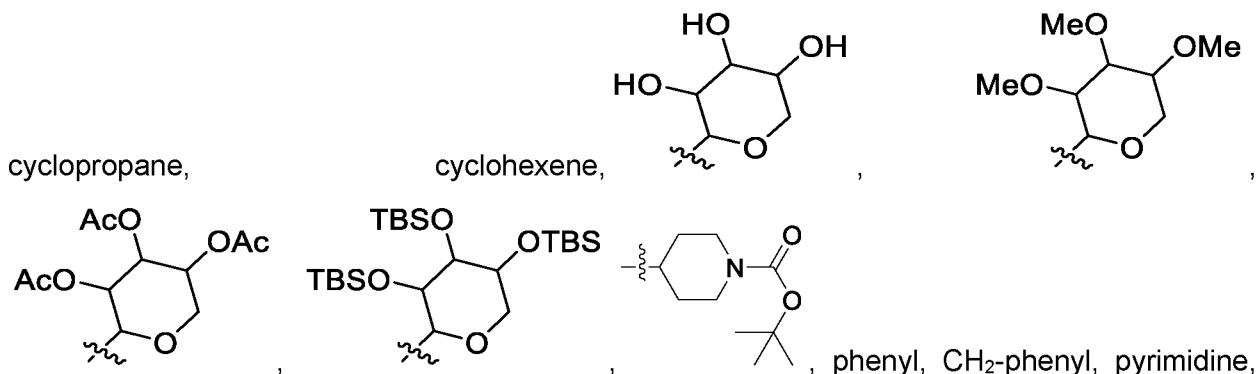
[0095] In some embodiments, X^1 is selected from O, $OC(O)$, NH and $NC_{1-6}alkyl$. In some embodiments, X^1 is selected from O, $OC(O)$, NH and $NC_{1-4}alkyl$. In some embodiments, X^1 is O. In some embodiments, X^1 is $OC(O)$. In some embodiments, X^1 is NH. In some embodiments, X^1 is NCH_3 .

[0096] In some embodiments, ---- is a double bond and R^1 is absent.

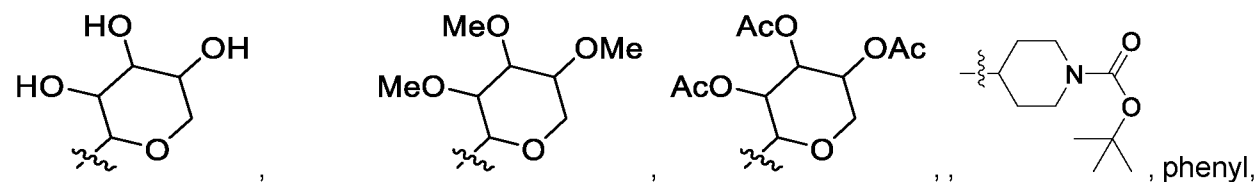
[0097] In some embodiments, ---- is a single bond and R^1 is selected from H, $SO_2NR^4R^5$, $C_{1-6}alkyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{6-10}aryl$, $C_{5-10}heteroaryl$, $C_{1-6}alkylenearyl$, $C_{1-6}alkyleneheteroaryl$, $C_{1-6}alkyleneC_{3-8}cycloalkyl$ and $C_{1-6}alkyleneC_{3-8}heterocycloalkyl$, the latter nine groups being optionally substituted with one to three of OH, halo, $OC_{1-6}alkyl$, $OC(O)C_{1-6}alkyl$, $C(O)OC_{1-6}alkyl$, $OSi(CH_3)_2(C_{1-6}alkyl)$ and $C_{1-6}alkyl$.

[0098] In some embodiments, R^1 is selected from H, $SO_2NR^4R^5$, $C_{1-4}alkyl$, $C_{3-6}cycloalkyl$, $C_{3-8}heterocycloalkyl$, $C_{6-10}aryl$, $C_{5-8}heteroaryl$, $C_{1-4}alkylenearyl$, the latter six groups being optionally substituted with one to three of OH, $OC_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $C(O)OC_{1-4}alkyl$ and $C_{1-4}alkyl$.

[0099] In some embodiments, R^1 is selected from H, SO_2NH_2 , CH_3 , CH_2CH_3 , CH_2CH_2OH ,



and wherein the phenyl is optionally substituted with one or two of CH_3 . In some embodiments, R^1 is selected from H, SO_2NH_2 , CH_3 , CH_2CH_3 , CH_2CH_2OH , cyclopropane, cyclohexene,

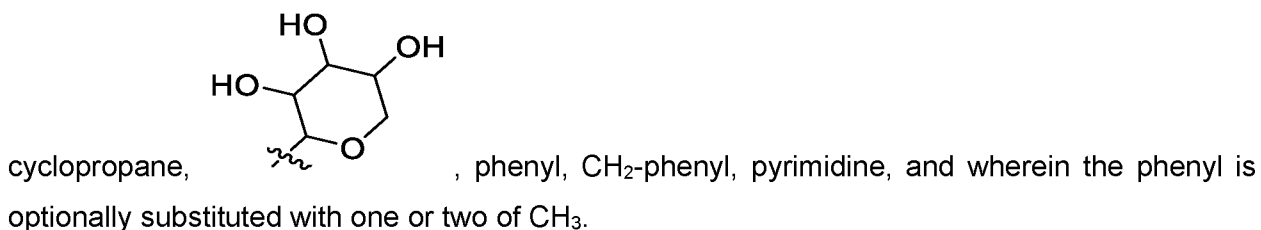


CH_2 -phenyl, pyrimidine, and wherein the phenyl is optionally substituted with one or two of CH_3 .

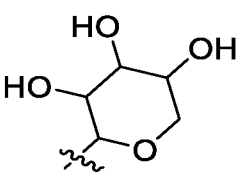
[00100] In some embodiments, R^1 is selected from H, $SO_2NR^4R^5$, C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{3-10} heterocycloalkyl, C_{6-10} aryl, C_{5-10} heteroaryl, C_{1-6} alkylenearyl, C_{1-6} alkyleneheteroaryl, C_{1-6} alkylene C_{3-8} cycloalkyl and C_{1-6} alkylene C_{3-8} heterocycloalkyl, the latter nine groups being optionally substituted with one to three of OH, halo, OC_{1-6} alkyl and C_{1-6} alkyl.

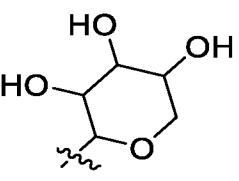
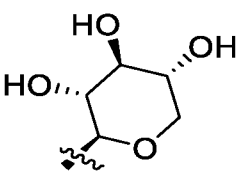
[00101] In some embodiments, R^1 is selected from H, $SO_2NR^4R^5$, C_{1-4} alkyl, C_{3-6} cycloalkyl, C_{3-8} heterocycloalkyl, C_{6-10} aryl, C_{5-8} heteroaryl, C_{1-4} alkylenearyl, the latter six groups being optionally substituted with one to three of OH and C_{1-4} alkyl.

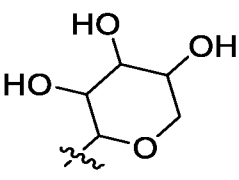
[00102] In some embodiments, R^1 is selected from H, SO_2NH_2 , CH_3 , CH_2CH_2OH ,

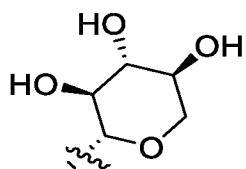


[00103] In some embodiments, R^1 is H.

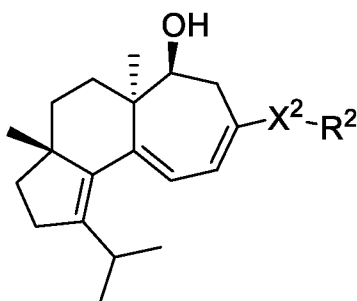
[00104] In some embodiments, R^1 is . In some embodiments, when R^1

is , R^1 has the following stereochemistry . In some

embodiments, when R^1 is , R^1 has the following stereochemistry

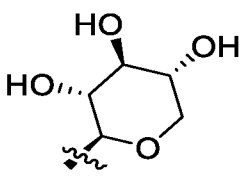


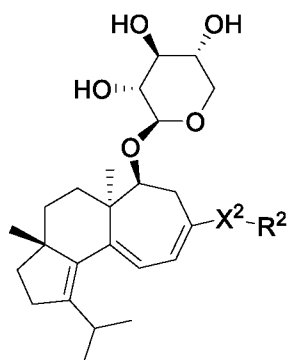
[00105] In some embodiments, --- is a single bond, X^1 is O, R^1 is H and the compound of Formula I has the following structure:



I

or a pharmaceutically acceptable salt and/or solvate, wherein X^2 and R^2 are as defined for Formula I.

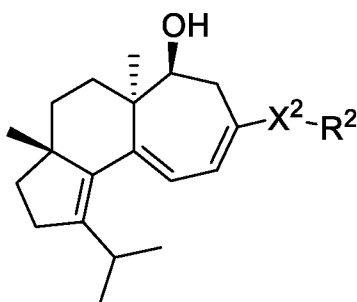
[00106] In some embodiments, --- is a single bond, X^1 is O, R^1 is  and the compound of Formula I has the following structure:



I

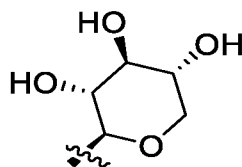
or a pharmaceutically acceptable salt and/or solvate, wherein X^2 and R^2 are as defined for Formula I.

[00107] In some embodiments, X^1 is O, R^1 is H and the compound of Formula I has the following structure:



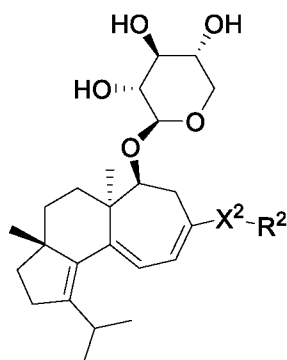
I

or a pharmaceutically acceptable salt and/or solvate, wherein X^2 and R^2 are as defined for Formula I.



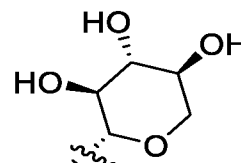
[00108] In some embodiments, X^1 is O, R^1 is

and the compound of Formula I has the following structure:

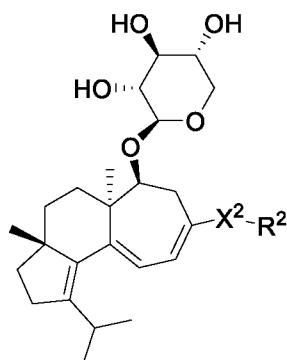


I

or a pharmaceutically acceptable salt and/or solvate, wherein X^2 and R^2 are as defined for Formula I.



[00109] In some embodiments, ---- is a single bond, X^1 is O, R^1 is the compound of Formula I has the following structure:



I

or a pharmaceutically acceptable salt and/or solvate, wherein X^2 and R^2 are as defined for Formula I.

[00110] In some embodiments, X^2 is selected from a direct bond, $C(O)$, $C(O)Y^1$, $C_{1-4}alkyleneY^1$, $C_{1-4}alkyleneY^1C(O)$, $C_{1-4}alkyleneY^1SO_2$, $C_{1-4}alkyleneY^1SO_2Y^2$ and $C_{1-4}alkyleneY^1C(O)Y^2$, wherein Y^1 and Y^2 are independently selected from NR^{10} and O.

[00111] In some embodiments, X^2 is selected from a direct bond, $C(O)$, $C(O)Y^1$, $C(NR^{11})$, Y^1SO_2 , $C_{1-4}alkyleneY^1$, $C_{1-4}alkyleneY^1C(O)$, $C_{1-4}alkyleneY^1SO_2$, $C_{1-4}alkyleneY^1SO_2Y^2$ and $C_{1-6}alkyleneY^1C(O)Y^2$, wherein Y^1 and Y^2 are independently selected from NR^{10} and O.

[00112] In some embodiments, X^2 is selected from a direct bond, C(O), C(O)O, C(O)NR¹⁰, C₁₋₂alkyleneNR¹⁰, C₁₋₂alkyleneNR¹⁰C(O), C₁₋₂alkyleneOC(O), C₁₋₂alkyleneNR¹⁰SO₂, C₁₋₂alkyleneOSO₂NR¹⁰, C₁₋₂alkyleneOSO₂, C₁₋₂alkyleneOC(O)NR¹⁰, C₁₋₂alkyleneOC(O)O and C₁₋₂alkyleneNR¹⁰C(O)NR¹⁰.

[00113] In some embodiments, X^2 is selected from a direct bond, C(O), C(O)O, C(O)NH, C(O)NCH₃, CH₂NH, CH₂NCH₃, CH₂OC(O), CH₂NHC(O), CH₂NH-SO₂, CH₂OSO₂NH, CH₂OC(O)NH, CH₂OSO₂, CH₂OC(O)O and CH₂NHC(O)NH.

[00114] In some embodiments, X^2 is selected from a direct bond, C(O), C(O)O, C(O)NH, C(O)NCH₃, C(O)NH, C(NH), C(NSO₂Ph), C(NPh), NHSO₂, CH₂NH, CH₂O, CH₂NCH₃, CH₂OC(O), CH₂NHC(O), CH₂NHSO₂, CH₂NHSO₂NCH₃, CH₂N(CH₃)SO₂NCH₃, CH₂OSO₂NH, CH₂OSO₂NCH₃, CH₂OC(O)NH, CH₂OSO₂, CH₂OC(O)O and CH₂NHC(O)NH, wherein all available hydrogen atoms are optionally and independently replaced with a fluorine. In some embodiments, R² is selected from H, C₁₋₅alkyl, C₂₋₅alkenyl, C₂₋₅alkynyl, C₃₋₈cycloalkyl, C₃₋₈heterocycloalkyl, C₆₋₁₀aryl, C₆₋₁₀heteroaryl, C₁₋₅alkylenearyl, C₁₋₅alkyleneheteroaryl, C₁₋₅alkyleneC₃₋₈cycloalkyl, C₁₋₅alkyleneC₃₋₈heterocycloalkyl and phenylC(O)phenylNR¹⁰C(O)R⁶, the latter twelve groups being optionally substituted with one to three of OH, C₁₋₄alkyl, OC₁₋₄alkyl, NR⁷R⁸, NR⁹C(O)OC₁₋₆alkyl, and halo.

[00115] In some embodiments, R² is selected from H, C₁₋₁₀alkyl, C₂₋₅alkenyl, C₂₋₅alkynyl, C₃₋₈cycloalkyl, C₃₋₈heterocycloalkyl, C₆₋₁₀aryl, C₆₋₁₀heteroaryl, C₁₋₅alkylenearyl, C₁₋₅alkyleneheteroaryl, C₁₋₅alkyleneC₃₋₈cycloalkyl, C₁₋₅alkyleneC₃₋₈heterocycloalkyl and phenylC(O)phenylNR¹⁰C(O)R⁶, the latter twelve groups being optionally substituted with one to three of OH, =O, C₁₋₄alkyl, OC₁₋₄alkyl, NR⁷R⁸, NR⁹C(O)OC₁₋₆alkyl, and halo, and wherein all available hydrogen atoms are optionally and independently replaced with a fluorine.

[00116] In some embodiments, R² is selected from H, C₁₋₅alkyl, C₂₋₅alkynyl, C₃₋₈cycloalkyl, C₃₋₈heterocycloalkyl, phenyl, C₆₋₁₀heteroaryl, C₁₋₂alkylenephenyl, C₁₋₄alkyleneC₃₋₆heterocycloalkyl and phenylC(O)phenylNR¹⁰C(O)R⁶, the latter nine groups being optionally substituted with one to three of OH, C₁₋₂alkyl, OC₁₋₂alkyl, NR⁷R⁸, NR⁹C(O)OC₁₋₆alkyl, fluoro or chloro.

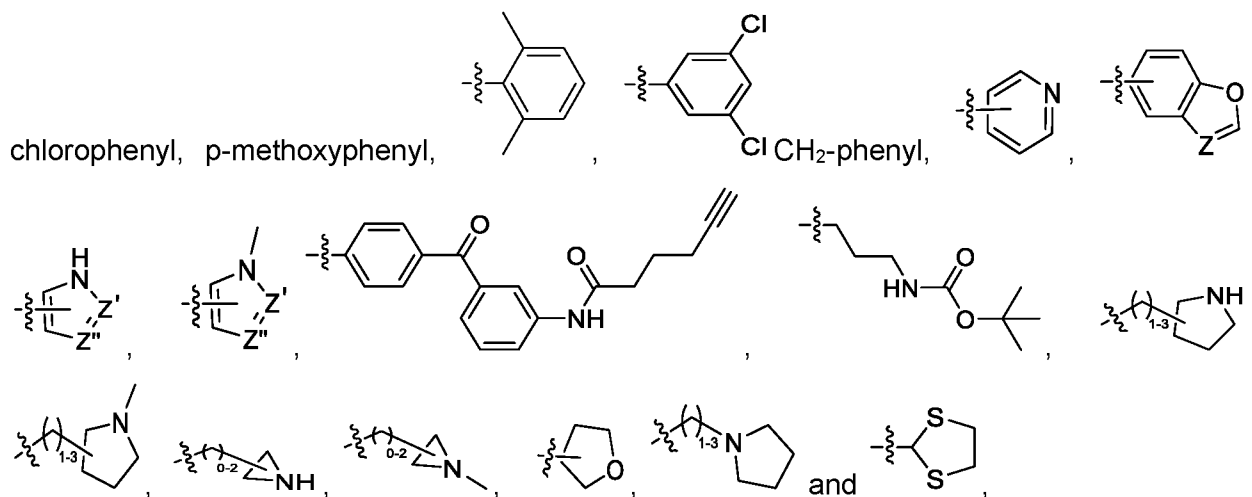
[00117] In some embodiments, R² is selected from H, C₁₋₁₀alkyl, C₂₋₅alkynyl, C₃₋₈cycloalkyl, C₃₋₈heterocycloalkyl, phenyl, C₆₋₁₀heteroaryl, C₁₋₂alkylenephenyl, C₁₋₄alkyleneC₃₋₈cycloalkyl, C₁₋₄alkyleneC₃₋₆heterocycloalkyl and phenylC(O)phenylNR¹⁰C(O)R⁶, the latter ten groups being optionally substituted with one to three of OH, =O, C₁₋₂alkyl, OC₁₋₂alkyl, NR⁷R⁸, NR⁹C(O)OC₁₋

alkyl, fluoro or chloro, and wherein all available hydrogen atoms are optionally and independently replaced with a fluorine.

[00118] In some embodiments, R² is selected from H, C₁₋₅alkyl, C₂₋₅alkynyl, C₃₋₈cycloalkyl, C₃₋₈heterocycloalkyl, phenyl, C₆₋₁₀heteroaryl, C₁₋₂alkylenepheryl, C₁₋₄alkyleneC₃₋₆heterocycloalkyl and phenylC(O)phenylNHC(O)C₁₋₆alkynyl, the latter nine groups being optionally substituted with one or two of OH, CH₃, OCH₃, NH₂, N(CH₃)₂, NHCH₃, NHC(O)OC(CH₃)₃ or chloro.

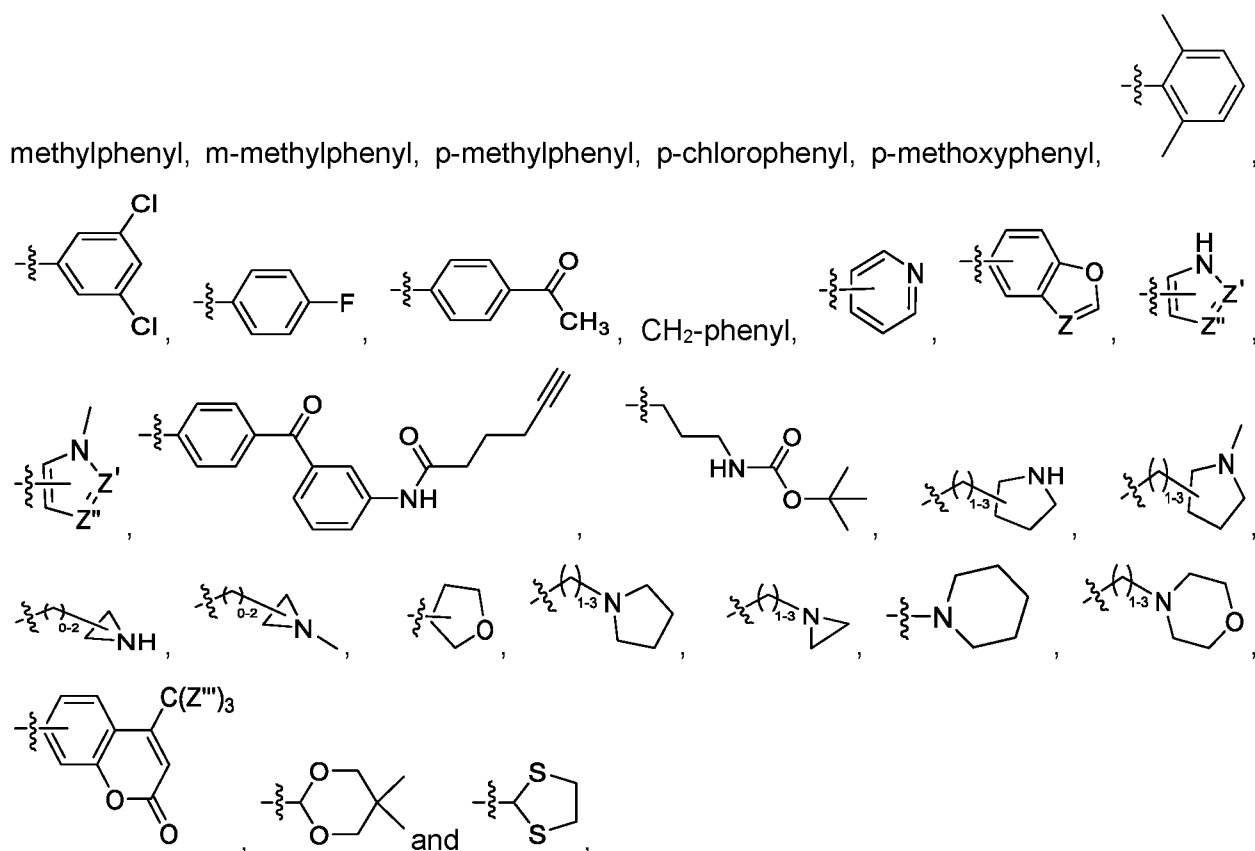
[00119] In some embodiments, R² is selected from H, C₁₋₈alkyl, C₂₋₅alkynyl, C₃₋₈cycloalkyl, C₃₋₈heterocycloalkyl, phenyl, C₆₋₁₀heteroaryl, C₁₋₂alkylenepheryl, C₁₋₂alkyleneC₃₋₈cycloalkyl, C₁₋₄alkyleneC₃₋₆heterocycloalkyl and phenylC(O)phenylNHC(O)C₁₋₆alkynyl, the latter ten groups being optionally substituted with one to three of OH, =O, CH₃, OCH₃, NH₂, N(CH₃)₂, NHCH₃, NHC(O)OC(CH₃)₃, fluoro or chloro, and wherein all available hydrogen atoms are optionally and independently replaced with a fluorine.

[00120] In some embodiments, R² is selected from H, CH₃, CH(CH₃)NH₂, CH₂NH₂, CH₂N(CH₃)₂, (CH₂)₂NH₂, (CH₂)₂NHCH₃, (CH₂)₂OH, CH₂C≡CH, (CH₂)₃NH₂, (CH₂)₃N(CH₃)₂, cyclopropyl, cyclobutyl, cyclohexyl, phenyl, o-methylphenyl, m-methylphenyl, p-methylphenyl, p-



wherein Z is selected from CH and N, and wherein at least one of Z' and Z'' is CH and the other one is N.

[00121] In some embodiments, R² is selected from H, CH₂OH, CH(OH)CH₃, CH(OH)CF₃, CH(OH)CH(CH₃)₂, CH(OH)CH₂CH(CH₃)₂, CH(OH)C(CH₃)₃, C(OH)(CH₃)₂, CH₃, CF₃, CHF₂, CH(CH₃)₂, CH₂CH₃, C(CH₃)₃, CH(CH₃)NH₂, CH₂NH₂, CH₂N(CH₃)₂, (CH₂)₂NH₂, (CH₂)₂NHCH₃, (CH₂)₂OH, CH₂C≡CH, (CH₂)₃NH₂, (CH₂)₃N(CH₃)₂, cyclopropyl, cyclobutyl, cyclohexyl, CH(OH)cyclopropyl, CH(OH)cyclobutyl, CH(OH)cyclohexyl, phenyl, CH(OH)phenyl, o-



wherein Z is selected from CH and N, wherein at least one of Z' and Z'' is CH and the other one is N, and wherein Z''' is selected from F and H.

[00122] In some embodiments, R⁴ and R⁵ are selected from H and C₁₋₄alkyl. In some embodiments, R⁴ and R⁵ are selected from H and C₁₋₂alkyl. In some embodiments, R⁴ and R⁵ are selected from H and CH₃. In some embodiments, both R⁴ and R⁵ are H.

[00123] In some embodiments, R⁶ is selected from C₁₋₈alkyl, C₂₋₈alkenyl and C₂₋₈alkynyl. In some embodiments, R⁶ is selected from C₁₋₆alkynyl.

[00124] In some embodiments, R⁷, R⁸, R⁹ and R¹⁰ are independently selected from H and C₁₋₃alkyl. In some embodiments, R⁷, R⁸, R⁹ and R¹⁰ are independently selected from H and CH₃.

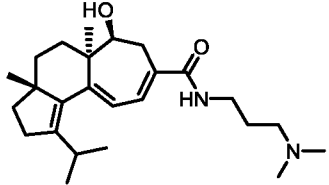
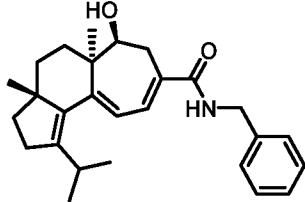
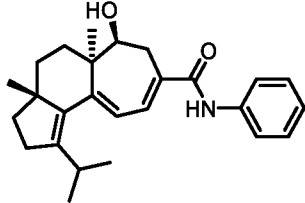
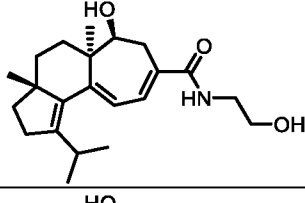
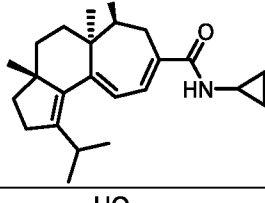
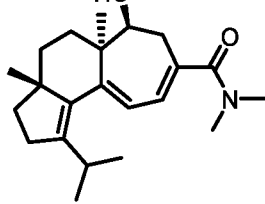
[00125] In some embodiments, R⁷, R⁸, and R⁹ are independently selected from H and C₁-alkyl. In some embodiments, R⁷, R⁸, and R⁹ are independently selected from H and CH₃.

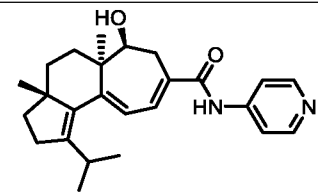
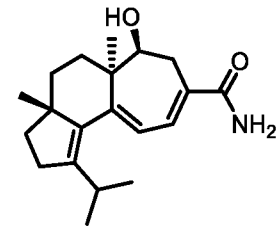
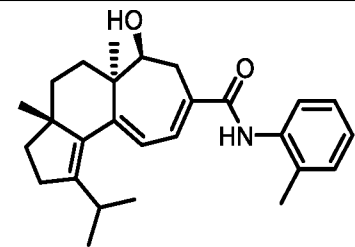
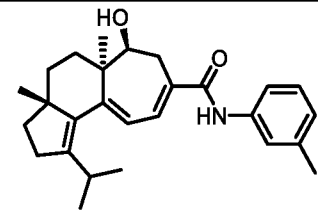
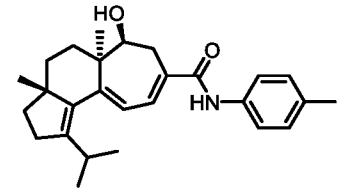
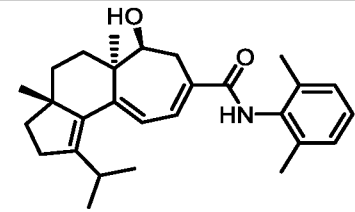
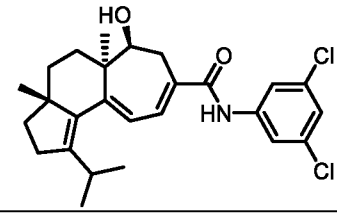
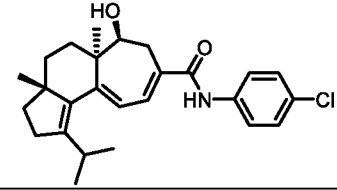
[00126] In some embodiments, R¹⁰ is selected from H and C₁₋₄alkyl. In some embodiments, R¹⁰ is selected from H and C₁₋₂alkyl. In some embodiments, R¹⁰ is H. In some embodiments, R¹⁰ is CH₃.

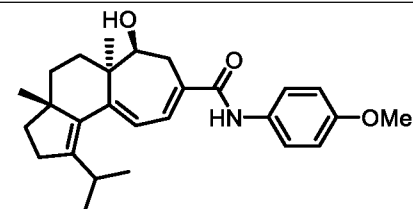
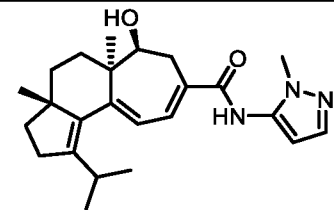
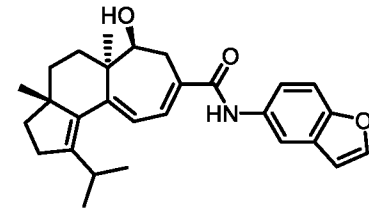
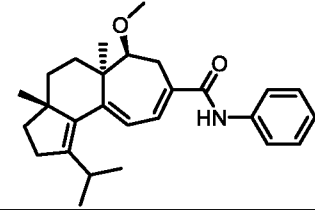
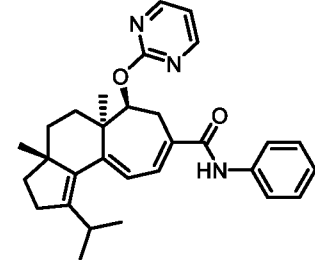
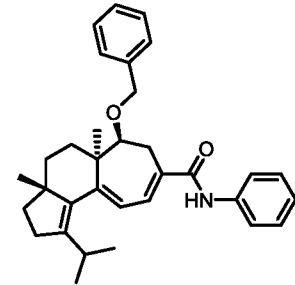
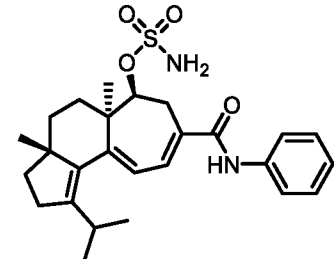
[00127] In some embodiments R^{11} is selected from H, C_{1-4} alkyl, Ph and SO_2Ph . In some embodiments R^{11} is SO_2Ph .

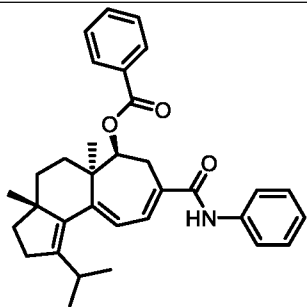
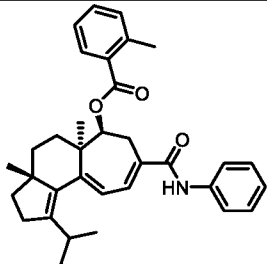
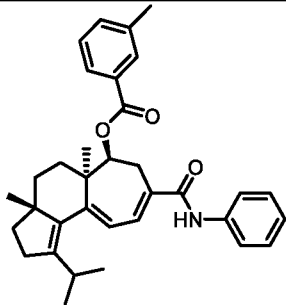
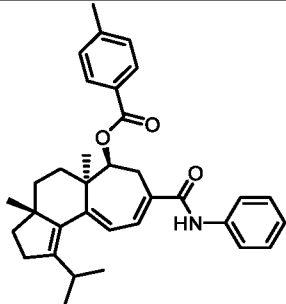
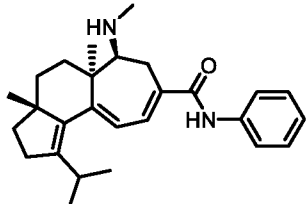
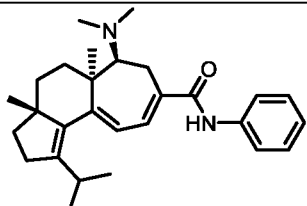
[00128] In some embodiments, the compound of Formula I is selected from the compounds listed in Table 1 below, or a pharmaceutically acceptable salt and/or solvate thereof:

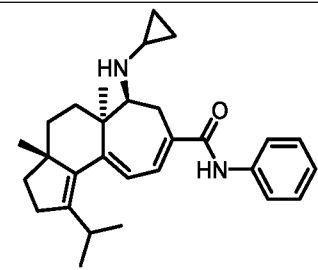
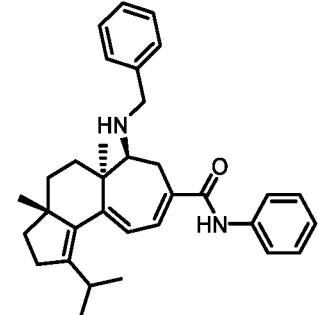
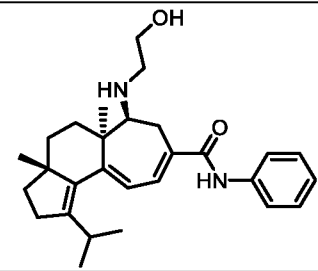
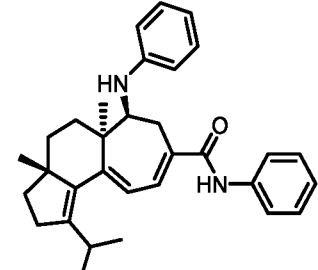
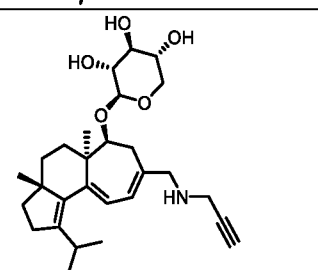
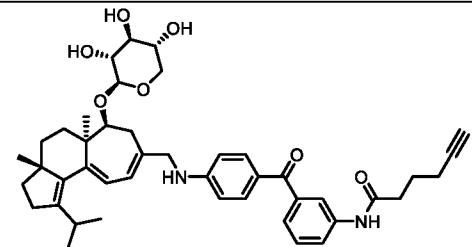
Table 1

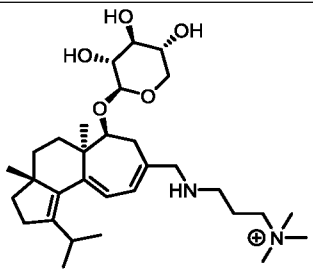
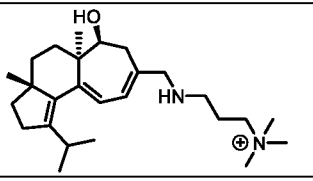
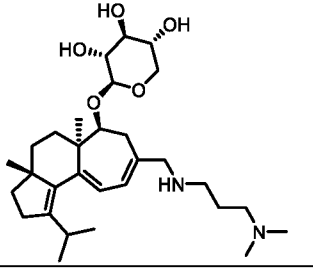
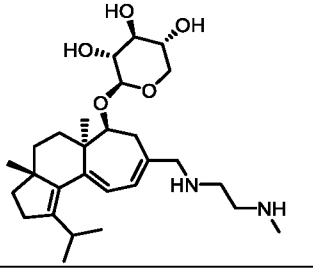
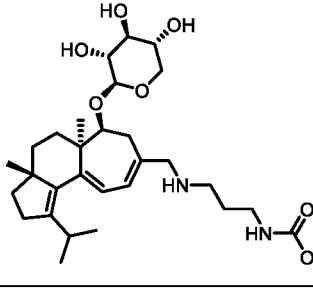
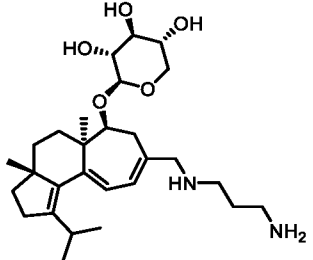
Compound ID	Compound
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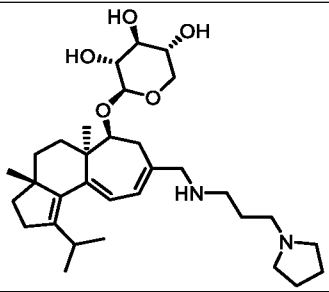
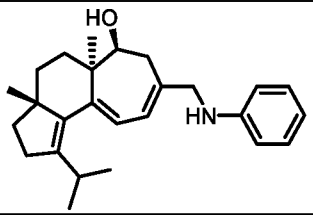
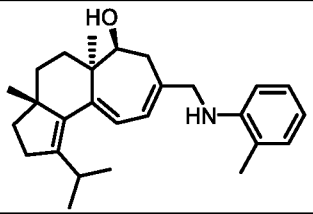
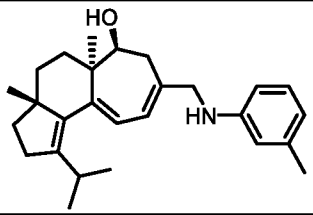
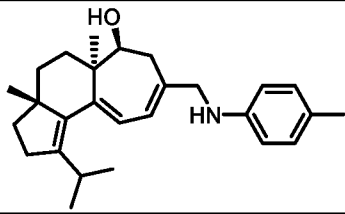
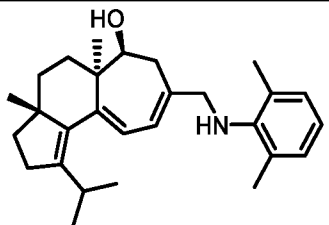
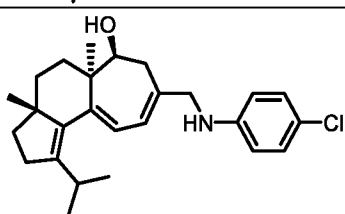
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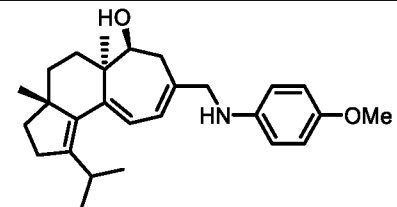
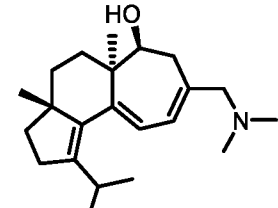
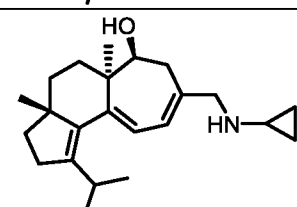
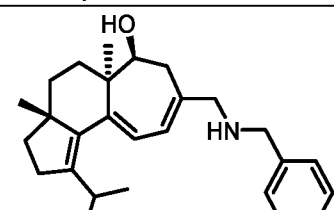
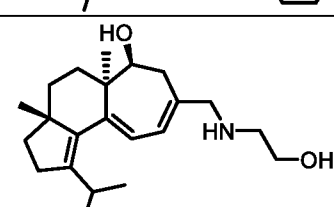
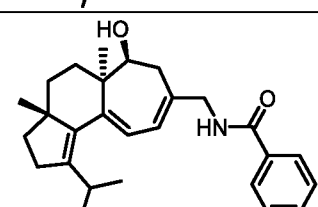
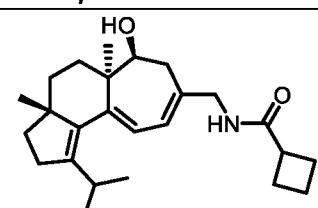
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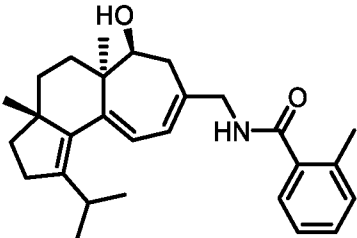
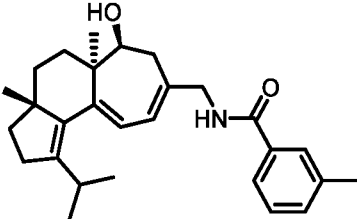
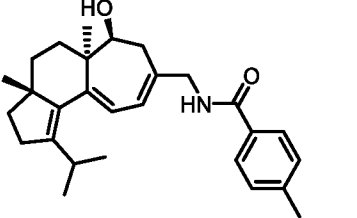
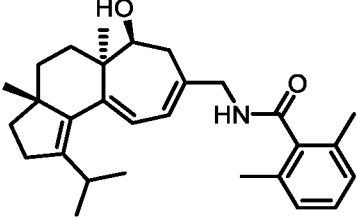
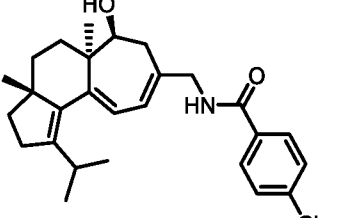
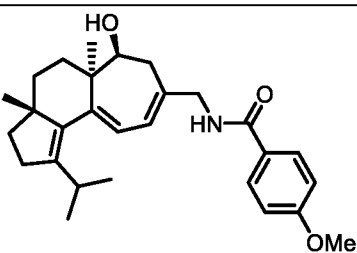
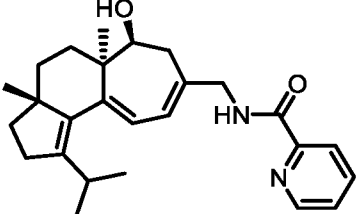
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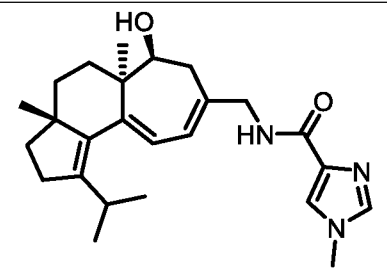
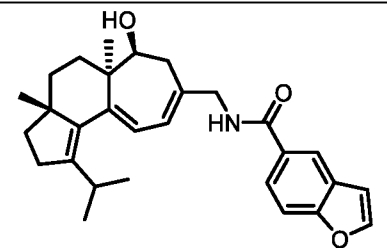
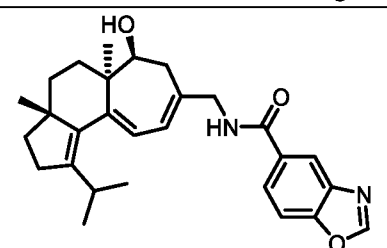
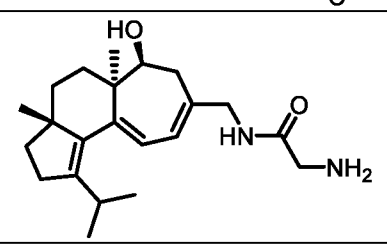
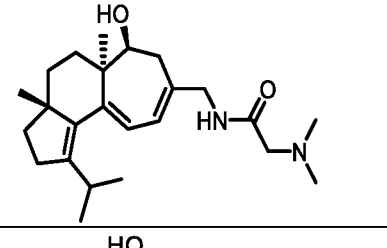
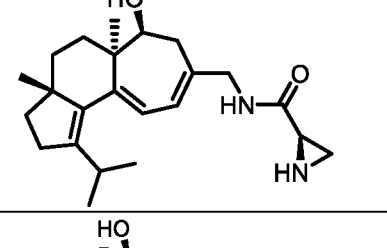
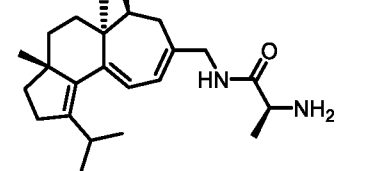
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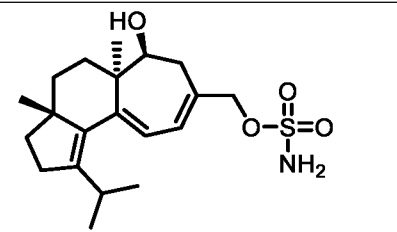
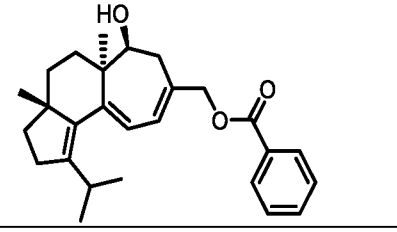
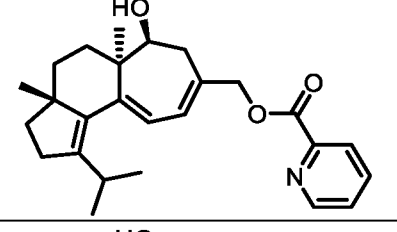
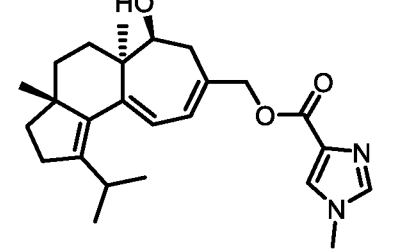
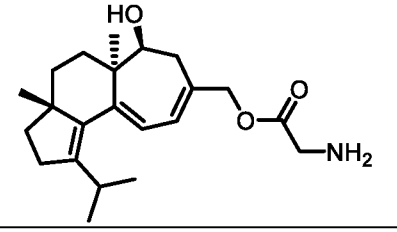
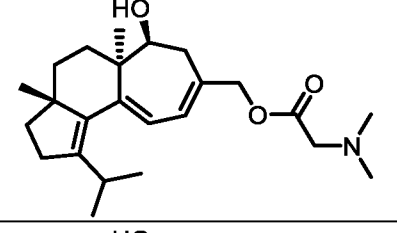
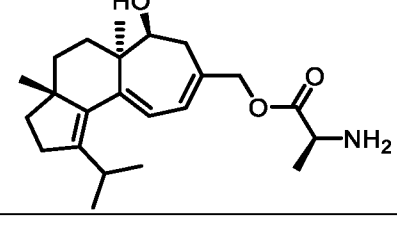
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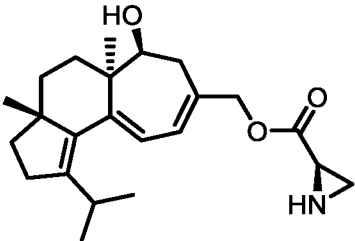
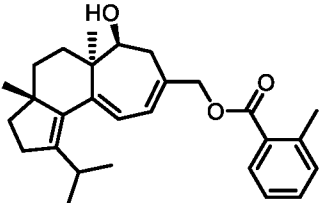
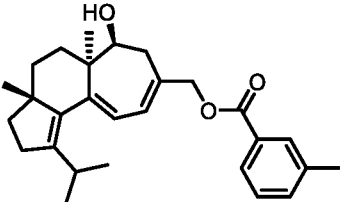
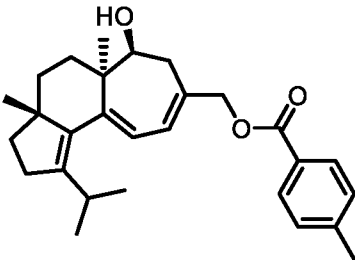
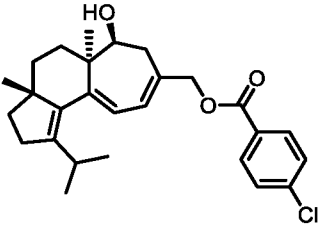
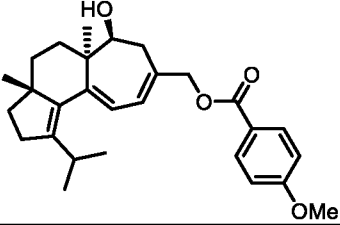
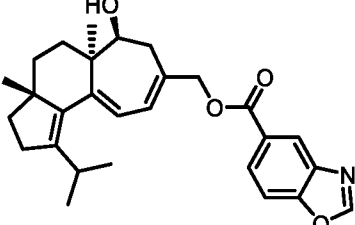
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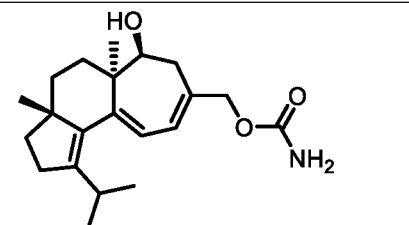
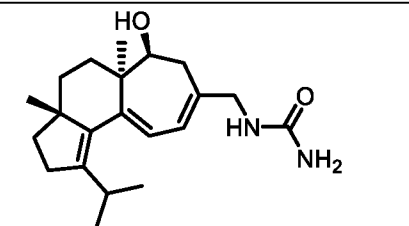
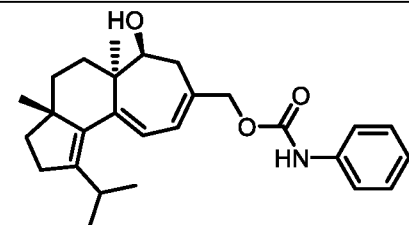
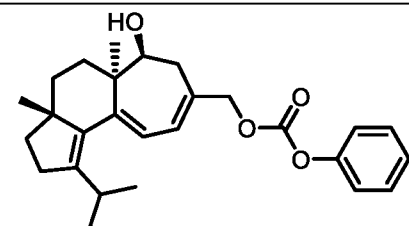
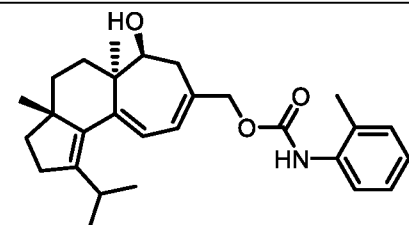
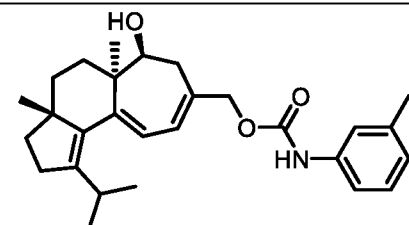
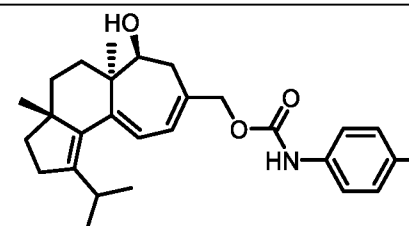
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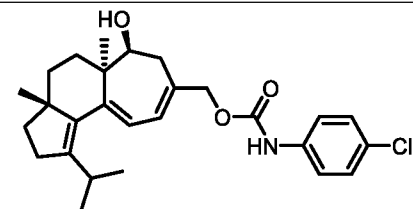
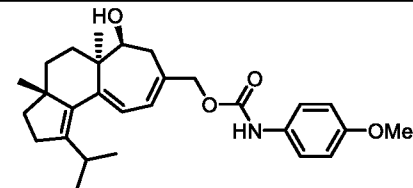
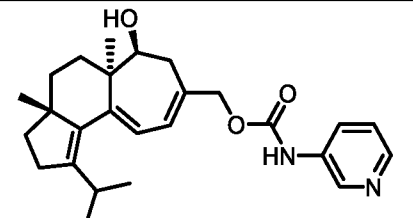
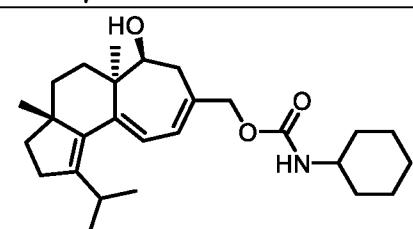
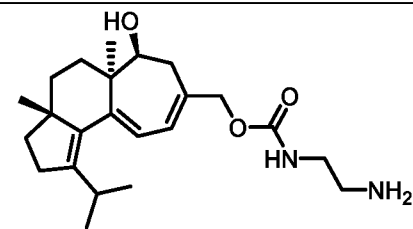
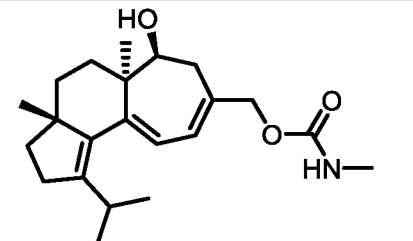
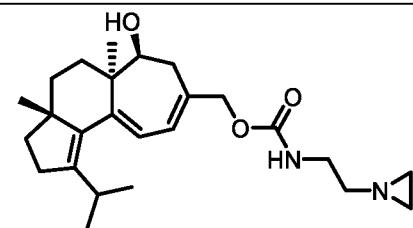
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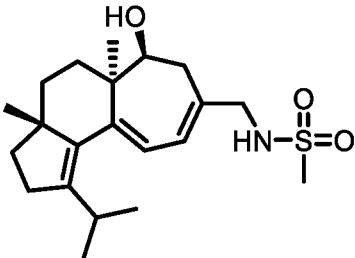
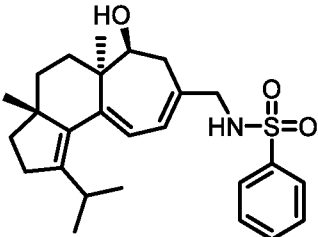
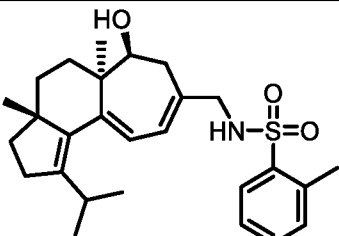
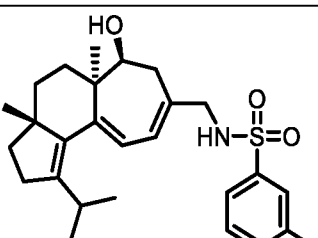
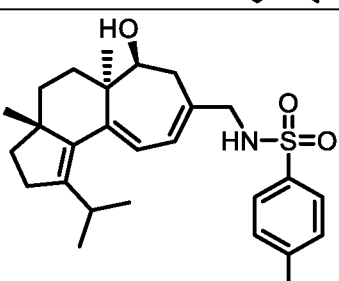
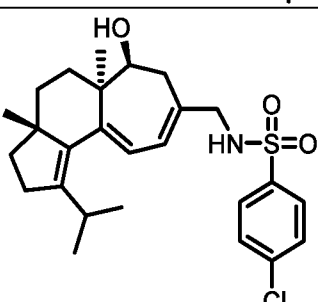
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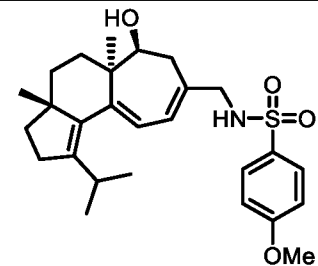
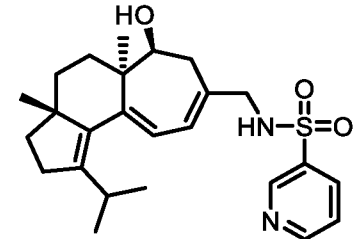
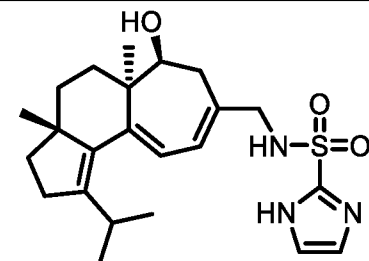
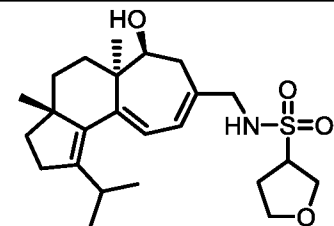
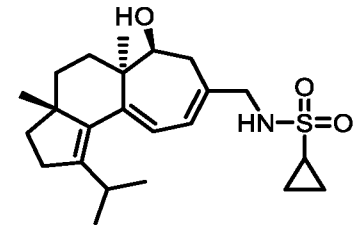
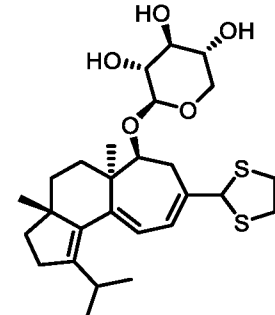
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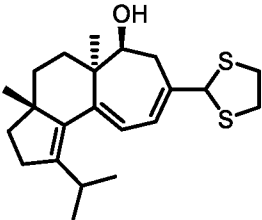
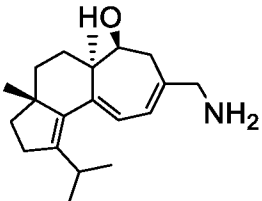
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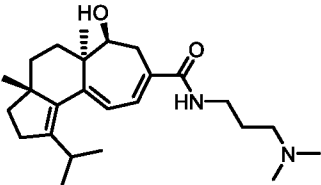
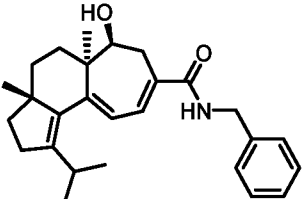
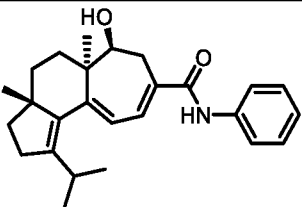
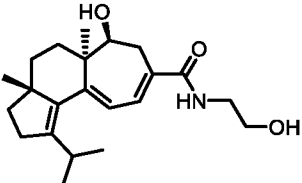
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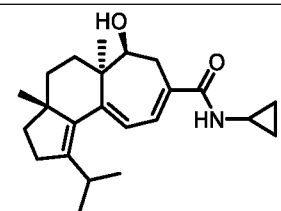
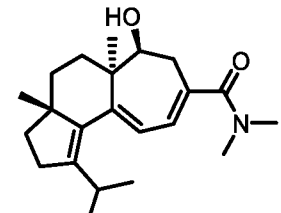
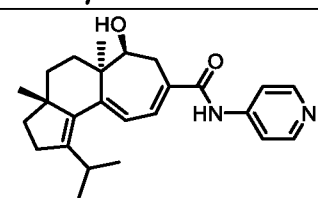
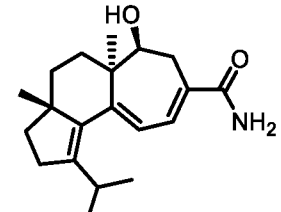
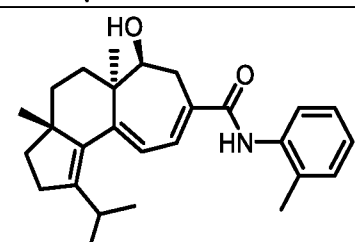
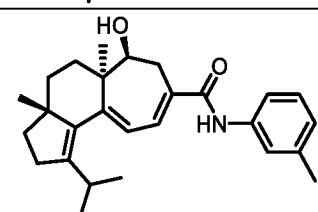
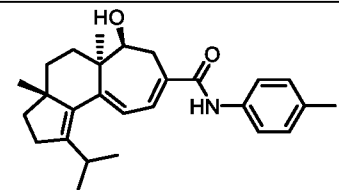
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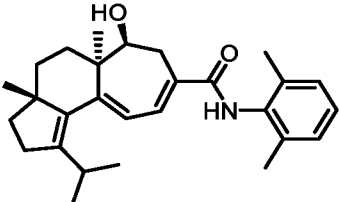
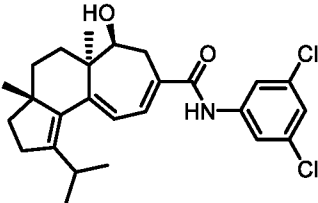
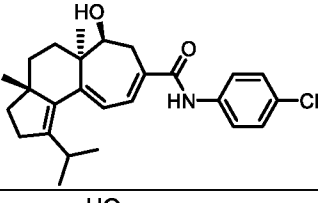
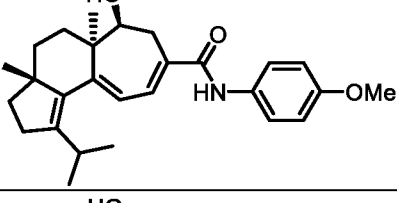
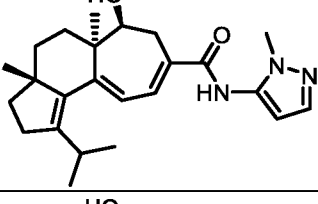
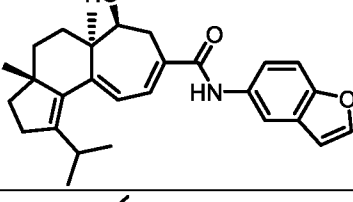
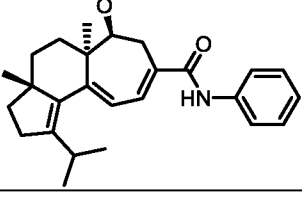
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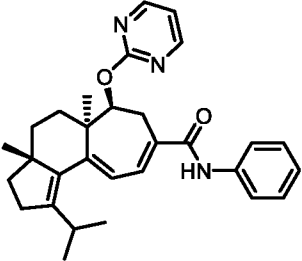
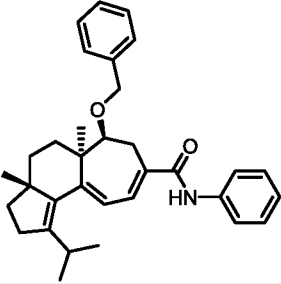
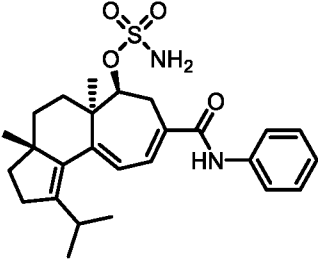
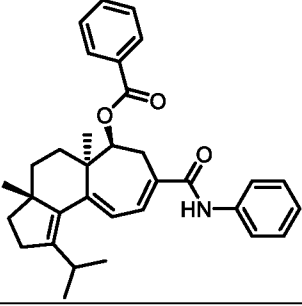
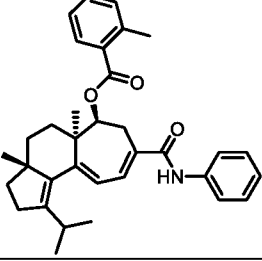
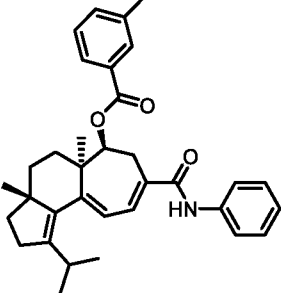
[00129] In some embodiments, the compound of Formula I is selected from the compounds listed in Table 2 below, or a pharmaceutically acceptable salt and/or solvate thereof:

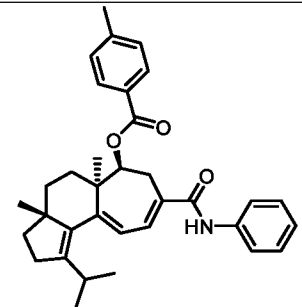
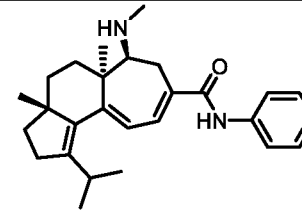
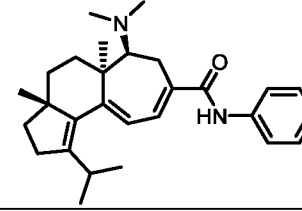
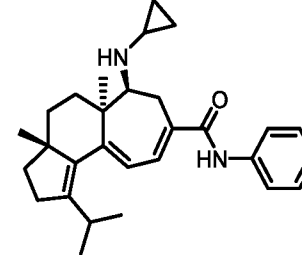
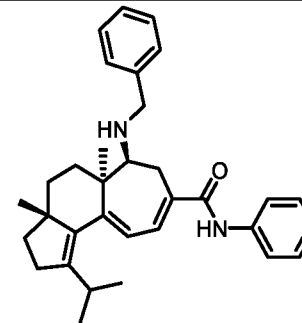
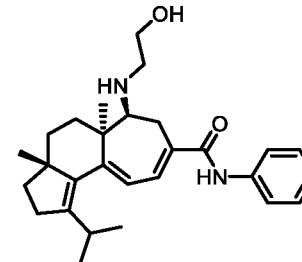
Table 2

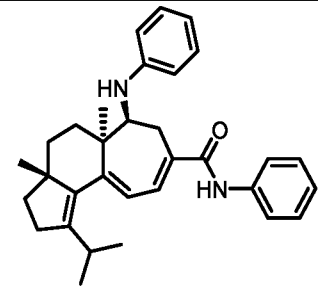
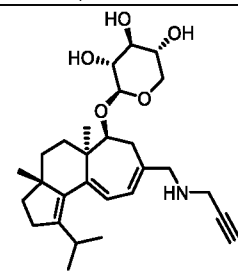
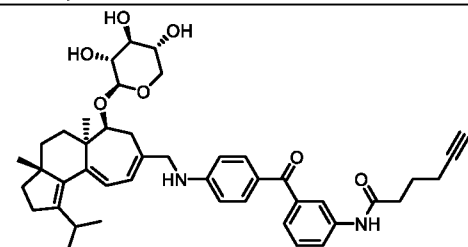
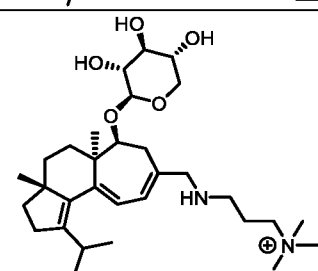
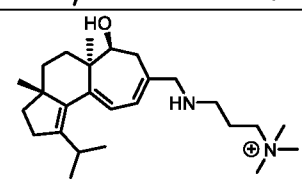
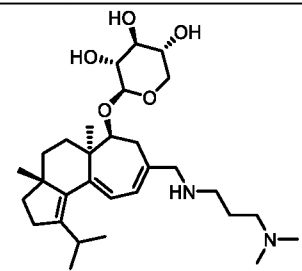
Compound ID	Compound
I-1	
I-2	
I-3	
I-4	

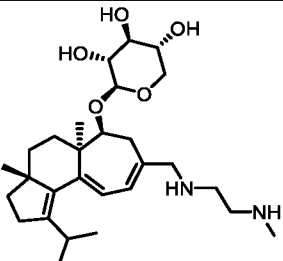
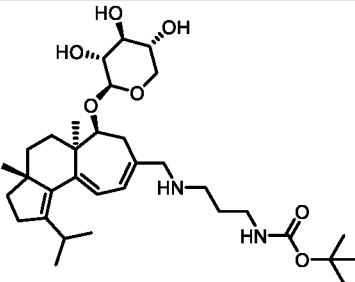
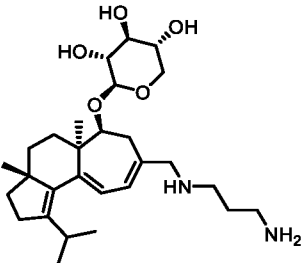
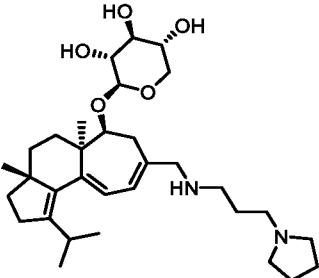
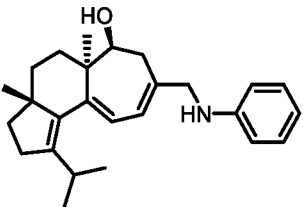
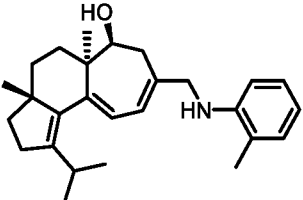
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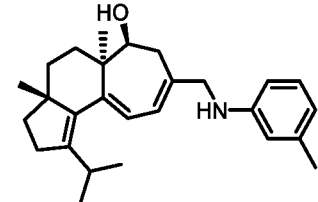
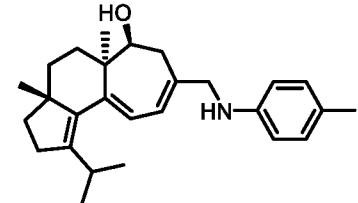
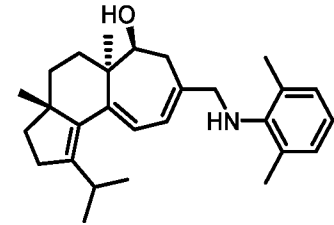
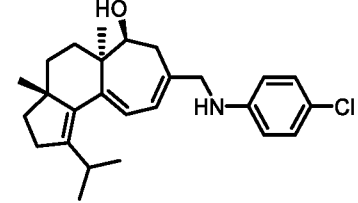
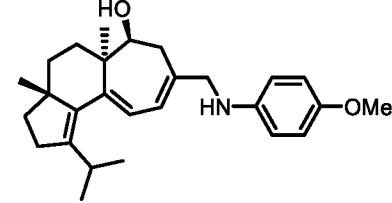
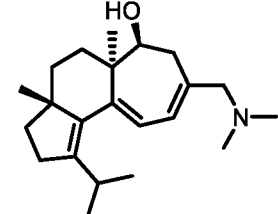
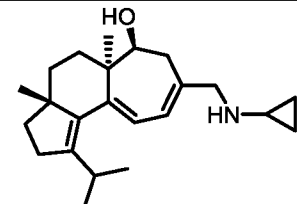
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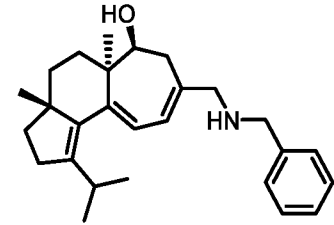
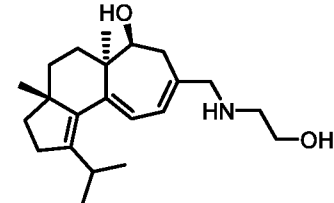
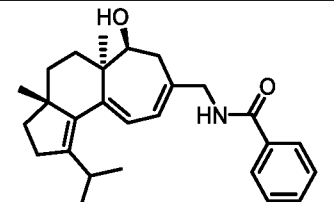
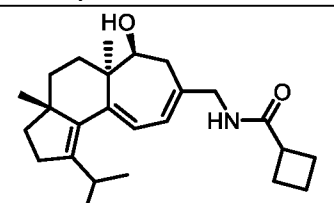
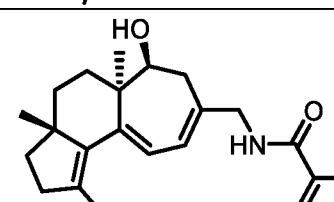
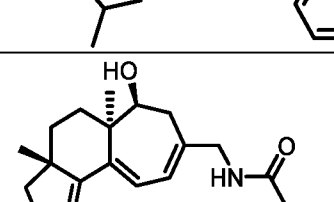
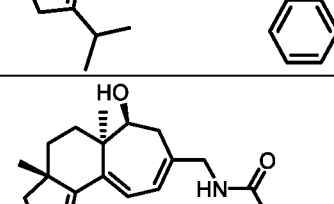
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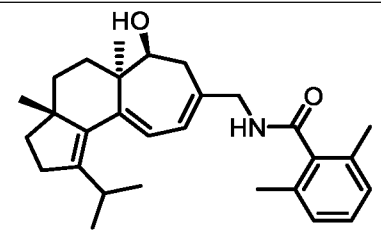
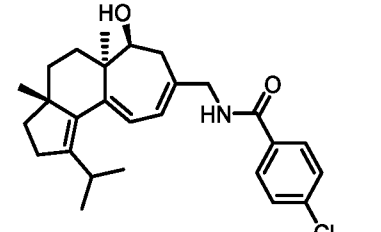
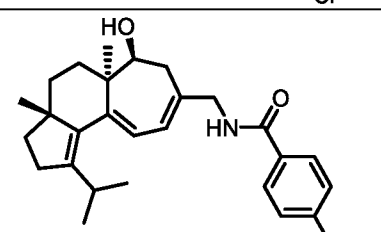
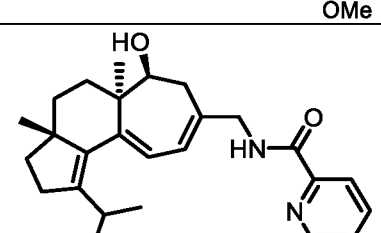
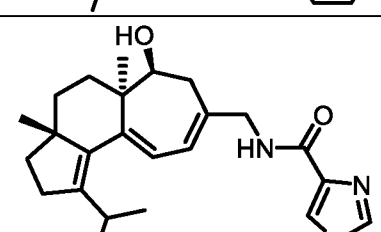
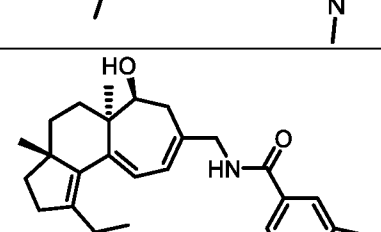
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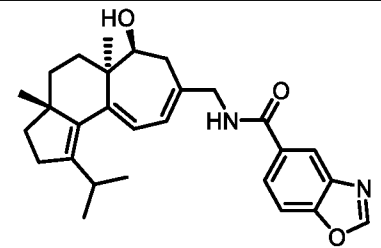
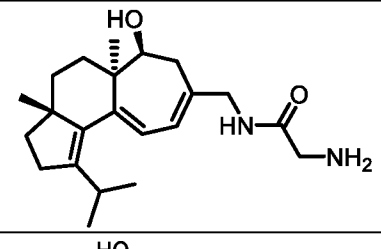
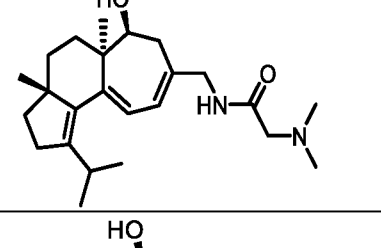
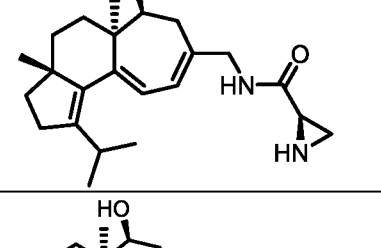
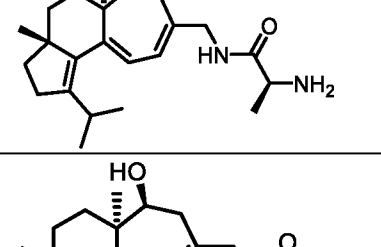
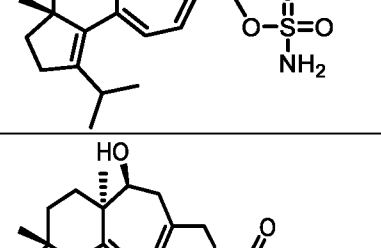
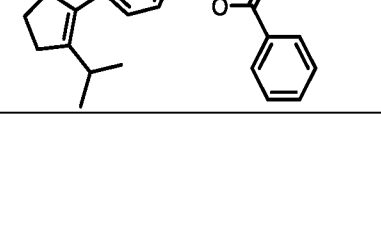
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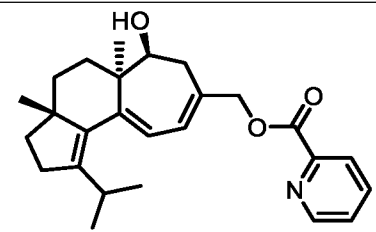
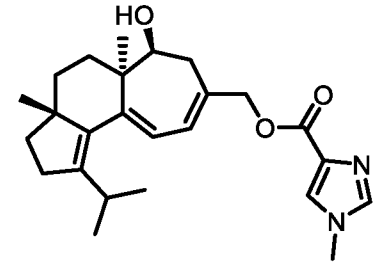
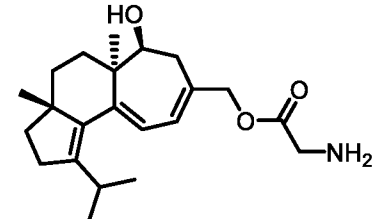
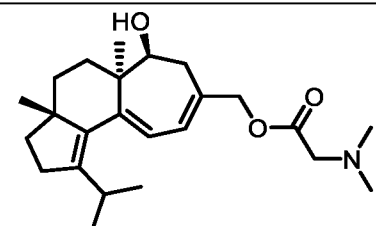
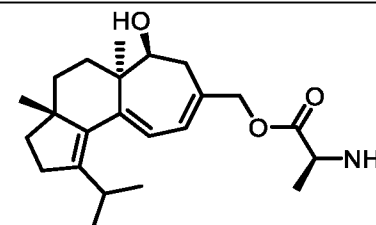
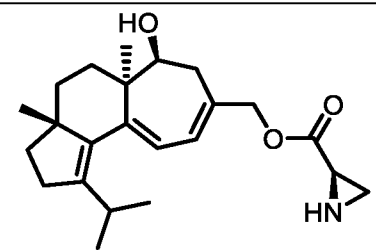
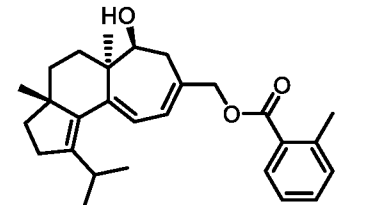
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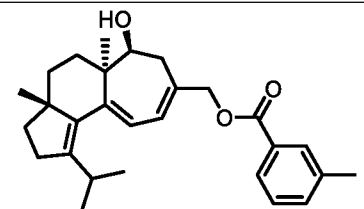
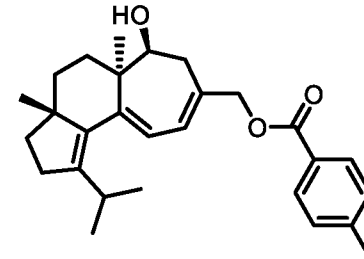
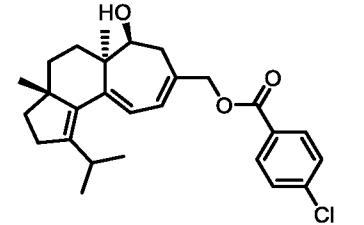
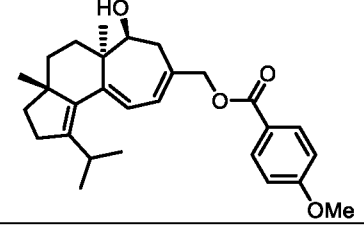
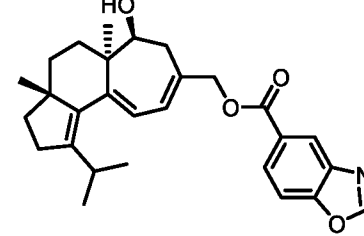
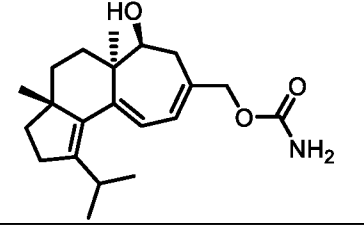
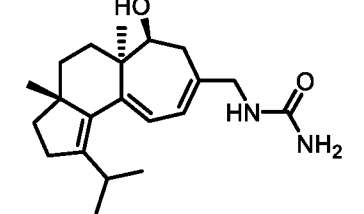
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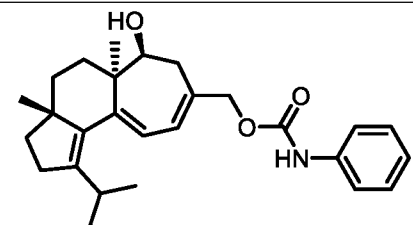
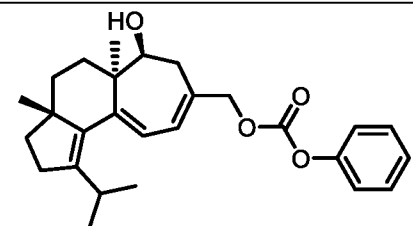
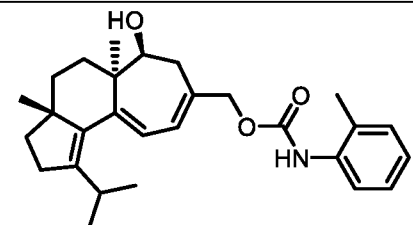
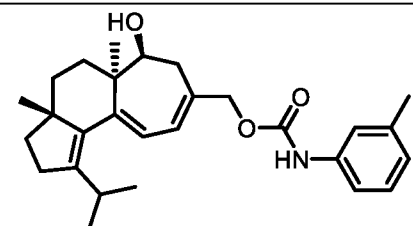
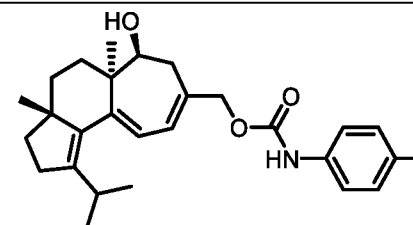
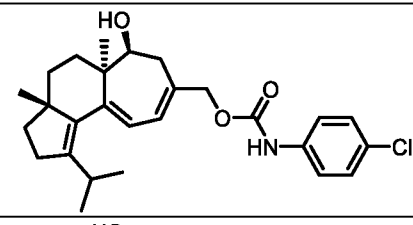
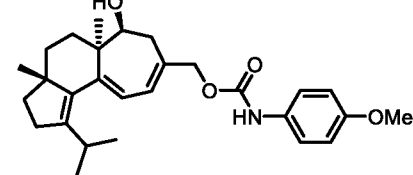
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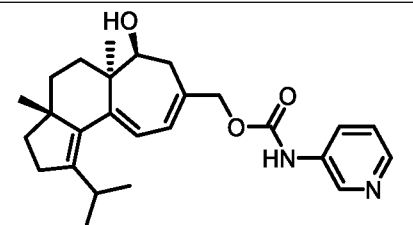
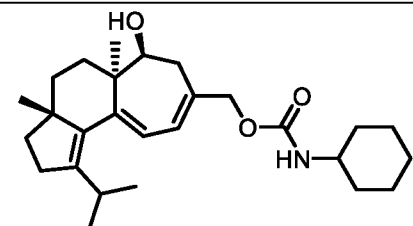
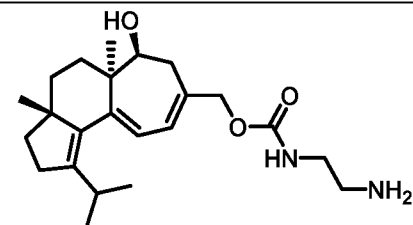
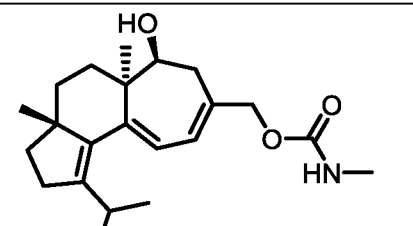
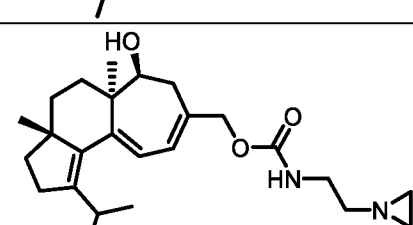
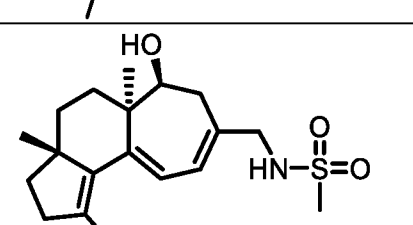
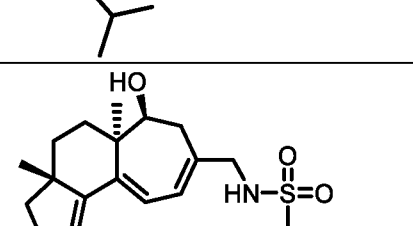
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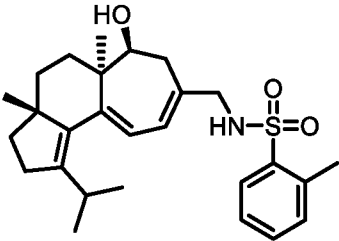
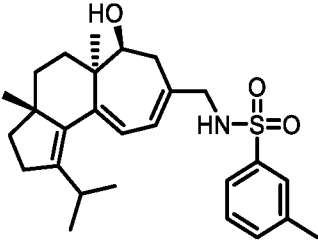
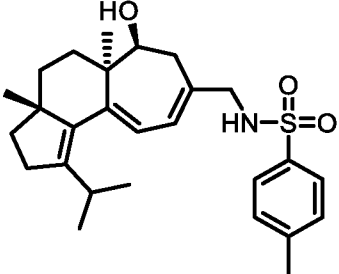
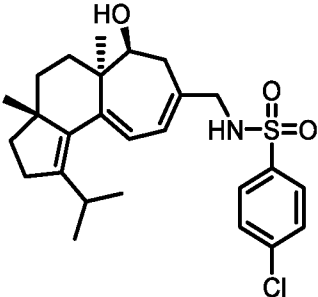
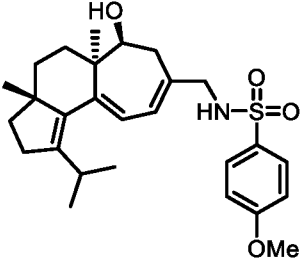
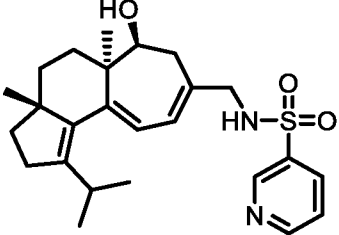
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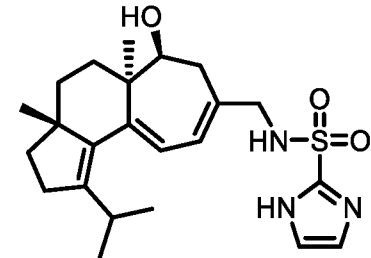
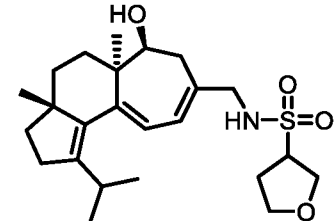
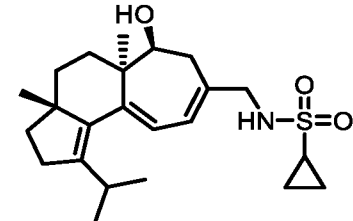
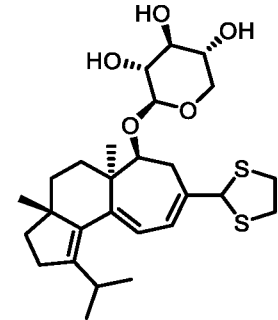
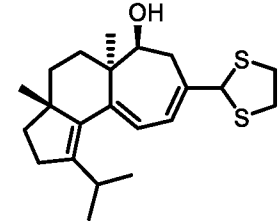
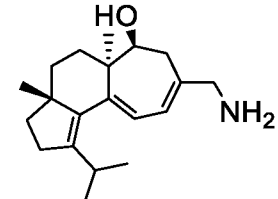
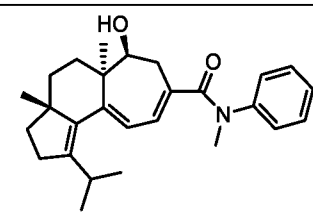
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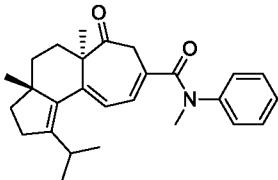
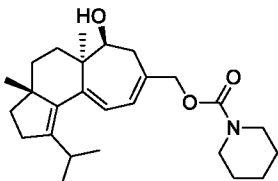
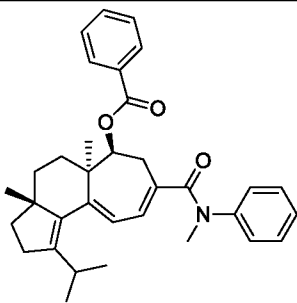
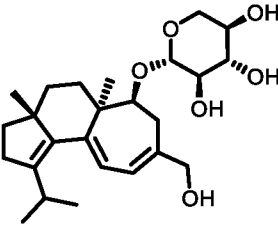
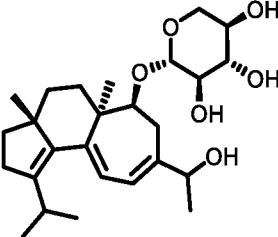
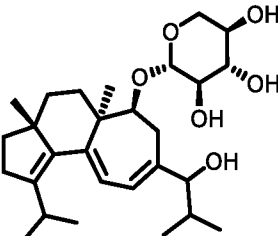
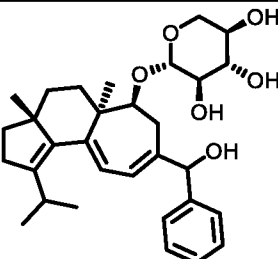
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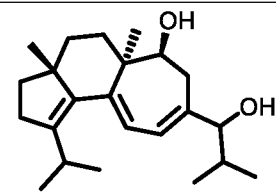
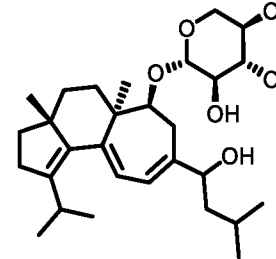
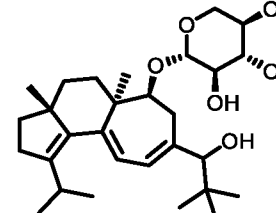
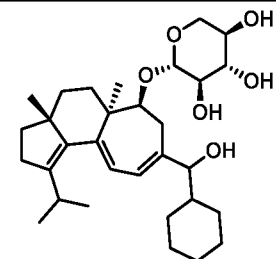
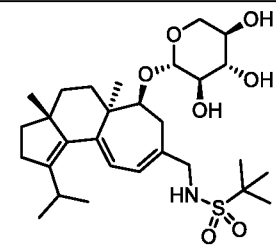
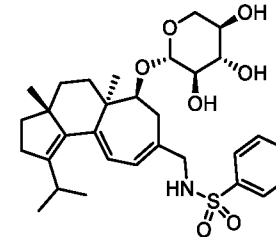
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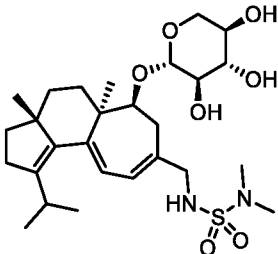
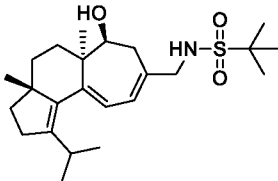
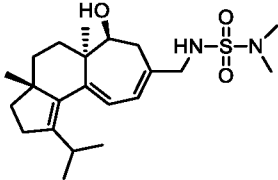
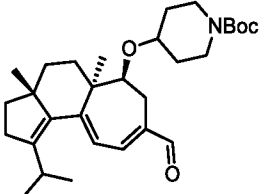
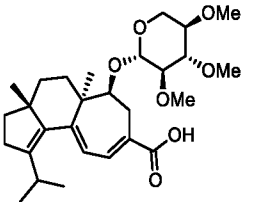
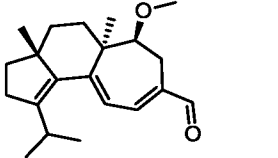
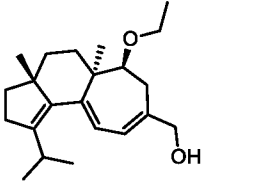
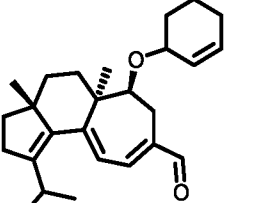
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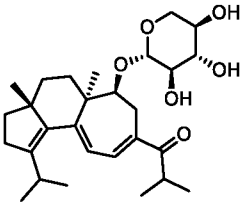
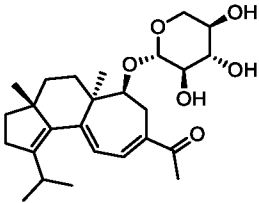
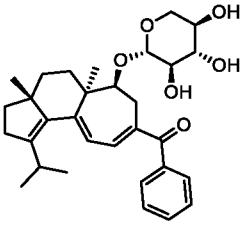
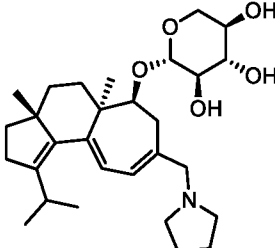
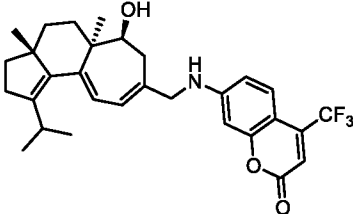
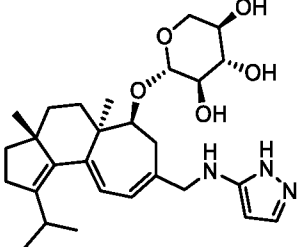
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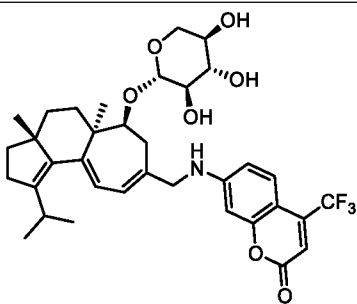
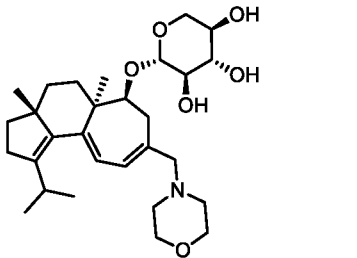
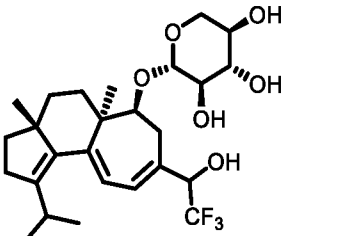
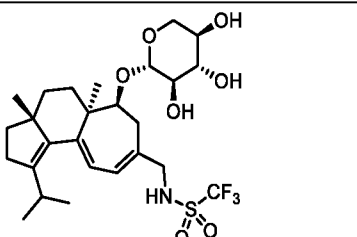
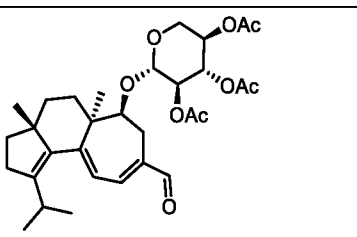
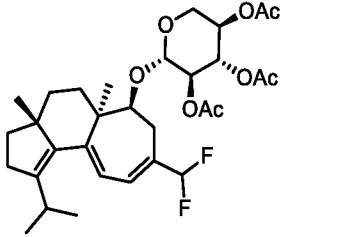
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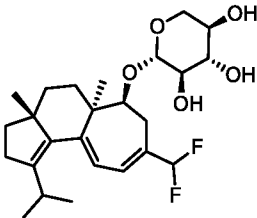
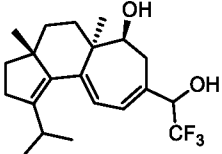
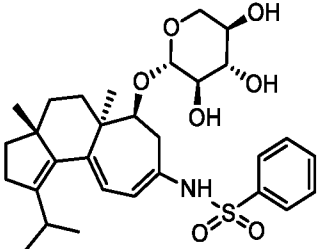
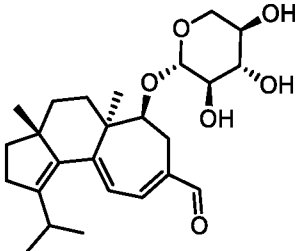
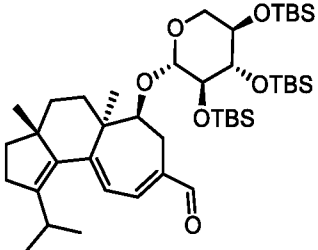
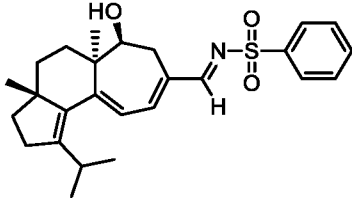
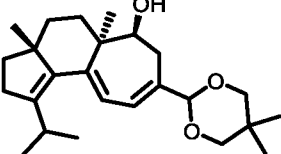
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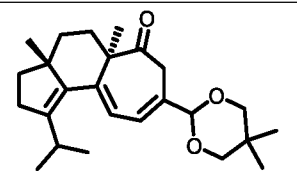
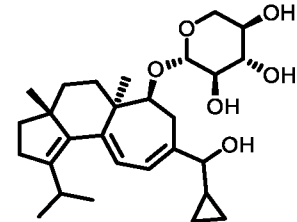
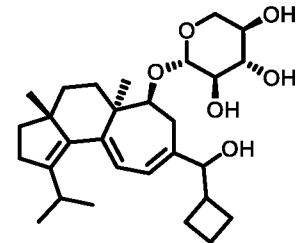
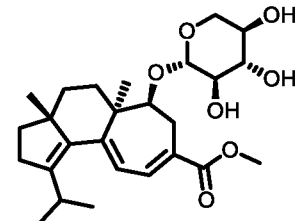
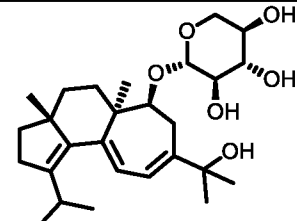
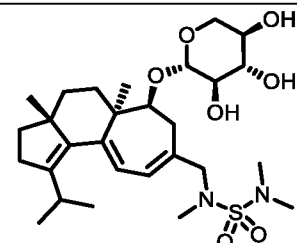
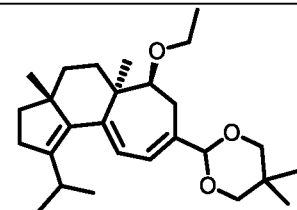
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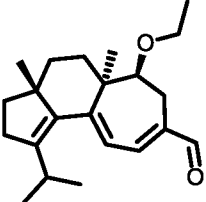
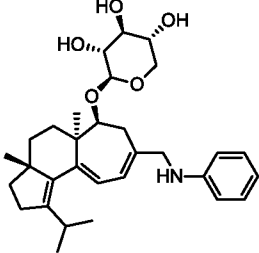
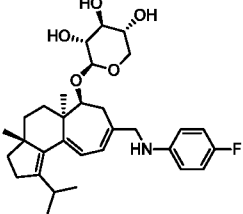
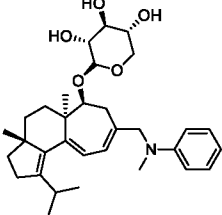
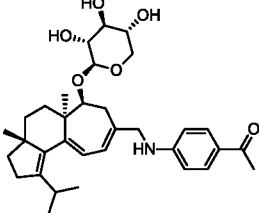
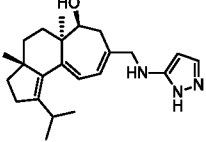
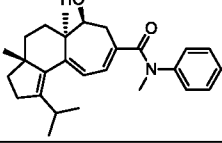
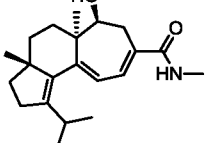
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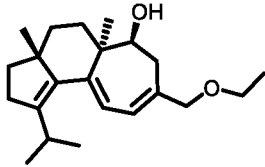
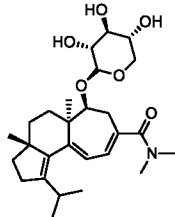
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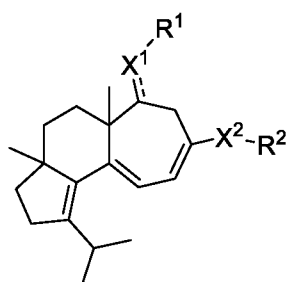
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[00130] The present application also includes compositions and uses of compounds of Formula II, or a pharmaceutically acceptable salt and/or solvate thereof:



II

wherein:

---- is a single or double bond;

when ---- is a double bond, X^1 is selected from O and NR^3 and R^1 is absent;

when ---- is a single bond, X^1 is selected from O, $OC(O)$ and NR^3 and R^1 is selected from H, $SO_2NR^4R^5$, C_{1-10} alkyl, C_{3-10} cycloalkyl, C_{3-10} heterocycloalkyl, C_{6-10} aryl, C_{5-10} heteroaryl, C_{1-10} alkylenearyl, C_{1-10} alkyleneheteroaryl, C_{1-10} alkylene C_{3-8} cycloalkyl and C_{1-10} alkylene C_{3-8} heterocycloalkyl, the latter nine groups being optionally substituted with one or more of OH, halo, OC_{1-6} alkyl, $OC(O)C_{1-6}$ alkyl, $C(O)OC_{1-6}$ alkyl, $OSi(CH_3)_2C_{1-6}$ alkyl and C_{1-6} alkyl;

X^2 is selected from a direct bond, $C(O)$, $C(O)Y^1$, $C(NR^{11})$, Y^1SO_2 , C_{1-6} alkylene Y^1 , C_{1-6} alkylene $Y^1C(O)$, C_{1-6} alkylene Y^1-SO_2 , C_{1-6} alkylene $Y^1-SO_2Y^2$ and C_{1-6} alkylene $Y^1C(O)Y^2$;

R^2 is selected from H, C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-10} cycloalkyl, C_{3-10} heterocycloalkyl, C_{6-10} aryl, C_{6-10} heteroaryl, C_{1-10} alkylenearyl, C_{1-10} alkyleneheteroaryl, C_{1-10} alkylene C_{3-8} cycloalkyl, C_{1-10} alkylene C_{3-8} heterocycloalkyl and phenyl $C(O)$ phenyl $Y^3C(O)R^6$, the latter twelve groups being

optionally substituted with one or more of OH, =O, C₁₋₆alkyl, OC₁₋₆alkyl, NR⁷R⁸, NR⁹C(O)OC₁₋₆alkyl, and halo;

Y¹, Y² and Y³ are independently selected from NR¹⁰ and O;

R³, R⁴, R⁵, R⁷, R⁸, and R⁹ are independently selected from H and C₁₋₆alkyl;

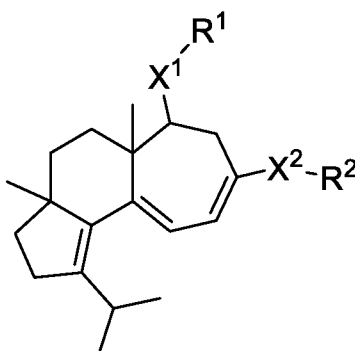
R⁶ is selected from C₁₋₁₀alkyl, C₂₋₁₀alkenyl and C₂₋₁₀alkynyl;

R¹⁰ is selected from H and C₁₋₆alkyl;

R¹¹ is selected from H, C₁₋₆alkyl, C₆₋₁₀aryl and SO₂C₆₋₁₀aryl; and

wherein all available hydrogen atoms are optionally and independently replaced with a fluorine or chlorine atom.

[00131] The present application also includes compositions and uses of compounds of Formula II, or a pharmaceutically acceptable salt and/or solvate thereof:



II

wherein X¹ is selected from O, OC(O) and NR³;

R¹ is selected from H, SO₂NR⁴R⁵, C₁₋₁₀alkyl, C₃₋₁₀cycloalkyl, C₃₋₁₀heterocycloalkyl, C₆₋₁₀aryl, C₅₋₁₀heteroaryl, C₁₋₁₀alkylenearyl, C₁₋₁₀alkyleneheteroaryl, C₁₋₁₀alkyleneC₃₋₈cycloalkyl and C₁₋₁₀alkyleneC₃₋₈heterocycloalkyl, the latter nine groups being optionally substituted with one or more of OH, halo, OC₁₋₆alkyl and C₁₋₆alkyl;

X² is selected from a direct bond, C(O), C(O)Y¹, C₁₋₆alkyleneY¹, C₁₋₆alkyleneY¹C(O), C₁₋₆alkyleneY¹-SO₂, C₁₋₆alkyleneY¹-SO₂Y² and C₁₋₆alkyleneY¹C(O)Y²;

R² is selected from H, C₁₋₁₀alkyl, C₂₋₁₀alkenyl, C₂₋₁₀alkynyl, C₃₋₁₀cycloalkyl, C₃₋₁₀heterocycloalkyl, C₆₋₁₀aryl, C₆₋₁₀heteroaryl, C₁₋₁₀alkylenearyl, C₁₋₁₀alkyleneheteroaryl, C₁₋₁₀alkyleneC₃₋₈cycloalkyl,

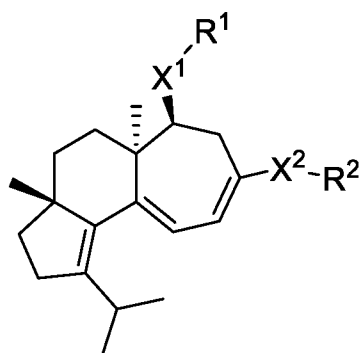
C_{1-10} alkylene C_{3-8} heterocycloalkyl and phenylC(O)phenylY³C(O)R⁶, the latter twelve groups being optionally substituted with one or more of OH, C_{1-6} alkyl, OC₁₋₆alkyl, NR⁷R⁸, NR⁹C(O)OC₁₋₆alkyl, and halo;

Y¹, Y² and Y³ are independently selected from NR¹⁰ and O;

R³, R⁴, R⁵, R⁷, R⁸, R⁹ and R¹⁰ are independently selected from H and C_{1-6} alkyl; and

R⁶ is selected from C_{1-10} alkyl, C_{2-10} alkenyl and C_{2-10} alkynyl.

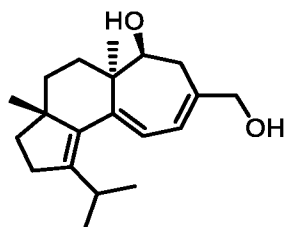
[00132] In some embodiments, the compounds of Formula II have the following relative stereochemistry:



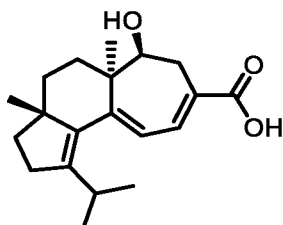
II.

[00133] The embodiments for R¹, R², X¹ and X² of the compounds of Formula II are as defined above for the compounds of Formula I.

[00134] In some embodiments, the compound of Formula II is selected from:

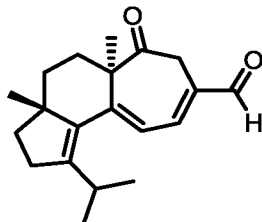


II-1;



; and

II-2



II-3,

or a pharmaceutically acceptable salt and/or solvate thereof.

[00135] In some embodiments, the pharmaceutically acceptable salt is an acid addition salt or a base addition salt. The selection of a suitable salt may be made by a person skilled in the art. Suitable salts include acid addition salts that may, for example, be formed by mixing a solution of a compound with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, acetic acid, trifluoroacetic acid, or benzoic acid. Additionally, acids that are generally considered suitable for the formation of pharmaceutically useful salts from basic pharmaceutical compounds are discussed, for example, by P. Stahl et al, Camille G. (eds.) and Handbook of Pharmaceutical Salts. Properties, Selection and Use. (2002) Zurich: Wiley VCH; S. Berge et al, Journal of Pharmaceutical Sciences 1977 66(1) 1-19; P. Gould, International J. of Pharmaceutics (1986) 33 201-217; Anderson et al, The Practice of Medicinal Chemistry (1996), Academic Press, New York; and in The Orange Book (Food & Drug Administration, Washington, D.C. on their website).

[00136] An acid addition salt suitable for, or compatible with, the treatment of subjects is any non-toxic organic or inorganic acid addition salt of any basic compound. Basic compounds that form an acid addition salt include, for example, compounds comprising an amine group. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric, nitric and phosphoric acids, as well as acidic metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids which form suitable salts include mono-, di- and tricarboxylic acids. Illustrative of such organic acids are, for example, acetic, trifluoroacetic, propionic, glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, benzoic, hydroxybenzoic, phenylacetic, cinnamic, mandelic, salicylic, 2-phenoxybenzoic, p-toluenesulfonic acid and other sulfonic acids such as methanesulfonic acid, ethanesulfonic acid and 2-hydroxyethanesulfonic acid. In some embodiments, exemplary acid addition salts also include acetates, ascorbates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates,

fumarates, hydrochlorides, hydrobromides, hydroiodides, lactates, maleates, methanesulfonates (“mesylates”), naphthalenesulfonates, nitrates, oxalates, phosphates, propionates, salicylates, succinates, sulfates, tartarates, thiocyanates, toluenesulfonates (also known as tosylates) and the like. In some embodiments, the mono- or di-acid salts are formed and such salts exist in either a hydrated, solvated or substantially anhydrous form. In general, acid addition salts are more soluble in water and various hydrophilic organic solvents and generally demonstrate higher melting points in comparison to their free base forms. The selection criteria for the appropriate salt will be known to one skilled in the art. Other non-pharmaceutically acceptable salts such as but not limited to oxalates may be used, for example in the isolation of compounds of the application for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

[00137] A base addition salt suitable for, or compatible with, the treatment of subjects is any non-toxic organic or inorganic base addition salt of any acidic compound. Acidic compounds that form a basic addition salt include, for example, compounds comprising a carboxylic acid group. Illustrative inorganic bases which form suitable salts include lithium, sodium, potassium, calcium, magnesium or barium hydroxide as well as ammonia. Illustrative organic bases which form suitable salts include aliphatic, alicyclic or aromatic organic amines such as isopropylamine, methylamine, trimethylamine, picoline, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Exemplary organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine. The selection of the appropriate salt may be useful, for example, so that an ester functionality, if any, elsewhere in a compound is not hydrolyzed. The selection criteria for the appropriate salt will be known to one skilled in the art. In some embodiments, exemplary basic salts also include ammonium salts, alkali metal salts such as sodium, lithium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases (for example, organic amines) such as dicyclohexylamine, abutyl amine, choline and salts with amino acids such as arginine, lysine and the like. Basic nitrogen containing groups may be quarternized with agents such as lower alkyl halides (e.g., methyl, ethyl and butyl chlorides, bromides and iodides), dialkyl sulfates (e.g., dimethyl, diethyl and dibutyl sulfates), long chain halides (e.g., decyl, lauryl and stearyl chlorides, bromides and iodides), aralkyl halides (e.g., benzyl and phenethyl bromides) and others. Compounds carrying an acidic moiety can be mixed with suitable pharmaceutically acceptable salts to provide, for example,

alkali metal salts (e.g., sodium or potassium salts), alkaline earth metal salts (e.g., calcium or magnesium salts) and salts formed with suitable organic ligands such as quaternary ammonium salts. Also, in the case of an acid (-COOH) or alcohol group being present, pharmaceutically acceptable esters can be employed to modify the solubility or hydrolysis characteristics of the compound.

[00138] All such acid salts and base salts are intended to be pharmaceutically acceptable salts within the scope of the application and all acid and base salts are considered equivalent to the free forms of the corresponding compounds for purposes of the application. In addition, when a compound of the application contains both a basic moiety, such as, but not limited to an aliphatic primary, secondary, tertiary or cyclic amine, an aromatic or heteroaryl amine, pyridine or imidazole and an acidic moiety, such as, but not limited to tetrazole or carboxylic acid, zwitterions ("inner salts") may be formed and are included within the terms "salt(s)" as used herein. It is understood that certain compounds of the application may exist in zwitterionic form, having both anionic and cationic centers within the same compound and a net neutral charge. Such zwitterions are included within the application.

[00139] Solvates of compounds of the application include, for example, those made with solvents that are pharmaceutically acceptable. Examples of such solvents include water (resulting solvate is called a hydrate) and ethanol and the like. Suitable solvents are physiologically tolerable at the dosage administered.

[00140] In some embodiments, compounds of the present application have at least one chiral center and therefore exist as enantiomers and/or diastereomers. It is to be understood that all such isomers and mixtures thereof in any proportion are encompassed within the scope of the present application. It is to be further understood that while the stereochemistry of the compounds may be as shown in any given compound listed herein, such compounds may also contain certain amounts (for example, less than 20%, suitably less than 10%, more suitably less than 5%) of compounds of the present application having an alternate stereochemistry. It is intended that any optical isomers, as separated, pure or partially purified optical isomers or racemic mixtures thereof are included within the scope of the present application.

[00141] In some embodiments, the compounds of the present application can also include tautomeric forms, such as thioketo-enol tautomers and the like. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution. It is intended that any tautomeric forms which the compounds form, as well as mixtures thereof, are included within the scope of the present application.

[00142] In some embodiments, the compounds of the present application exist in varying amorphous and polymorphic forms and it is contemplated that any amorphous forms, polymorphs, or mixtures thereof, which form are included within the scope of the present application.

[00143] The compounds of the present application are suitably formulated in a conventional manner into compositions using one or more carriers. Accordingly, the present application also includes a composition comprising one or more compounds of the application and a carrier. The compounds of the application are suitably formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. Accordingly, the present application further includes a pharmaceutical composition comprising one or more compounds of the application and a pharmaceutically acceptable carrier.

[00144] A compound of the application is suitably used on their own but will generally be administered in the form of a composition in which the one or more compounds of the application (the active ingredient) is in association with an acceptable carrier.

[00145] In some embodiments, the compounds of the application are administered to a subject in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. In some embodiments, the compound of the application is administered, for example, by oral, parenteral, buccal, sublingual, nasal, rectal, patch, pump or transdermal administration and the pharmaceutical compositions are formulated accordingly. Administration can be by means of a pump for periodic or continuous delivery. Conventional procedures and ingredients for the selection and preparation of suitable compositions are described, for example, in Remington's Pharmaceutical Sciences (2000 - 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

[00146] Parenteral administration includes intravenous, intra-arterial, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary (for example, by use of an aerosol), intrathecal, rectal and topical (including the use of a patch or other transdermal delivery device) modes of administration. In some embodiments, the parenteral administration is carried out by continuous infusion over a selected period of time.

[00147] In some embodiments, topical administration includes using solid supports (such as dressings and other matrices) and medicinal formulations (such as gels, mixtures, suspensions and ointments). In some embodiments, the solid support comprises a biocompatible membrane

or insertion into a treatment site. In another embodiment, the solid support comprises a dressing or matrix.

[00148] In some embodiments, topical administration includes liquid, ointment, cream, gel, hydrogel, cataplasm, pomade, liniment, milk, lotion, emulsion, spray, aerosol, collyrium, drops, powder forms of administration. In some embodiments, for topical administration, suitable excipients include, but are not limited to animal and plant oils, mineral oils, synthetic oils, ester oils, waxes, linear higher alcohols, fatty acids, surfactants, phospholipids, gelling and/or thickening agents, alcohol, polyols (including glycerine and propylene glycol), fillers such as clay minerals, soft-focus powders, preservatives, fragrances, pigments, purified water, polysaccharides, such as for example mannans, gluco mannans, galactomannans, fucomannans, proteoglycans, glucosaminoglycans, chitins and chitomannans. In some embodiments, topical administration includes a medical device comprising one or more compounds or compositions of the application. In some embodiments, the medical device is in the form of a dressing, bandage, transdermic medical device, controlled drug release medical device, or a drug-eluting stent. Suitable dressings include, without any limitation, hydrocolloid dressings, hydrocellular dressings, alginate dressings, hydrogel dressings, chitosan-based dressings, cellulose derivatives dressings and any other type of dressing. By "transdermic medical device" it is meant a device for slow liberation via transdermic process of a substance, such as for example adhesive patch. By "drug-eluting stent", also called "coated" or "medicated" stent, it is meant a stent that has been coated with the active substance, such as for example "protein 156A".

[00149] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists.

[00150] In some embodiments, the compound of the application is orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it is enclosed in hard or soft shell gelatin capsules, or it is compressed into tablets, or it is incorporated directly with the food of the diet. In some embodiments, for oral therapeutic administration, the compound is incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, caplets, pellets, granules, lozenges, chewing gum, powders, syrups, elixirs, wafers, aqueous solutions and suspensions, and the like. In the case of tablets, carriers that are used include lactose, corn starch, sodium citrate and salts of phosphoric acid. Pharmaceutically acceptable excipients include binding agents (e.g., pregelatinized maize starch,

polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). In some embodiments, the tablets are coated by methods well known in the art. In the case of tablets, capsules, caplets, pellets or granules for oral administration, pH sensitive enteric coatings, such as Eudragits™ designed to control the release of active ingredients are optionally used. Oral dosage forms also include modified release, for example immediate release and timed-release, formulations. Examples of modified-release formulations include, for example, sustained-release (SR), extended-release (ER, XR, or XL), time-release or timed-release, controlled-release (CR), or continuous-release (CR or Contin), employed, for example, in the form of a coated tablet, an osmotic delivery device, a coated capsule, a microencapsulated microsphere, an agglomerated particle, e.g., as of molecular sieving type particles, or, a fine hollow permeable fiber bundle, or chopped hollow permeable fibers, agglomerated or held in a fibrous packet. Timed-release compositions can be formulated, e.g. liposomes or those wherein the active compound is protected with differentially degradable coatings, such as by microencapsulation, multiple coatings, etc. Liposome delivery systems include, for example, small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines. For oral administration in a capsule form, useful carriers or diluents include lactose and dried corn starch.

[00151] In some embodiments, liquid preparations for oral administration take the form of, for example, solutions, syrups or suspensions, or they are suitably presented as a dry product for constitution with water or other suitable vehicle before use. When aqueous suspensions and/or emulsions are administered orally, the compound of the application is suitably suspended or dissolved in an oily phase that is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added. In some embodiments, such liquid preparations for oral administration are prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters or ethyl alcohol); and preservatives (e.g., methyl or propyl p-hydroxybenzoates or sorbic acid). Useful diluents include lactose and high molecular weight polyethylene glycols.

[00152] It is also possible to freeze-dry the compounds of the application and use the lyophilizates obtained, for example, for the preparation of products for injection.

[00153] In some embodiments, the compound of the application is administered parenterally. Solutions of a compound of the application can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. A person skilled in the art would know how to prepare suitable formulations. For parenteral administration, sterile solutions of the compounds of the application are usually prepared, and the pH of the solutions are suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic. For ocular administration, ointments or droppable liquids may be delivered by ocular delivery systems known to the art such as applicators or eye droppers. Such compositions can include mucomimetics such as hyaluronic acid, chondroitin sulfate, hydroxypropyl methylcellulose or polyvinyl alcohol, preservatives such as sorbic acid, EDTA or benzyl chromium chloride, and the usual quantities of diluents or carriers. For pulmonary administration, diluents or carriers will be selected to be appropriate to allow the formation of an aerosol.

[00154] In some embodiments, the compounds of the application are formulated for parenteral administration by injection, including using conventional catheterization techniques or infusion. In some embodiments, the formulations for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. In some embodiments, the compositions take such forms as sterile suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilizing and/or dispersing agents. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. Alternatively, the compounds of the application are suitably in a sterile powder form for reconstitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[00155] In some embodiments, the compositions for nasal administration are conveniently be formulated as aerosols, drops, gels and powders.

[00156] In some embodiments, for intranasal administration or administration by inhalation, the compounds of the application are conveniently delivered in the form of a solution, dry powder formulation or suspension from a pump spray container that is squeezed or pumped by the patient or as an aerosol spray presentation from a pressurized container or a nebulizer. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge

or refill for use with an atomising device. Alternatively, the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form comprises an aerosol dispenser, it will contain a propellant which can be a compressed gas such as compressed air or an organic propellant such as fluorochlorohydrocarbon. Suitable propellants include but are not limited to dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, heptafluoroalkanes, carbon dioxide or another suitable gas. In the case of a pressurized aerosol, the dosage unit is suitably determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of a compound of the application and a suitable powder base such as lactose or starch. The aerosol dosage forms can also take the form of a pump-atomizer.

[00157] Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, wherein the active ingredient is formulated with a carrier such as sugar, acacia, tragacanth, or gelatin and glycerine. Compositions for rectal administration are conveniently in the form of suppositories containing a conventional suppository base such as cocoa butter.

[00158] Suppository forms of the compounds of the application are useful for vaginal, urethral and rectal administrations. Such suppositories will generally be constructed of a mixture of substances that is solid at room temperature but melts at body temperature. The substances commonly used to create such vehicles include but are not limited to theobroma oil (also known as cocoa butter), glycerinated gelatin, other glycerides, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol. See, for example: Remington's Pharmaceutical Sciences, 16th Ed., Mack Publishing, Easton, PA, 1980, pp. 1530-1533 for further discussion of suppository dosage forms.

[00159] In some embodiments, the compounds of the application are coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide-phenol, polyhydroxy-ethylaspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. In some embodiments, the compounds of the application are coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid,

polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and crosslinked or amphipathic block copolymers of hydrogels.

[00160] In some embodiments, the compounds of the application are coupled with viral, non-viral or other vectors. In some embodiments, the viral vectors include retrovirus, lentivirus, adenovirus, herpesvirus, poxvirus, alphavirus, vaccinia virus or adeno-associated viruses. In some embodiments, the non-viral vectors include nanoparticles, cationic lipids, cationic polymers, metallic nanoparticles, nanorods, liposomes, micelles, microbubbles, cell-penetrating peptides, or lipospheres. In some embodiments, the nanoparticles include silica, lipid, carbohydrate, or other pharmaceutically acceptable polymers.

[00161] In some embodiments, depending on the mode of administration, the pharmaceutical composition will comprise from about 0.05 wt% to about 99 wt% or about 0.10 wt% to about 70 wt%, of the compounds of the application, and from about 1 wt% to about 99.95 wt% or about 30 wt% to about 99.90 wt% of one or more pharmaceutically acceptable carriers, all percentages by weight being based on the total composition.

[00162] In some embodiments, the pharmaceutical composition is formulated for oral administration. In some embodiments, the compounds of the application are administered in a dose of about 0.01 mg/kg body weight to about 250 mg/kg body weight, about 0.1 mg/kg to about 230 mg/kg, about 1 mg/kg to about 210 mg/kg, about 30 mg/kg to about 200 mg/kg, about 50 mg/kg to about 180 mg/kg, about 60 mg/kg, to about 150 mg/kg, about 80 mg/kg to about 120 mg/kg once daily or twice daily.

[00163] In some embodiments, a compound of the application is administered with another therapeutic agent simultaneously or sequentially in separate unit dosage forms or together in a single unit dosage form. Accordingly, the present application provides a single unit dosage form comprising one or more compounds of the application, an additional therapeutic agent, and a pharmaceutically acceptable carrier. In some embodiments, the additional therapeutic agent is ErA.

[00164] In the above, the term “a compound” also includes embodiments wherein one or more compounds are referenced.

III. Methods and Uses of the Application

[00165] Exemplary compounds of the application were tested for their potential for neurotrophic activity in human cells using a neurite outgrowth assay in rat pheochromocytoma (PC12) cells. Exemplary compounds showed the ability to induce nerve growth factor (NGF)

production in this assay. The neurotrophic activity *in vitro* was comparable to Erinacine A (ErA). Further, exemplary compounds of the application showed reduced *in vitro* cytotoxicity and improved physiochemical properties in comparison to ErA.

[00166] Exemplary compounds of the application have been shown to induce the biosynthesis of NGF. ErA has also been shown to possess potent stimulatory effects on the biosynthesis of BDNF. NGF and BDNF are associated with CNS and PNS related diseases, disorders or conditions such as for example, Alzheimer's disease and Parkinson's disease. Therefore, in some embodiments, the compounds of the present application are used, for example, for the treatment of various CNS and/or PNS related diseases, disorders or conditions and have reduced toxicity, and improved pharmacokinetic properties.

[00167] As such, the compounds of the application are useful for treating diseases, disorders or conditions by inducing biosynthesis of NGF and/or BDNF. Therefore, the compounds of the present application are useful as medicaments. Accordingly, the application also includes a compound of the application for use as a medicament.

[00168] The present application includes a method of treating a disease, disorder or condition that benefits from induction of biosynthesis of NGF and/or BDNF, the method comprising administering a therapeutically effective amount of one or more compounds of the application to a subject in need thereof.

[00169] The present application also includes a use of one or more compounds of the application for treatment of a disease, disorder or condition that benefits from induction of biosynthesis of NGF and/or BDNF as well as a use of one or more compounds of the application for the preparation of a medicament for treatment of a disease, disorder or condition that benefits from induction of biosynthesis of NGF and/or BDNF. The application further includes one or more compounds of the application for use in treating a disease, disorder or condition that benefits from induction of biosynthesis of NGF and/or BDNF.

[00170] In some embodiments, the compounds of the application are useful for treating a central nervous system (CNS) or a peripheral nervous system (PNS) disorder or condition in a subject in need of such therapy.

[00171] Therefore, the present application also includes a method for treating a central nervous system (CNS) or a peripheral nervous system (PNS) disease, disorder or condition comprising administering a therapeutically effective amount of one or more compounds of the application to a subject in need thereof. The present application further includes a use of one or

more compounds of the application for treating CNS and/or PNS disease, disorder or condition, a use of one or more compounds of the application for preparation of a medicament for treating CNS and/or PNS disease, disorder or condition, as well as one or more compounds of the application for use in treating CNS and/or PNS disease, disorder or condition.

[00172] The present application also includes a method for treating a central nervous system (CNS) or a peripheral nervous system (PNS) disease, disorder or condition by inducing biosynthesis of NGF and/or BDNF, the method comprising administering a therapeutically effective amount of one or more compounds of the application to a subject in need thereof. The present application further includes a use of one or more compounds of the application for treating CNS and/or PNS disease, disorder or condition by inducing biosynthesis of NGF and/or BDNF, a use of one or more compounds of the application for preparation of a medicament for treating CNS and/or PNS disease, disorder or condition by inducing biosynthesis of NGF and/or BDNF, as well as one or more compounds of the application for use in treating CNS and/or PNS disease, disorder or condition by inducing biosynthesis of NGF and/or BDNF.

[00173] In some embodiments the CNS disease, disorder or condition is selected from neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS, also known as motor neurone disease), ataxia-telangiectasia, progressive bulbar palsy, progressive muscular atrophy, dementia with Lewy bodies, multiple system atrophy, spinocerebellar ataxia type 1 (SCA 1), a retinal degenerative disease, an age-related neurodegenerative disorder, and spinal cord injury.

[00174] In some embodiments, the PNS disease, disorder or condition is selected from acute motor axonal neuropathy, Charcot-Marie-Tooth disease types 1A, 1B and 1X, Guillain-Barré syndrome, Lambert-Eaton syndrome, diabetic neuropathy, chemotherapy induced peripheral neuropathy, cisplatin neuropathy, familial amyloid neuropathy, diphtheritic neuropathy, neuropathy with IgM1 anti-myelin-associated glycoprotein, pyridoxine neuropathy, Refsum's disease, and neuropathy associated with Leprosy and Botulism, and the like.

[00175] In some embodiments, the method and the use of the present application includes administering a therapeutically effective amount of ErA, or a pharmaceutically acceptable salt and/or solvate thereof.

[00176] ErA was tested for its ability to promote or improve wound healing processes. Results showed ErA's ability to accelerate migration of fibroblasts and endothelial cells and to promote new blood vessel formation. Further, treatment of diabetic mice with ErA showed

accelerated wound closure and lower levels of the pro-inflammatory cytokine IL-1 β . Finally, ErA-treated wounds showed induced expression of Ki67, CD31 and fibronectin, markers indicating the ongoing process of repair and regeneration. Thus, the studies herein demonstrated ErA's ability to regulate wound healing processes, and can be used to promote or improve wound healing in a subject.

[00177] Therefore, in further aspects of the present application, ErA and compounds of the application are useful for promoting or improving wound healing.

[00178] Therefore, the present application also includes a method of promoting or improving wound healing, the method comprising administering a therapeutically effective amount of ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, to a subject in need thereof.

[00179] The present application also includes a use of ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, for promoting or improving wound healing as well as a use of ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, for the preparation of a medicament for promoting or improving wound healing. The application further includes ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, for use in promoting or improving wound healing.

[00180] Wounds refer to any injury to a tissue such as, for example, acute, chronic and burn wounds. Examples of wounds include, but are not limited to, burns, incisions, excisions, lacerations, abrasions, puncture or penetrating wounds, surgical wounds, contusions, hematomas, crushing injuries, and/or ulcers.

[00181] In some embodiments, wound healing comprises healing acute burn wounds.

[00182] In some embodiments, chronic wounds comprise diabetic ulcers, pressure ulcers, or burns.

[00183] In some embodiments, the subject is a mammal. In some embodiments, the subject is human. In some embodiments, the subject is a non-human animal. Accordingly, the compounds, methods and uses of the present application are directed to both human and veterinary diseases, disorders and conditions.

[00184] In some embodiments, the ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, are administered with an additional therapeutic agent simultaneously or sequentially in separate unit dosage forms or together in a single unit dosage form. Accordingly, the present application provides a single unit dosage form comprising ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, an additional therapeutic agent, and a pharmaceutically acceptable carrier.

[00185] Effective amounts may vary according to factors such as the disease state, age, sex and/or weight of the subject. The amount of a given compound or compounds that will correspond to such an amount will vary depending upon various factors, such as the given compound(s), the pharmaceutical formulation, the route of administration, the type of condition, disease or disorder, the identity of the subject being treated, and the like, but can nevertheless be routinely determined by one skilled in the art. The effective amount is one that following treatment therewith manifests as an improvement in or reduction of any disease symptom.

[00186] The dosage of the ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, can vary depending on many factors such as the pharmacodynamic properties of the compound(s), the mode of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the frequency of the treatment and the type of concurrent treatment, if any, and the clearance rate of the compound(s) in the subject to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response. Dosages will generally be selected to maintain a serum level of compound(s) from about 0.01 µg/cc to about 1000 µg/cc, or about 0.1 µg/cc to about 100 µg/cc. As a representative example, oral dosages of ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, will range between about 1 mg per day to about 1000 mg per day for an adult, suitably about 10 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600mg or about 650 mg of per day. For parenteral administration, a

representative amount is from about 0.001 mg/kg to about 10 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 0.01 mg/kg to about 1 mg/kg or about 0.1 mg/kg to about 1 mg/kg will be administered. For oral administration, a representative amount is from about 0.001 mg/kg to about 10 mg/kg or about 0.1 mg/kg to about 10 mg/kg. For administration in suppository form, a representative amount is from about 0.1 mg/kg to about 10 mg/kg. In some embodiments of the application, compositions are formulated for oral administration and the compound(s) are suitably in the form of tablets or suppositories containing 0.25, 0.5, 0.75, 1.0, 5.0, 10.0, 20.0, 25.0, 30.0, 40.0, 50.0, 60.0, 70.0, 75.0, 80.0, 90.0, 100.0, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 mg of the compound(s) per tablet. The ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, may be administered in a single daily, weekly or monthly dose or the total daily dose may be divided into two, three, four, five or six daily doses.

[00187] In some embodiments, the pharmaceutical composition is formulated for oral administration.

[00188] In some embodiments, the pharmaceutical composition is formulated for topical administration.

[00189] In some embodiments, the ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, are administered in a dose of about 0.01 mg/kg body weight to about 250 mg/kg body weight, about 0.1 mg/kg to about 230 mg/kg, about 1 mg/kg to about 210 mg/kg, about 30 mg/kg to about 200 mg/kg, about 50 mg/kg to about 180 mg/kg, about 60 mg/kg, to about 150 mg/kg, about 80 mg/kg to about 120 mg/kg and values therebetween in a single daily, weekly or monthly dose or the total daily dose may be divided into two, three, four, five or six daily doses.

[00190] In some embodiments, ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, are administered at least once a week. However, in another embodiment, the one or more compounds are administered from about one time per two weeks, three weeks or one month. In another embodiment, ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, are administered about one time per week to about once daily. In another embodiment, the ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or

more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, are administered 2, 3, 4, 5 or 6 times daily. The length of the treatment period depends on a variety of factors, such as the severity of the disease, disorder or condition, the age of the subject, the concentration and/or the activity of ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, and/or a combination thereof. It will also be appreciated that the effective dosage of ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, used for the treatment may increase or decrease over the course of a particular treatment regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration is required. For example, the compound(s) are administered to the subject in an amount and for duration sufficient to treat the subject.

[00191] In some embodiments, for promoting or improving wound healing, the ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, are administered for about 1-2 hours, about 2-4 hours, about 4-6 hours, about 6-8, or about 24 hours or longer. In some embodiments, the ErA, or a pharmaceutically acceptable salt and/or solvate thereof, and/or one or more compounds of the application, or a pharmaceutically acceptable salt and/or solvate thereof, are administered before, during, or immediately following wounding, for example, within about 180, about 120, about 90, about 60, or about 30 days, or within about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, or about 2 days or less, or within about 24, about 12, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2 hours or within about 60, about 45, about 30, about 15, about 10, about 5, about 4, about 3, about 2, about 1 minute following wounding.

[00192] All references to “a compound” above, also include embodiments where one or more compounds are administered or used. As a reminder, the term “compound(s) of the application” or “compound(s) of the present application” and the like as used herein refers to a compound of Formula I or salts and/or solvates thereof, or a compound of Formula II or salts and/or solvates thereof. It should be noted that the compounds of Formula II, or salts and/or solvates thereof, are the same as the compounds of Formula I, or salts and/or solvates thereof, with the exception that compounds wherein X^1-R^1 is OH, and X^2-R^2 is CH_2OH , $C(O)OH$ or $C(O)H$ are included within the compounds of Formula II, or salts and/or solvates thereof.

IV. Methods of Preparing the Compounds of the Application

[00193] Compounds of the application can be prepared by various standard chemistries known in the art. The choice of particular structural features and/or substituents may influence the selection of one process over another. The selection of a particular process to prepare a given compound of the application is within the purview of the person of skill in the art. Some starting materials for preparing compounds of the application are available from commercial chemical sources. Other starting materials, for example as described below, are readily prepared from available precursors using straightforward transformations that are well known in the art.

[00194] In an exemplary embodiment, compounds of the application are prepared using a semi-synthetic route starting from the naturally occurring erinacine A is converted to Erinacine aglycon by first protection of the aldehyde group followed by reacting the resulting protected compound under known conditions for sugar hydrolysis. In some embodiments, the aldehyde group of the ErA aglycon is further converted to, for example, a carboxylic acid using a suitable oxidizing agent (such as sodium chlorite), and/or the alcohol group of the ErA aglycon is converted to ketone group using a suitable oxidizing agent (such as for example Dess–Martin periodinane). In some embodiments, the carboxylic acid group is converted to an amide group by reaction with amines of formula $\text{NHR}^{10}\text{R}^2$ in the presence of suitable coupling reagents (such as HATU and DIPEA). In some embodiments, the R^{10} and R^2 are both H, the corresponding amide is first converted to a primary amine with any suitable reducing agent (such as LAH), and then reacted with a compound of formula HO-C(O)-R^2 group in the presence of suitable coupling reagents (such as HATU and DIPEA) to provide the corresponding “reverse amide”. In some embodiments, the aldehyde group of ErA is modified either by reductive amination to create a series of differently substituted amines (primary, secondary, tertiary, and quaternary) or by dithiolane substitution.

[00195] Alternatively, in some embodiments, ErA is reacted with a compound of formula $\text{NHR}^{10}\text{R}^2$ under reductive amination conditions in the presence of a suitable reducing agent, such as NaCNBH_3 .

[00196] Salts of the compound of Formula I, or a solvate thereof, are generally formed by dissolving the neutral compound in an inert organic solvent and adding either the desired acid or base and isolating the resulting salt by either filtration or other known means.

[00197] The formation of solvates of the compound of Formula I, or a salt thereof, will vary depending on the compound and the solvate. In general, solvates are formed by dissolving the compound in the appropriate solvent and isolating the solvate by cooling or using an antisolvent.

The solvate is typically dried or azeotroped under ambient conditions. The selection of suitable conditions to form a particular solvate can be made by a person skilled in the art.

[00198] Throughout the processes described herein it is to be understood that, where appropriate, suitable protecting groups will be added to, and subsequently removed from, the various reactants and intermediates in a manner that will be readily understood by one skilled in the art. Conventional procedures for using such protecting groups as well as examples of suitable protecting groups are described, for example, in "*Protective Groups in Organic Synthesis*", T.W. Green, P.G.M. Wuts, Wiley-Interscience, New York, (1999). It is also to be understood that a transformation of a group or substituent into another group or substituent by chemical manipulation can be conducted on any intermediate or final product on the synthetic path toward the final product, in which the possible type of transformation is limited only by inherent incompatibility of other functionalities carried by the molecule at that stage to the conditions or reagents employed in the transformation. Such inherent incompatibilities, and ways to circumvent them by carrying out appropriate transformations and synthetic steps in a suitable order, will be readily understood to one skilled in the art. Examples of transformations are given herein, and it is to be understood that the described transformations are not limited only to the generic groups or substituents for which the transformations are exemplified. References and descriptions of other suitable transformations are given in "Comprehensive Organic Transformations – A Guide to Functional Group Preparations" R.C. Larock, VHC Publishers, Inc. (1989). References and descriptions of other suitable reactions are described in textbooks of organic chemistry, for example, "*Advanced Organic Chemistry*", March, 4th ed. McGraw Hill (1992) or, "*Organic Synthesis*", Smith, McGraw Hill, (1994). Techniques for purification of intermediates and final products include, for example, straight and reversed phase chromatography on column or rotating plate, recrystallisation, distillation and liquid-liquid or solid-liquid extraction, which will be readily understood by one skilled in the art.

[00199] The products of the processes of the application may be isolated according to known methods, for example, the compounds may be isolated by evaporation of the solvent, by filtration, centrifugation, and/or chromatography or other suitable method.

[00200] One skilled in the art will recognize that where a reaction step of the present application is carried out in a variety of solvents or solvent systems, said reaction step may also be carried out in a mixture of the suitable solvents or solvent systems.

[00201] The following non-limiting examples are illustrative of the present application:

EXAMPLES

Example 1: Erinacine A*Detection of Erinacine A in the lion's mane mycelium extract.*

[00202] To detect the presence of Erinacine A (ErA) in the *Hericium erinaceus* mycelial extract, an established protocol provided by Grape King Bio Ltd. was followed that utilizes high-performance liquid chromatography (HPLC) and detects ErA at absorption maximum of 340 nm [K. F. Lee, 2014]. Briefly, 0.1 gram of *H. erinaceus* mycelia was extracted with 5 ml of 85% ethanol using ultrasonic bath for 60 min. The supernatant was centrifuged and analyzed using liquid chromatography-tandem mass spectrometer with electrospray ionization (LC-MS-ESI), where separation was achieved using a mobile phase consisting of 0.1% formic acid and acetonitrile at a flow rate of 1.0 mL/min.

Purification of Erinacine A from lion's mane mycelium extract.

[00203] ErA enriched *H. erinaceus* mycelium (Grape King Bio Ltd.) was extracted with 95% ethanol using the Dionex Accelerated Solvent Extractor (ASE). The extract was centrifuged and dried using a rotary evaporator. It was re-dissolved in ethyl acetate (EtOAc) followed by addition of deionized water and mixed in an ultrasonic bath for 20 min. The EtOAc layer was rinsed with 5% sodium chloride solution, separated using a separating funnel and dried with silica gel. Dried extract was then loaded onto a silica gel column (RediSep Gold® Silica) and purified using flash chromatography system (Teledyne ISCO) employing a linear gradient (35–100% hexane/EtOAc-0.1% acetic acid). Eluted fractions with an absorption peak at 340 nm were collected and dried under vacuum. Dried samples were dissolved in dimethylsulfoxide (DMSO) and subjected to reverse-phase chromatography using a C18 column (RediSep Rf C18, Teledyne) and linear gradient of 5–100% water/acetonitrile-0.1% formic acid. Eluted fractions with peak at 340 nm were collected and lyophilised. The purity and identity of the compound was further confirmed by LC-MS-ESI analysis, using a mobile phase consisting of 0.1% formic acid and acetonitrile at a flow rate of 1.0 mL/min. Its chemical structure was elucidated by two-dimensional (2D) nuclear magnetic resonance NMR spectroscopy.

In vitro neurite outgrowth assay.

[00204] U87 cells which are human primary glioblastoma cells were seeded in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) in 48-well plates at a density of 1.8×10^5 cells per ml [A. Zabłocka, 2015]. After 24 hrs, wells were washed once with serum free DMEM and 250 µl of fresh serum free media was added. Plates were further incubated

for 24 hr followed by treatment with ErA (0.1–1 μ M) or DMSO (0.5%). After 48 hours of exposure, the conditioned supernatants were harvested by centrifugation and added to PC12 cells seeded in collagen coated 96-well plates (10,000 cells per well). PC12 cells were allowed to differentiate in response to the secreted NGF and images were acquired under the microscope after 48 h using an inverted light microscope (EVOS M7000). Serum-free medium supplemented with recombinant NGF (Abcam) served as a positive control.

In vitro blood-brain barrier (BBB) assay

[00205] The BBB permeation assay was conducted using the PAMPA-BBB kit (BioAssay Systems) as per the manufacturer's instructions. Briefly, 10 mM stock solutions for ErA and analogs were prepared in DMSO. These were then diluted in PBS (pH 7.4) to a concentration of 500 μ M. Equilibrium standards for each test compound and permeability controls were also prepared in phosphate-buffered saline (PBS) at concentration 200 μ M, to be used for analysis the next day. A DMSO blank control was included as well. 300 μ L of PBS was added to the wells in the acceptor plate, and 5 μ L of BBB lipid solution was carefully added to the well membranes of the donor plate. 200 μ L of test compounds and permeability controls were then added to the donor plate and incubated at room temperature for 27 hours. At the end of incubation, 100 μ L of liquid from the acceptor plate wells and equilibrium standards was transferred into a UV plate and the absorbance spectrum was measured 200-500 nm at 10 nm intervals to ascertain the peak absorbance for each test compound. The permeability rate (Pe) for each respective test compound and PC was calculated using the obtained absorbance values, employing the equation: $Pe = C \times -\ln(1 - \frac{ODA}{ODE})$ cm/s; ODA – Absorbance of the acceptor solution minus blank; ODE – Absorbance of the equilibrium standard solution minus blank; $C = 5.14 \times 10^{-6}$. The results were obtained as average $\pm$ SD based on duplicate measurements.

In vitro toxicity testing of Erinacine A

[00206] Peripheral Blood Mononuclear Cells (PBMCs) at 2×10^6 cells per ml were resuspended in Roswell Park Memorial Institute medium (RPMI) with 10% fetal bovine serum (FBS) and added to tissue culture treated 96-well plates for 24 hrs [T. Bhandu, 2020]. Cells were then incubated in the presence of varying concentrations of ErA at 37°C in the presence of 5% CO₂. Control cells were treated with 0.5 % of dimethyl sulphoxide (DMSO) as in drug. After 24 hours, 20 μ L of PrestoBlue™ Cell Viability Reagent (ThermoFisher Scientific) was added to each well and incubated for 2 hrs [M. Xu, 2015]. Fluorescence was measured at excitation/emission

of 560/590 nm in a plate reader (Biotek Synergy). Fluorescence values were expressed as percentage and inhibition with respect to solvent control was plotted.

Example 2: Erinacine A analogues

[00207] A combination of a Structure-Activity Relationship (SAR) analysis and a semi-synthesis approach was employed to optimize the therapeutic potential of ErA by fine-tuning of its chemical structure. Novel ErA analogs with enhanced efficacy, reduced toxicity, and improved pharmacokinetic properties for the treatment of CNS related disorders were developed. ErA was purified in bulk quantities from ErA enriched *H. erinaceus* mycelium and served as the starting material for the study. On the ErA molecule, aldehyde group and sugar moiety pose as two potential sites for chemical modifications (Figure 1), and substitutions at these sites were obtained as explained in further detail below.

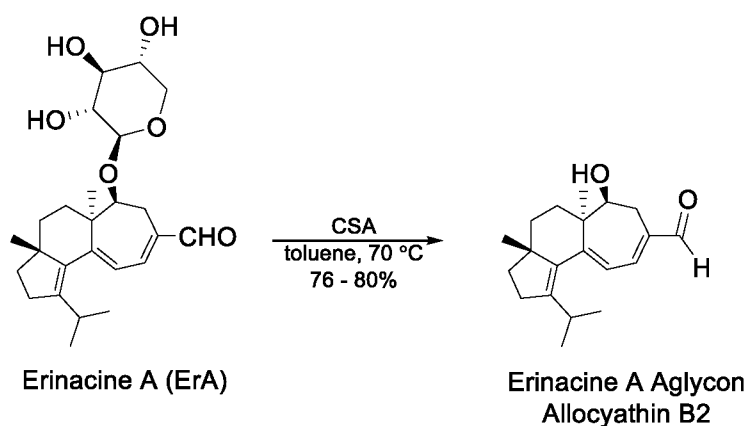
[00208] As the first strategy, the sugar moiety on ErA was hydrolysed in the presence of toluene producing ErA aglycon.

[00209] As the second strategy, the aldehyde group of ErA was modified either by reductive amination to create a series of differently substituted amines (primary, secondary, tertiary, and quaternary) or by dithiolane substitution. Analogs were further tested for their cytotoxicity against primary eukaryotic cells, *in vitro* ability to diffuse across the BBB, and their neurite outgrowth potential using rat pheochromocytoma (PC12) cells.

Method of Synthesis of analogs of Erinacine A

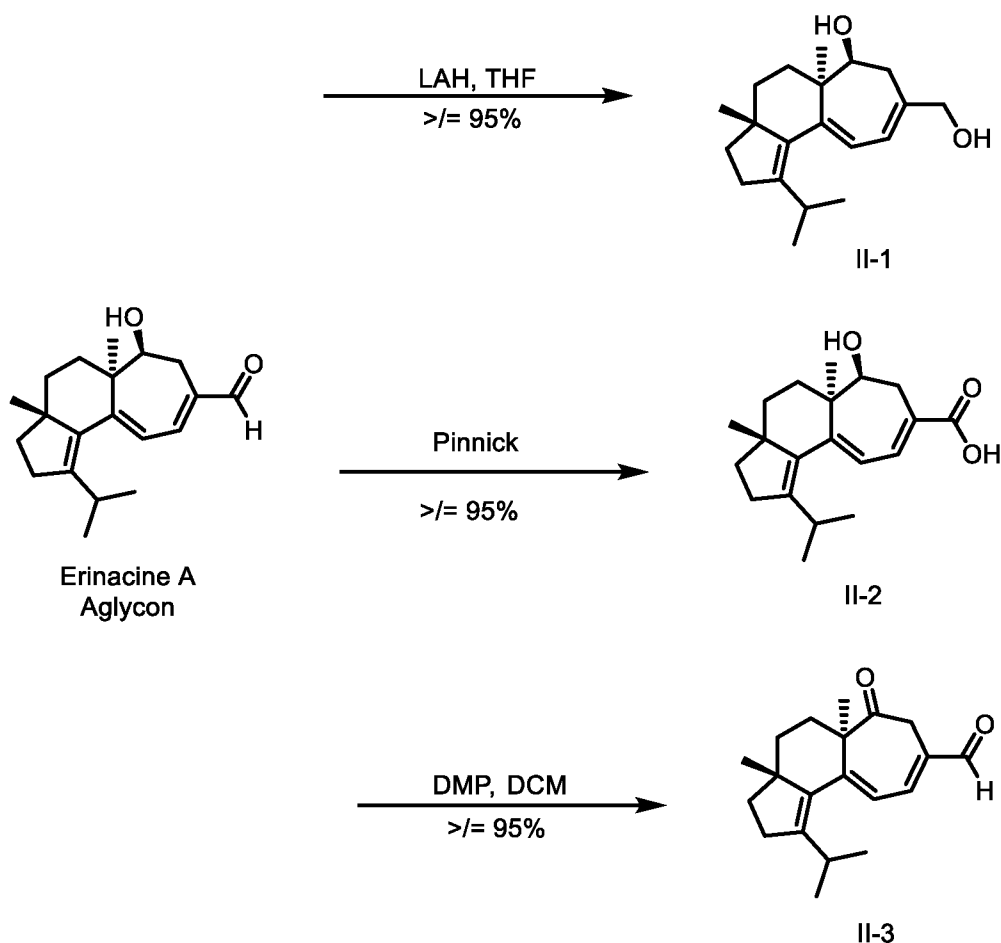
General Procedure I

[00210] Erinacine A aglycon also known as Allocyathin B2 in literature was synthesised as shown in Scheme 1.



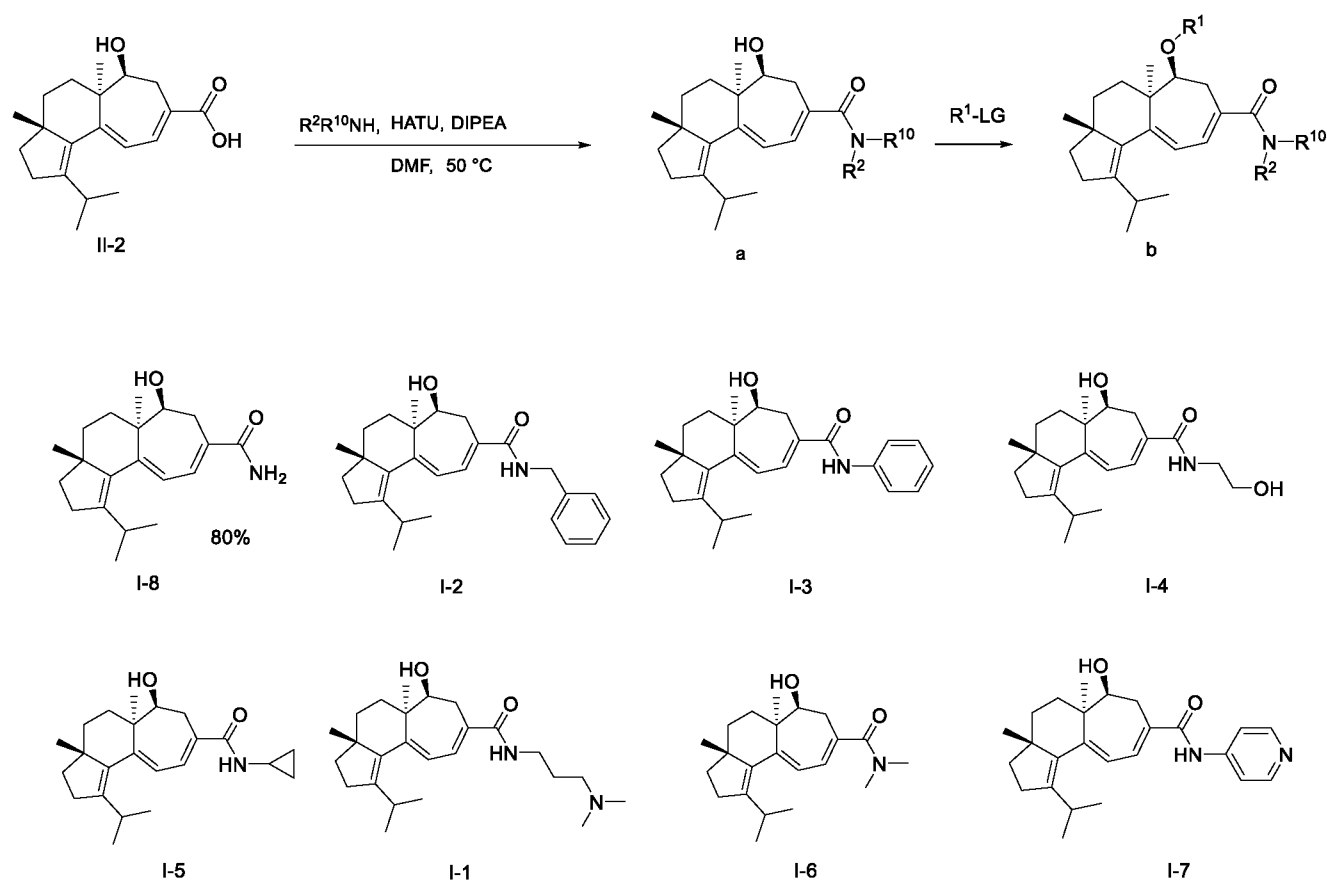
Scheme 1

[00211] The functional groups such as hydroxyl, aldehyde and double bonds in Erinacine A aglycon are sites of manipulation towards Structure Activity Relationship (SAR) studies. Further manipulation of the functional groups of Erinacine A aglycon were carried out as described in Scheme 2 below. Reduction of the aldehyde produced the primary alcohol (II-1), oxidation of the aldehyde produced the carboxylic acid Cyathin D (II-2) and oxidation of the alcohol to ketone produced Anhydrocyathin B₃ (II-3).



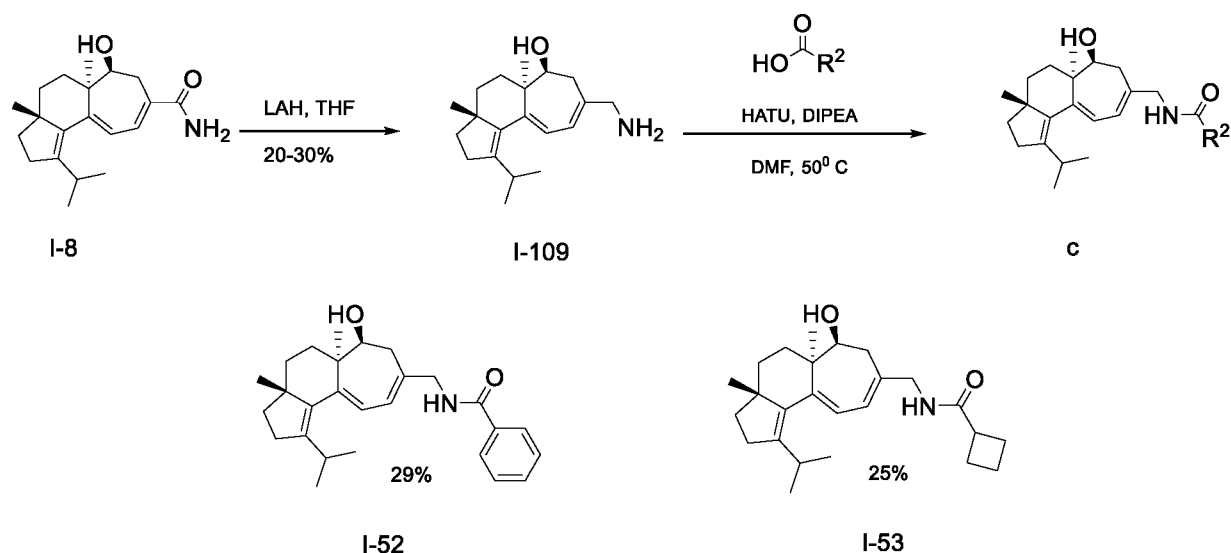
Scheme 2

[00212] Amide coupling reactions were further carried out on compound II-2 by reaction with $R^{10}R^2NH$ to obtain compound a, which represents a variety of substituted exemplary amides, in which X^1 is O, R^1 is H; X^2 is $C(O)NR^{10}$, and R^2 and R^{10} are as defined for compound of Formula I (Scheme 3). Further, the OH group can undergo esterification by reaction with R^1 -LG (wherein LG is a leaving group) to obtain compound b, in which R^1 , R^2 and R^{10} are as defined for compound of Formula I. Several exemplary amides are also shown in Scheme 3 below.



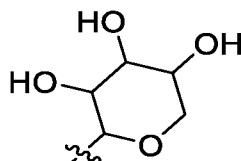
Scheme 3

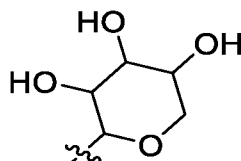
[00213] The amide in compound I-8, which can be obtained for example by the synthesis shown in Scheme 3 above and in which X^1-R^1 is OH, X^2 is C(O)NH and R^2 is H was reduced to a primary amine with the aim to use it as a functional handle (Scheme 4). The yield of primary amine “I-109” was observed to be low and the compound was found to be unstable. It was further decided to proceed with amide coupling reactions for compound “I-109” with HO-C(O)- R^2 to form compound **c**, which represents exemplary compounds of the application in which X^1-R^1 is OH, X^2 is CH₂NHC(O) and R^2 is as defined for compounds of Formula I. Exemplary ErA analogs I-52 and I-53 which can be prepared by said amide coupling reactions are also shown in Scheme 4 below.

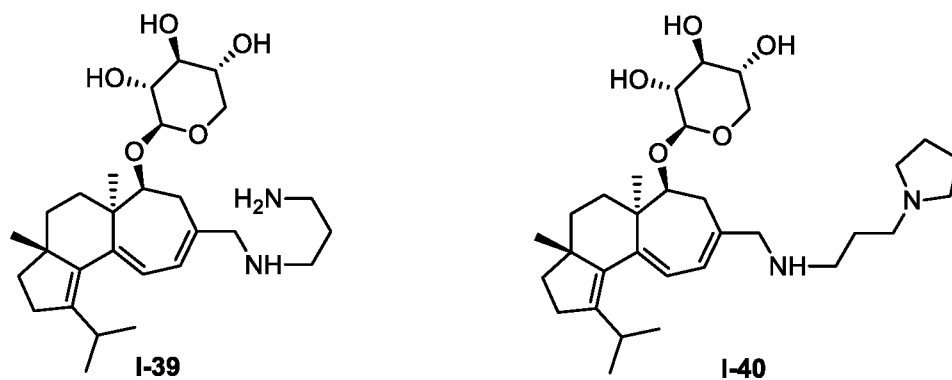


Reductive amination of the ErA aldehyde

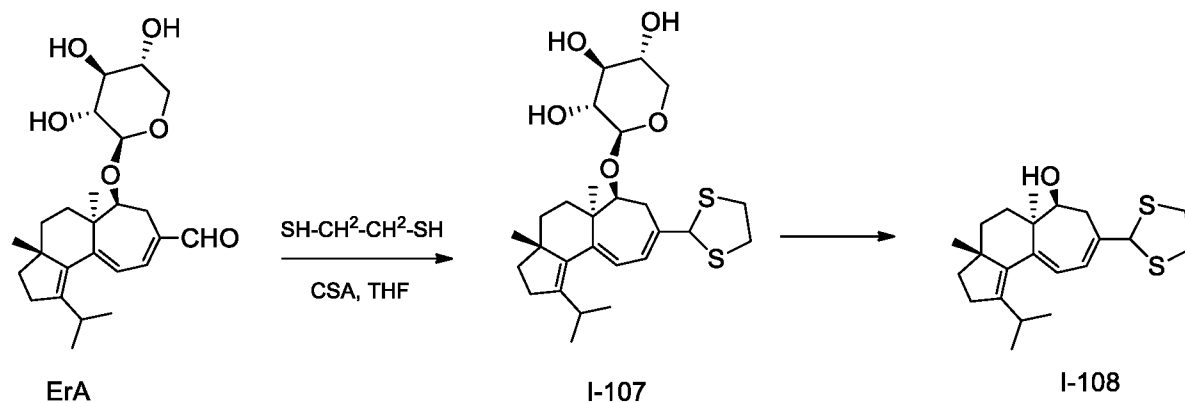
[00214] Stepwise (indirect) reductive amination of ErA with $\text{NH}_2\text{-R}^2$ compounds can be



employed to obtain compound d, in which $\text{X}^1\text{-R}^1$ is , X^2 is CH_2NH and R^2 is as defined for compounds of Formula I. Exemplary ErA analogues I-32 and I-33 and I-34, I-36 to I-40 were obtained using this synthesis (Scheme 5). Analogs I-34 and I-36 to I-40 were particularly aimed at improving the solubility of the parent compound. Based on these and other studies the reactions with diamines were chosen [K. Koteva, 2023]. Briefly, to a solution of ErA (1eq) in dimethylformamide (DMF) was added a solution of the corresponding amine (3eq) in methanol and the reaction was carried out for 2 h at 70°C with stirring. Then NaCNBH_3 (3eq) was added to each reaction and was heated at 70°C for 4 more hours and then stirred overnight. The MeOH was removed under nitrogen and each reaction was then purified using C18 flash chromatography. The Boc protecting group of analog I-38 was removed in 20% trifluoroacetic acid (TFA) in DCM, for 30 min at room temperature to afford analog I-39. Analog I-107 was obtained wherein the aldehyde of ErA was protected with ethanedithiol to form thioacetal in the presence of catalytic amount of camphor sulfonic acid (CSA) [R. N. Yadav, 2018]. I-108 was further synthesised upon sugar hydrolysis of I-107 (Scheme 6).

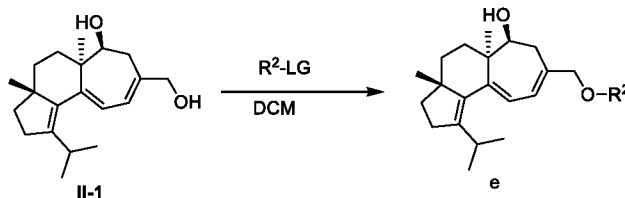


Scheme 5



Scheme 6.

[00215] Scheme 7 below demonstrates esterification or etherification of compound II-1 with a suitable R²-LG (LG is a leaving group) compound to prepare compound e in which X¹-R¹ is OH, X² is CH₂-O and R² is as defined for compounds of Formula I (such as exemplary compounds I-112 and I-69).



Scheme 7

Synthesis of the compounds of the present application.

Erinacine A Aglycon

[00216] To a round bottom flask containing Erinacine A (1.0 equiv.) was added toluene (0.022M) followed by CSA (5.5 equiv.) and the reaction mixture was heated to 70 °C for 1.5 hours. Toluene was removed and the crude mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried with Na₂SO₄, filtered and concentrated. The crude was purified by flash column chromatography providing aglycon erinacine A as a waxy yellow oil (78%). ¹H NMR (400 MHz, CDCl₃) δ 9.38 (s, 1H), 6.75 (dd, *J* = 8.2, 2.6 Hz, 1H), 5.87 (d, *J* = 8.2 Hz, 1H), 3.65 (ddd, *J* = 10.0, 5.7, 1.7 Hz, 1H), 3.10 (dd, *J* = 18.3, 5.8 Hz, 1H), 2.76 (sept, *J* = 6.9 Hz, 1H), 2.82-2.71 (m, 2H), 2.37-2.33 (m, 2H), 1.69-1.57 (m, 4H),

1.54 (d, $J = 9.9$ Hz, 1H), 1.27 (dt, $J = 13.9, 3.7$ Hz, 1H), 0.98 (d, $J = 6.9$ Hz, 3H), 0.93-0.89 (m, 9H).

//-1

[00217] To a round bottom flask containing aglycon ErA (1.0 equiv.) under an argon gas atmosphere was added ether and the solution was cooled to -78°C . LAH (4.0 equiv.) was added and the reaction mixture was stirred for 30 minutes followed by 30 minutes at room temperature. The reaction mixture was cooled in an ice/water bath and diluted with ether. Water was slowly added followed by 15% NaOH and the reaction mixture was stirred for 15 minutes at room temperature. Na_2SO_4 was added and the reaction mixture was stirred for an additional 15 minutes. The solids were filtered, and the filtrate was concentrated under reduced pressure providing the primary alcohol as a clear oil (94%). ^1H NMR (700 MHz, CDCl_3) δ 6.04 (ddt, $J = 8.1, 2.8, 1.4$ Hz, 1H), 5.59 (d, $J = 8.0$ Hz, 1H), 4.20 – 4.05 (m, 2H), 3.59 (dd, $J = 10.1, 6.2$ Hz, 1H), 2.83 (hept, $J = 6.9$ Hz, 1H), 2.72 (br d, $J = 17.6$ Hz, 1H), 2.59 (dd, $J = 17.5, 6.0$ Hz, 1H), 2.46 (dt, $J = 13.9, 9.3$ Hz, 1H), 2.38 – 2.29 (m, 2H), 1.99 (d, $J = 11.4$ Hz, 1H), 1.70 (ddd, $J = 12.3, 7.4, 4.8$ Hz, 1H), 1.66 – 1.55 (m, 4H), 1.24 (dt, $J = 13.8, 3.5$ Hz, 1H), 1.00 (d, $J = 7.0$ Hz, 3H), 0.97 – 0.90 (m, 9H).

//-3

[00218] DMP (1.2 equiv.) was added to a solution of aglycon ErA (1.0 equiv.) in DCM (0.02M) at room temperature under an argon gas atmosphere. The reaction was quenched with saturated Na_2CO_3 and extracted with DCM. The crude material was purified by prep TLC providing the ketone as a clear oil (57%). ^1H NMR (700 MHz, CDCl_3) δ 9.49 (s, 1H), 6.93 (dd, $J = 6.3, 1.7$ Hz, 1H), 6.07 (d, $J = 6.3$ Hz, 1H), 3.84 (d, $J = 11.3$ Hz, 1H), 3.29 (d, $J = 11.3$ Hz, 1H), 2.88 (sept, $J = 6.9$ Hz, 1H), 2.43 (dd, $J = 9.3, 5.6$ Hz, 2H), 2.06 (td, $J = 13.1, 4.4$ Hz, 1H), 1.79 (dt, $J = 12.5, 5.6$ Hz, 1H), 1.75-1.63 (m, 3H), 1.50 (dt, $J = 13.6, 4.0$ Hz, 1H), 1.20 (s, 3H), 1.07 (d, $J = 6.9$ Hz, 3H), 1.03 (s, 3H), 1.01 (d, $J = 6.8$ Hz, 3H).

//-2

[00219] Aglycon ErA (1.0 equiv.) was dissolved in *t*-BuOH (0.02M) followed by the addition of 2-methyl-2-butene (118 equiv.). A freshly prepared solution of sodium chlorite (6.6 equiv.) in a 20% w/w aqueous NaH_2PO_4 (0.35g in 1.8 ml H_2O) was added to the aglycon erinacine A solution. The reaction was then diluted with water and extracted with ethyl acetate. The crude mixture was purified by prep TLC providing the carboxylic acid as a white solid (95%). ^1H NMR (700 MHz, CDCl_3) δ 7.34 (dd, $J = 8.6, 2.7$ Hz, 1H), 5.80 (d, $J = 8.5$ Hz, 1H), 3.67 (br d, $J = 5.6$ Hz, 1H), 3.22 (dd, $J = 18.4, 5.9$ Hz, 1H), 2.83 (sept, $J = 6.8$ Hz, 1H), 2.74 (br d, $J = 18.4$ Hz, 1H), 2.53-2.49 (m,

1H), 2.43-2.36 (m, 2H), 1.72 (ddd, $J = 12.4, 7.6, 4.6$, 1H), 1.67-1.61 (m, 3H), 1.32 (dt, $J = 13.9, 3.7$ Hz, 1H), 1.03 (d, $J = 6.9$ Hz, 3H), 0.98-0.95 (m, 9H).

I-8

[00220] To a round bottom flask was added the starting acid – II-2 (1.0 equiv.), ammonium chloride (3.0 equiv.), and HATU (1.5 equiv.) under an argon gas atmosphere. DMF was added followed by DIPEA (4.0 equiv.) and the reaction was stirred at room temperature for 30 minutes. The solution was then heated to 50 °C for two hours and diluted with water at room temperature. The aqueous phase was extracted with ether, and the combined organic phases were washed with saturated NaHCO₃, water, and brine. The crude material was purified by prep TLC providing the final product as a yellow solid (88%). ¹H NMR (700 MHz, CDCl₃) δ 6.87 (dd, $J = 8.4, 2.6$ Hz, 1H), 5.73 (d, $J = 8.4$ Hz, 1H), 5.66 (br s, 2H), 3.68 (d, $J = 5.8$ Hz, 1H), 3.04 (dd, $J = 17.8, 6.0$ Hz, 1H), 2.85-2.78 (m, 2H), 2.47 (dt, $J = 13.9, 9.2$ Hz, 1H), 2.39-2.37 (m, 2H), 1.93 (br s, 1H), 1.72 (ddd, $J = 12.3, 7.4, 4.7$ Hz, 1H), 1.65-1.60 (m, 3H), 1.31 (dt, $J = 14.0, 3.7$ Hz, 1H), 1.01 (d, $J = 6.9$ Hz, 3H), 0.97-0.94 (m, 9H).

I-109

[00221] To a round bottom flask under an argon gas atmosphere was added the starting amide – I-8 (1.0 equiv.) and THF (0.1M). The solution was cooled in an ice/water bath followed by the addition of LAH (6.0 equiv.). The reaction mixture was stirred in the ice/water bath for five minutes then heated to 45 °C for 20 hours. The reaction mixture was cooled in an ice/water bath followed by the slow addition of water and 1M NaOH. The mixture was stirred for 15 minutes followed by the addition of MgSO₄. The reaction mixture was filtered and washed with ether. The filtrate was poured into a separatory funnel and washed with 5% HCl (5x). The combined aqueous layers were basified using saturated NaHCO₃ and extracted with ether. The combined organic layers were dried with Na₂SO₄, filtered and concentrated under reduced pressure to provide the primary amine as a white solid (34%). Product is highly unstable. ¹H NMR (700 MHz, CDCl₃) δ 5.94 (d, $J = 7.9$ Hz, 1H), 5.58 (d, $J = 7.9$ Hz, 1H), 3.57 (dd, $J = 6.1, 1.4$ Hz, 1H), 3.29 (AB_q, $J = 15.9$ Hz, 2H), 2.84 (sept, $J = 6.8$ Hz, 1H), 2.73 (br d, $J = 17.5$ Hz, 1H), 2.53 (dd, $J = 17.3, 6.0$ Hz, 1H), 2.48-2.45 (m, 1H), 2.36-2.32 (m, 2H), 1.69-1.67 (m, 6H), 1.63-1.60 (m, 1H), 1.23-1.19 (m, 1H), 1.00 (d, $J = 6.9$ Hz, 3H), 0.95-0.93 (m, 9H).

General Procedure I-A:

[00222] To a round bottom flask under an argon gas atmosphere was added the starting amine – I-109 (1.0 equiv.), carboxylic acid (1.1 equiv.), and HATU (1.5 equiv.). DMF was added

(0.1M) followed by DIPEA (2.0 equiv.). The reaction was stirred at room temperature and once complete, the solution was diluted with water and extracted with ether. The combined organic layers were washed with saturated NaHCO₃, water, and brine, dried with Na₂SO₄, filtered and concentrated under reduced pressure. The crude material was purified by prep TLC.

I-52

[00223] Following general procedure I-A, compound **I-52** was obtained as a beige solid (29%). ¹H NMR (700 MHz, CDCl₃) δ 7.81-7.80 (m, 2H), 7.52-7.49 (m, 1H), 7.45-7.43 (m, 2H), 6.36 (br s, 1H), 6.01 (br d, *J* = 7.6 Hz, 1H), 5.56 (d, *J* = 8.0 Hz, 1H), 4.17 (dd, *J* = 14.9, 6.2 Hz, 1H), 4.04 (dd, *J* = 14.9, 5.0 Hz, 1H), 3.61 (dd, *J* = 6.3, 1.3 Hz, 1H), 2.83 (sept, *J* = 6.9 Hz, 1H), 2.76 (br d, *J* = 17.3 Hz, 1H), 2.61 (dd, *J* = 17.2, 6.3 Hz, 1H), 2.44-2.40 (m, 1H), 2.35-2.33 (m, 2H), 1.71 (ddd, *J* = 12.2, 6.9, 4.9 Hz, 1H), 1.64-1.58 (m, 4H), 1.28-1.26 (m, 1H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.95-0.94 (m, 9H).

I-53

[00224] Following general procedure I-A, compound **I-53** was obtained as a beige solid (25%). ¹H NMR (700 MHz, CDCl₃) δ 5.90 (br d, *J* = 7.7 Hz, 1H), 5.53 (d, *J* = 8.0 Hz, 1H), 5.50 (br s, 1H), 3.95 (dd, *J* = 15.0, 6.3 Hz, 1H), 3.83 (dd, *J* = 15.0, 5.2 Hz, 1H), 3.56 (d, *J* = 6.1 Hz, 1H), 3.03 (p, *J* = 8.6 Hz, 1H), 2.81 (sept, *J* = 6.8 Hz, 1H), 2.68 (br d, *J* = 17.4 Hz, 1H), 2.51 (dd, *J* = 17.4, 6.2 Hz, 1H), 2.42 (dt, *J* = 13.8, 9.3 Hz, 1H), 2.35-2.28 (m, 4H), 2.19-2.14 (m, 2H), 2.00-1.93 (m, 2H), 1.90-1.85 (m, 1H), 1.71-1.68 (m, 1H), 1.62-1.57 (m, 3H), 1.26-1.21 (m, 1H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.94-0.92 (m, 9H).

General procedure I-B:

[00225] To a round bottom flask containing the starting carboxylic acid – II-2 (1.0 equiv.) under an argon gas atmosphere was added DMF (0.1M). To the solution was added amine (1.2 equiv.) followed by HATU (1.2 equiv.) and DIPEA (2.0 equiv.) and the reaction was warmed to 50 °C. Upon completion of the reaction, the crude material was purified directly by prep TLC providing the amide product. The reaction was diluted with water at room temperature. The aqueous phase was extracted with ether, and the combined organic phases were washed with saturated NaHCO₃, water, and brine. The crude material was purified by C-18 flash chromatography. 10→100% acetonitrile:water.

I-2

[00226] Following general procedure I-B, compound **I-2** was obtained as a white solid (58%). ¹H NMR (700 MHz, CDCl₃) δ 7.36-7.28 (m, 5H), 6.80 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.06 (br t, *J* = 5.7 Hz, 1H), 5.70 (d, *J* = 8.4 Hz, 1H), 4.52 (d, *J* = 5.6 Hz, 2H), 3.67 (dd, *J* = 10.2, 5.9 Hz, 1H), 3.04 (dd, *J* = 17.8, 6.0 Hz, 1H), 2.83-2.78 (m, 2H), 2.47 (dt, *J* = 13.9, 9.3 Hz, 1H), 2.39-2.36 (m, 2H), 1.91 (d, *J* = 10.6 Hz, 1H), 1.71 (ddd, *J* = 12.4, 7.5, 4.6 Hz, 1H), 1.64-1.59 (m, 3H), 1.29 (dt, *J* = 13.9, 3.6 Hz, 1H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.96-0.93 (m, 9H).

I-3

[00227] Following general procedure I-B, compound **I-3** was obtained as a yellow solid (20%). ¹H NMR (700 MHz, CDCl₃) δ 7.54 (d, *J* = 7.9 Hz, 2H), 7.34 (t, *J* = 7.9 Hz, 2H), 7.11 (t, *J* = 7.4 Hz, 1H), 6.86 (dd, *J* = 8.3, 2.6 Hz, 1H), 5.76 (d, *J* = 8.3 Hz, 1H), 3.74 (d, *J* = 6.0 Hz, 1H), 3.14 (dd, *J* = 17.8, 6.0 Hz, 1H), 2.89-2.83 (m, 2H), 2.51-2.46 (m, 1H), 2.41-2.38 (m, 2H), 2.22 (t, *J* = 7.6 Hz, 1H), 1.73 (ddd, *J* = 12.3, 7.4, 4.7 Hz, 1H), 1.67-1.62 (m, 4H), 1.34 (dt, *J* = 13.5, 3.3 Hz, 1H), 1.03 (d, *J* = 6.9 Hz, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.96 (d, *J* = 6.8 Hz, 3H).

I-4

[00228] Following general procedure I-B, compound **I-4** was obtained as a white solid (65%). ¹H NMR (700 MHz, CDCl₃) δ 6.83 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.28 (br t, *J* = 5.9 Hz, 1H), 5.72 (d, *J* = 8.4 Hz, 1H), 3.78 (t, *J* = 4.9 Hz, 2H), 3.72 (sept, *J* = 6.7 Hz, 1H), 3.68-3.66 (m, 1H), 3.51 (q, *J* = 5.2 Hz, 2H), 3.02 (dd, *J* = 17.7, 6.0 Hz, 1H), 2.84-2.78 (m, 2H), 2.47 (dt, *J* = 14.1, 9.4 Hz, 1H), 2.41-2.34 (m, 2H), 1.72 (ddd, *J* = 12.3, 7.5, 4.6 Hz, 1H), 1.66-1.60 (m, 4H), 1.30 (dt, *J* = 15.2, 4.4 Hz, 1H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.98-0.94 (m, 9H).

I-5

[00229] Following general procedure I-B, compound **I-5** was obtained as a white solid (66%). ¹H NMR (700 MHz, CDCl₃) δ 6.74 (dd, *J* = 8.4, 2.6 Hz, 1H), 5.91 (br s, 1H), 5.70 (d, *J* = 8.3 Hz, 1H), 3.65 (dd, *J* = 10.3, 5.8 Hz, 1H), 2.98 (dd, *J* = 17.7, 6.0 Hz, 1H), 2.82-2.75 (m, 2H), 2.46 (dt, *J* = 13.9, 9.3 Hz, 1H), 2.40-2.34 (m, 2H), 1.88 (d, *J* = 10.6 Hz, 1H), 1.71 (ddd, *J* = 12.4, 7.5, 4.6 Hz, 1H), 1.64-1.59 (m, 4H), 1.29 (dt, *J* = 14.0, 3.5 Hz, 1H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.96-0.93 (m, 9H), 0.82-0.80 (m, 2H), 0.54-0.52 (m, 2H).

I-1

[00230] Following general procedure I-B, compound **I-1** was obtained as a yellow solid (44%). ¹H NMR (700 MHz, *d*⁴-MeOH) δ 6.68 (dd, *J* = 8.0, 1.8 Hz, 1H), 5.70 (d, *J* = 8.0 Hz, 1H), 3.67 (br d, *J* = 6.0 Hz, 1H), 3.34-3.32 (m, 2H), 2.93-2.86 (m, 2H), 2.79-2.73 (m, 3H), 2.60 (s, 6H),

2.41 (dd, $J = 9.3, 5.7$ Hz, 2H), 2.35 (td, $J = 13.7, 4.8$ Hz, 1H), 1.89-1.84 (m, 2H), 1.78-1.74 (m, 1H), 1.73-1.65 (m, 3H), 1.40-1.37 (m, 1H), 1.04 (d, $J = 7.0$ Hz, 3H), 1.01 (s, 3H), 1.00 (s, 3H), 0.99 (d, $J = 6.8$ Hz, 3H).

I-6

[00231] Following general procedure I-B, compound I-6 was obtained as a clear oil (77%). ^1H NMR (700 MHz, CDCl_3) δ 6.06 (dd, $J = 8.1, 2.6$ Hz, 1H), 5.63 (d, $J = 8.0$ Hz, 1H), 3.67 (br s, 1H), 3.04 (br s, 6H), 2.87 (br d, $J = 17.7$ Hz, 1H), 2.83 (sept, $J = 6.9$ Hz, 1H), 2.74 (dd, $J = 17.8, 6.0$ Hz, 1H), 2.46 (dt, $J = 13.9, 9.4$ Hz, 1H), 2.37-2.32 (m, 2H), 2.22-2.20 (m, 1H), 1.71 (ddd, $J = 12.4, 7.0, 4.9$ Hz, 1H), 1.64-1.59 (m, 3H), 1.30 (dt, $J = 13.9, 3.3$ Hz, 1H), 0.99 (d, $J = 6.9$ Hz, 3H), 0.96-0.93 (m, 9H).

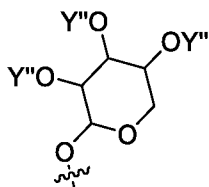
I-7

[00232] Following general procedure I-B, compound I-7 was obtained as a white solid (73%). ^1H NMR (700 MHz, CDCl_3) δ 8.49 (d, $J = 6.0$ Hz, 2H), 7.81 (s, 1H), 7.53-7.52 (m, 2H), 6.87 (dd, $J = 8.3, 2.6$ Hz, 1H), 5.77 (d, $J = 8.3$ Hz, 1H), 3.75 (dd, $J = 6.3, 1.5$ Hz, 1H), 3.71 (sept, $J = 6.7$ Hz, 1H), 3.13 (dd, $J = 17.6, 6.2$ Hz, 1H), 2.87-2.81 (m, 2H), 2.49-2.54 (m, 1H), 2.41-2.36 (m, 2H), 1.74 (ddd, $J = 12.3, 7.3, 4.8$ Hz, 1H), 1.68-1.62 (m, 3H), 1.35 (dt, $J = 13.8, 3.6$ Hz, 1H), 1.03 (d, $J = 6.9$ Hz, 3H), 1.00 (s, 3H), 0.98 (s, 3H), 0.96 (d, $J = 6.8$ Hz, 3H).

General Procedure II

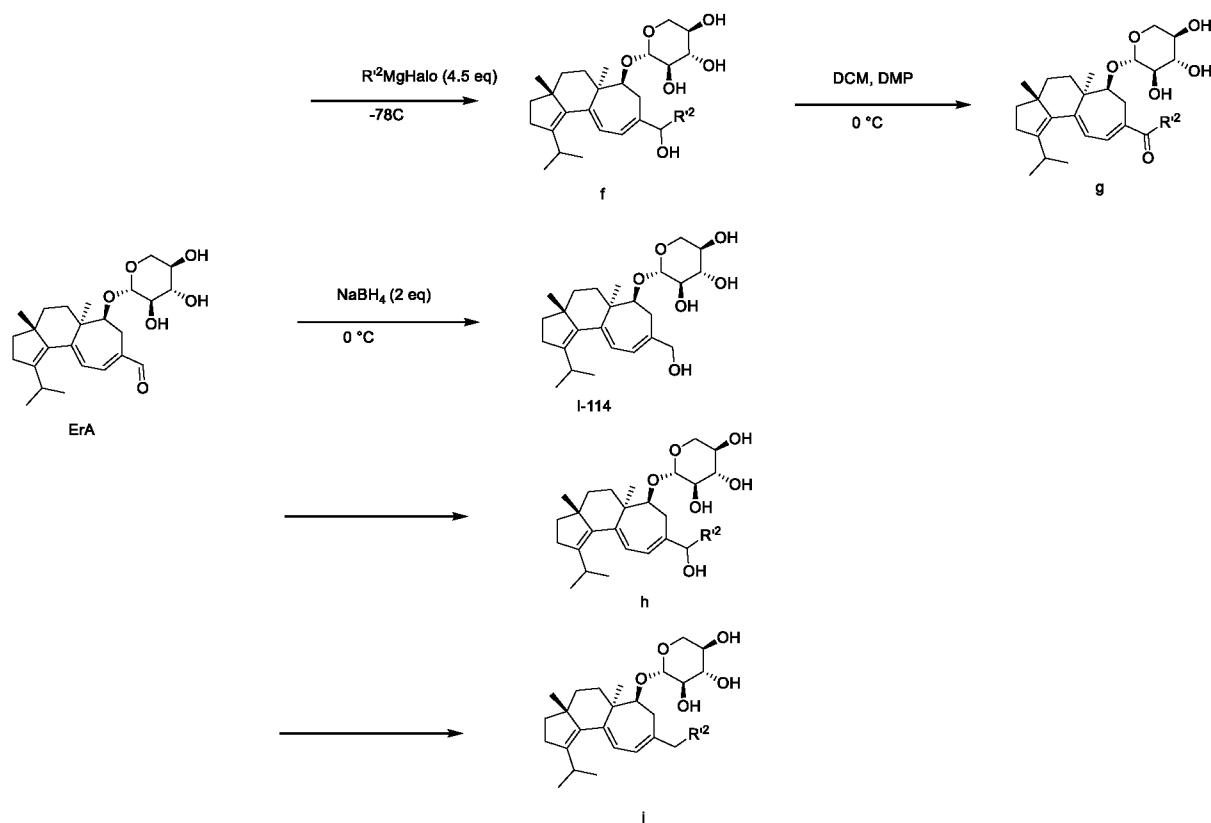
[00233] Exemplary ErA analogues of the present application were synthesized as shown in Schemes 8-12 below.

[00234] Scheme 8 below shows manipulation of the aldehyde group of the ErA to form



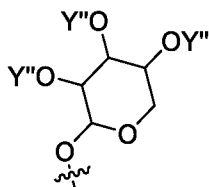
compounds in which $X^1\text{-R}^1$ is . Reduction of the aldehyde with Grignard reagent $\text{R}^{2'}\text{MgHalo}$ (Halo is halogen) produces secondary alcohol compounds of formula **f** (exemplary compounds I-115 to I-117, I-119 to I-121, 152 and 153), which can be further oxidized to produce ketone compounds of formula **g** (exemplary compounds I-132 to I-134); compound I-114 was produced by reacting ErA with NaBH_4 ; nucleophilic addition reaction with suitable $\text{R}^{2'}$ -containing reagents produces secondary alcohol compounds of formula **h** (exemplary compound I-140); and nucleophilic addition reaction with suitable $\text{R}^{2'}$ -containing reagents followed by acid treatment produces exemplary compounds of formula **i**, (exemplary compounds I-137 and I-138). $\text{R}^{2'}$

represents a part of R^2 substituent or R^2 substituent wherein R^2 is as defined for compounds of Formula I.

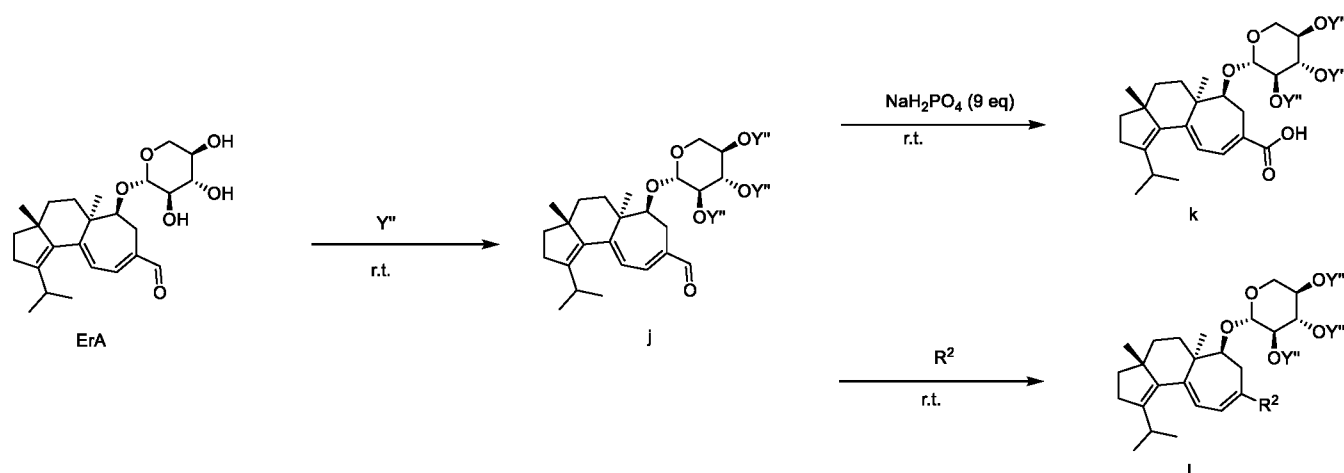


Scheme 8

[00235] Scheme 9 below shows the formation of exemplary compounds j to l, in which X^1 -

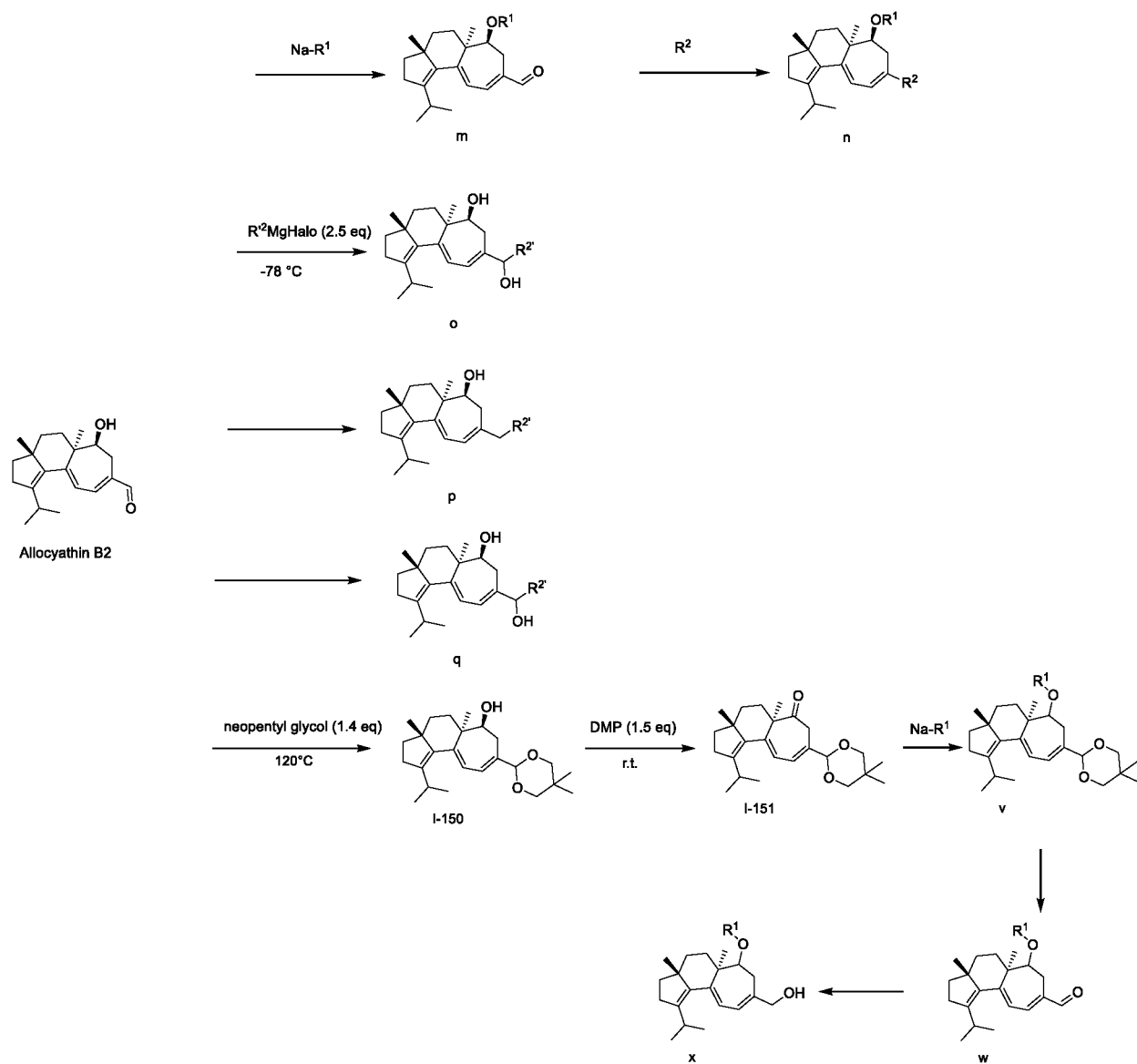


R^1 is and Y'' is a protecting group. Compound j is formed by reaction with the suitable reagent to introduce Y'' (exemplary compounds I-142, I-147 and I-148), which can be further oxidized to form compound k (exemplary compound I-128) or can undergo substitution reaction with a suitable R^2 -containing reagent to produce compounds of formula I, in which X^2 is a bond and R^2 is as defined for compounds of Formula I (exemplary compound I-143).



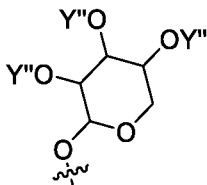
Scheme 9

[00236] Scheme 10 below shows manipulations of allocyathin B2. Williamson ether synthesis with Na- R^1 group, in which R^1 is as defined for compounds of Formula I produces compound **m** (exemplary compounds I-129, I-130), which can then undergo substitution reaction of the aldehyde group with a suitable R^2 -containing reagent to produce compound **n**, in which X^1 is a bond and R^2 is as defined for compounds of Formula I (exemplary compounds I-144, I-145). Reduction of the aldehyde with Grignard reagent $R^{2'}MgHalo$ (Halo is halogen) produces secondary alcohol compounds **o**, in which $R^{2'}$ is a part of the R^2 substituent as defined for compounds of Formula I (exemplary compound I-118); substitution reaction with a suitable $R^{2'}$ -containing reagent produces compound **p**, in which $R^{2'}$ is a part of the R^2 substituent as defined for compounds of Formula I (exemplary compounds I-136, I-49, I-149 and I-97); nucleophilic addition reaction with a suitable $R^{2'}$ -containing reagent produces compound **q**, in which $R^{2'}$ is a part of the R^2 substituent as defined for compounds of Formula I (exemplary compound I-145); and reaction of allocyathin B2 with neopentyl glycol produces compound I-150, which can be further oxidized to produce compound I-151. Williamson ether synthesis with Na- R^1 group with the compound I-151, in which R^1 is as defined for compounds of Formula I produces compound **v** (exemplary compound I-157). Compound **v** can further be treated with acid, such as HCl to obtain compound **w**, in which R^1 is as defined for compounds of Formula I (exemplary compound I-158), which can further react with $NaBH_4$ to obtain compound **x**, in which R^1 is as defined for compounds of Formula I (exemplary compound I-130).



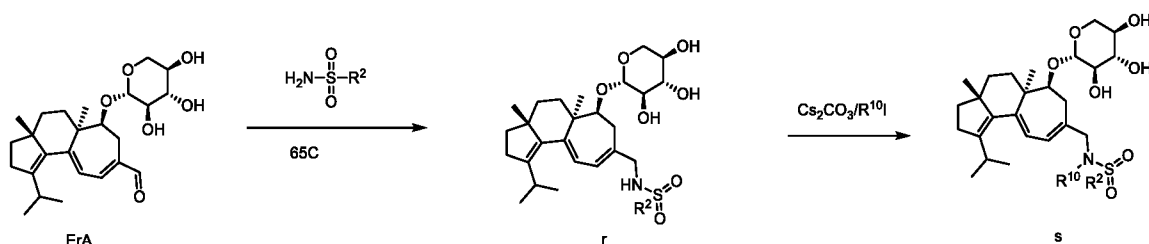
Scheme 10

[00237] Scheme 11 below shows the reaction of the ErA with a sulfonamide group to



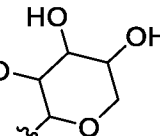
produce compound r, in which X¹-R¹ is and R² is as defined for compounds of Formula I (exemplary compounds I-122 to I-126, I-135, I-139, I-141 and I-146). Compound r with

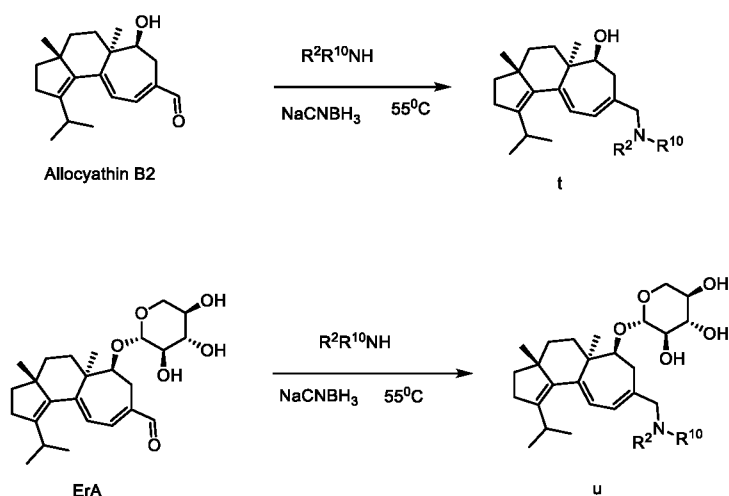
cesium carbonate and R^{10} -iodide can undergo amine alkylation reaction to obtain compound **s**, in which R^2 is as defined for compounds of Formula I (exemplary compound I-156).



Scheme 11

[00238] Reductive amination reactions are shown on Scheme 12 below, in which Allocyathin B2 or ErA reacted with $R^{10}R^2NH$ to produce compounds **t** and **u** respectively, which

represent exemplary compounds, in which X^1 is O, R^1 is H or ; X^2 is CH_2NR^{10} , and R^2 and R^{10} are as defined for compound of Formula I (exemplary compounds I-163, I-162, I-161, I-160, I-159).



Scheme 12

General procedure II-A (Addition of Grignard)

[00239] To a solution of Erinacine A (20.0 mg, 1.0 Eq, 46.2 μ mol) in dry tetrahydrofuran (THF) (2.20 mL) cooled to -78°C under a dry and inert atmosphere (argon) was added the

Grignard reagent (4.5 Eq) in THF. The reaction mixture was stirred at -78 °C for 20 min and was allowed to slowly warm up to r.t. for 12 hours. EtOAc (8 mL) was added, followed by sat. aq. NaHCO₃ sol. (8 mL), and the layers were separated. The aqueous phase was extracted with EtOAc (3x 5 mL). The organic layers were combined, dried over MgSO₄, filtered through a pad of silica and concentrated under reduced pressure. The material was pre-purified by flash column chromatography (silica gel), using a gradient of 0-10% MeOH in EtOAc. The obtained material was then purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (Ammonium Bicarbonate 10 mM).

General procedure II-B (Reductive Amination mediated by Ti(OEt)₄)

[00240] To a solution of Erinacine A (20.0 mg, 1.0 Eq, 46.2 µmol) in dry THF (1.00 mL) at r.t., under a dry and argon atmosphere, was added the corresponding amine or sulfonamide (1.1 Eq) followed by titaniummethoxide (19.2 µL, 2.0 Eq, 92.5 µmol). The reaction mixture was stirred at 70 °C for 12 hours. *i*PrOH (1.50 mL) was added to the reaction mixture cooled at r.t., followed by the addition of sodium tetrahydroborate (5.25 mg, 4.91 µL, 3.0 Eq, 139 µmol). The reaction mixture was allowed to stir at r.t. for 2 hours and then was concentrated under reduced pressure. The obtained residue was diluted in EtOAc (6 mL), followed by the addition of sat. aq. NaHCO₃ sol. (6 mL). pH was adjusted to 9 with a 1M NaOH sol. The layers were separated, and the aqueous layer was extracted with EtOAc (2x 2 mL). The organic layers were combined, dried over MgSO₄, filtered through a silica pad and concentrated under reduced pressure. The material was purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (Ammonium Bicarbonate 10 mM).

General procedure II-C (Silver-triflate mediated O-alkylation)

[00241] To a solution of Allocyathin B2 (40.0 mg, 1.0 Eq, 133 µmol) and 2,6-di-tert-butylpyridine (104 µL, 3.5 Eq, 466 µmol) in anhydrous 1,2 dichloroethane (DCE) (3.00 mL) cooled to 0 °C, under a dry and inert atmosphere (in the dark), was added silver(I) trifluoromethanesulfonate (103 mg, 3.0 Eq, 136 µmol) in one portion. The reaction mixture was allowed to stir at 0 °C for 30 min. Then was added the halogen-alkane reagent (1.0 Eq) at 0 °C. The reaction mixture was slowly warmed up to rt and stirred for 12 hours. The reaction mixture was quenched by adding dichloromethane (DCM) (2 mL), followed by sat. aq. NaHCO₃ sol. (1 mL). The mixture was dried over MgSO₄ and then filtered through a silica pad, concentrated under reduced pressure. The resulting material was purified by column chromatography on silica gel using a gradient of 0-15% EtOAc in Hexane.

General Procedure II-D – Reductive Amination mediated by NaCNBH₃

[00242] Chosen aldehyde (1 eq) and amine (3 eq) were charged into a sealed oven-dried reaction vial. Methanol (0.2M) was added to the vial followed by one drop of acetic acid. The vial was sealed and the mixture heated and stirred at 55 °C for 2 h. At this point, NaCNBH₃ (3 eq) was added in one portion to the reaction mixture and the vial re-sealed and heated at 55 °C until TLC indicated complete consumption of aldehyde. The reaction mixture was then cooled to rt and a few drops of saturated aqueous NaHCO₃ was added to quench remaining NaCNBH₃. The crude mixture was concentrated *in vacuo* and added directly to C-18 flash column for purification. 10→100% acetonitrile:water.

*Synthesis of exemplary compounds of the present application**I-114*

[00243] To a solution of Erinacine A (20.0 mg, 1.0 Eq, 46.2 µmol) in iPrOH (2.30 mL) cooled to 0 °C was added NaBH₄ (3.50 mg, 2.0 Eq, 92.5 µmol). The reaction mixture was stirred at 0 °C for 20 min and at rt for 30 min. The reaction mixture was concentrated under reduced pressure and then diluted with EtOAc (4 mL). Sat. aq. NaHCO₃ (1 mL) was added, and the layers were separated. The aqueous layer was extracted with EtOAc (2x 2 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The material was purified by column chromatography on silica gel using an isocratic gradient of EtOAc to afford compound **I-114** (20.0 mg, 46.0 µmol, 99.5 %) as a white foam. LCMS: rt= 1.57 min, ESI (+) m/z= 285.3 [M-Sugar-H₂O+H], allocyathin B2 fragment - OH. ¹H NMR (Acetone-*d*₆, 400 MHz): δ 5.86 (1H, d, *J* = 7.5 Hz), 5.51 (1H, d, *J* = 7.5 Hz), 4.46 (1H, d, *J* = 5.9 Hz), 4.21 (1H, t, *J* = 5.8 Hz), 4.04 (2H, d, *J* = 6.1 Hz), 3.70-3.91 (2H, m), 3.70 (1H, t, *J* = 5.7 Hz), 3.45-3.53 (2H, m), 3.25-3.38 (3H, m), 2.83-2.88 (1H, m), 2.62-2.68 (1H, m), 2.56 (1H, d, *J* = 16.3 Hz), 2.34 (2H, t, *J* = 7.5 Hz), 2.16-2.23 (1H, m), 1.46-1.73 (4H, m), 1.27-1.30 (1H, m), 0.94-1.02 (12H, m).

I-115

[00244] Following general procedure II-A, compound **I-115** was obtained as a white foam (20.0 mg, 44.6 µmol, 96.4 %) – as mixture of two diastereoisomers from erinacine A and methyl magnesium bromide. LCMS: rt= 1.63 + 1.65 min, ESI (+) m/z= 299.3 [M-Sugar]; 431.4 [M-H₂O+H]. ¹H NMR (Acetone-*d*₆, 400 MHz): δH 5.86 (1H, d, *J* = 7.3 Hz), 5.77 (1H, d, *J* = 7.2 Hz), 5.48 (2H, t, *J* = 8.5 Hz), 4.38 (2H, t, *J* = 8.2 Hz), 4.14-4.19 (4H, m), 4.04 (1H, s), 3.85-3.88 (2H, m), 3.58 (1H, d, *J* = 4.2 Hz), 3.55-3.47 (4H, m), 3.36-3.31 (2H, m), 3.29-3.20 (4H, m), 2.91 – 2.84

(2H, m), 2.77 (4H, s), 2.67 – 2.61 (4H, m), 2.33 (4H, t, $J = 7.7$ Hz), 1.78 – 1.68 (m, 2H), 1.67 – 1.53 (m, 9H), 1.25 (dd, $J = 6.4, 3.3$ Hz, 8H), 1.14 – 1.07 (m, 12H), 1.03 – 0.93 (m, 18H).

I-116

[00245] Following general procedure II-A, compound **I-116** was obtained as a white foam (20.0 mg, 42.0 μ mol, 90.8 %) - mixture of 2 diastereoisomers from erinacine A and isopropylmagnesium bromide. LCMS: $rt = 1.82$ min, ESI (+) $m/z = 499.4$ [M+Na], 459.5 [M-H₂O+H]. ¹H NMR (Acetone-*d*₆, 400 MHz): δ 5.79 (1H, d, $J = 7.1$ Hz), 5.73 (1H, d, $J = 7.4$ Hz), 5.48 (2H, dd, $J = 11.0, 7.3$ Hz), 4.34 (2H, d, $J = 7.1$ Hz), 4.11-4.18 (4H, m), 4.03-4.07 (2H, m), 3.85 (2H, dt, $J = 11.7, 5.8$ Hz), 3.69 (1H, t, $J = 5.8$ Hz), 3.45-3.57 (7H, m), 3.29-3.35 (3H, m), 3.17-3.27 (5H, m), 2.83-2.88 (2H, m), 2.77 (6H, s), 2.58-2.64 (4H, m), 2.31-2.35 (5H, m), 1.84-1.93 (3H, m), 1.54-1.73 (14H, m), 1.13-1.20 (4H, m), 1.08-1.11 (16H, m), 0.95-1.00 (28H, m), 0.81-0.93 (18H, m).

I-117

[00246] Following general procedure II-A, compound **I-117** was obtained as a white foam (23.0 mg, 44 μ mol, 95 %, 98% Purity) - mixture of two diastereomers from erinacine A and phenylmagnesium bromide. LCMS: $rt = 1.83$ and 1.86 min, ESI (+) $m/z = 493.5$ [M-H₂O+H]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.41 (dd, $J = 7.5, 4.7$ Hz, 4H), 7.33 (t, $J = 7.5$ Hz, 4H), 7.23 (dd, $J = 8.4, 6.4$ Hz, 2H), 6.05 (dd, $J = 14.7, 7.3$ Hz, 2H), 5.56 (t, $J = 6.8$ Hz, 2H), 5.19 (t, $J = 5.4$ Hz, 2H), 4.29 (d, $J = 3.8$ Hz, 1H), 4.26 (d, $J = 4.9$ Hz, 1H), 4.21 (d, $J = 6.7$ Hz, 1H), 4.15 (t, $J = 4.4$ Hz, 2H), 4.11 (d, $J = 5.0$ Hz, 1H), 4.03 (dd, $J = 4.8, 1.6$ Hz, 1H), 3.96 (d, $J = 5.1$ Hz, 0H), 3.83 (dd, $J = 11.6, 4.8$ Hz, 1H), 3.73 (dd, $J = 11.6, 4.7$ Hz, 1H), 3.51 (d, $J = 7.8$ Hz, 2H), 3.50 – 3.35 (m, 4H), 3.36 – 3.25 (m, 2H), 3.22 (q, $J = 7.8, 6.7$ Hz, 2H), 3.13 (ddd, $J = 16.4, 11.6, 9.0$ Hz, 2H), 2.89 (h, $J = 6.8$ Hz, 2H), 1.77 – 1.66 (m, 2H), 1.68 – 1.51 (m, 8H), 1.11 (t, $J = 7.0$ Hz, 4H), 1.07 – 0.93 (m, 24H).

I-140

[00247] To a solution of erinacine A (30.0 mg, 1 eq, 69.4 μ mol) and trimethyl(trifluoromethyl)silane (24.7 mg, 2.5 Eq, 173 μ mol) in dry THF (3.50 mL) cooled to 0 °C, was added Cesium fluoride (26.3 mg, 6.34 μ L, 2.5 Eq, 173 μ mol). The reaction mixture was allowed to stir at 0 °C for 20 min and then to warm up to rt and stirred for 12 hours. Full completion of the reaction was observed by TLC and LCMS. EtOAc (6 mL) was added followed by sat. aq. NaHCO₃ sol. (6 mL), and the layers were separated. The aqueous layer was extracted with EtOAc (2x 5 mL). The organic layers were combined, washed with brine (10 mL), dried over MgSO₄,

filtered and concentrated under reduced pressure. The material was purified by column chromatography on silica gel using a gradient of 0-100% EtOAc in hexane to afford 4 mg of the desired compound. This compound was the silylated intermediate. To a solution of this material in THF (3.50 mL) cooled to 0 °C was added tetrabutylammonium fluoride (27.2 mg, 29.8 μ L, 1.5 Eq, 104 μ mol) and the reaction was stirred at r.t. for 1 hour. At this point, the silylated intermediate was consumed according to LCMS and TLC. The reaction mixture was concentrated under reduced pressure. The residue was diluted in EtOAc (5 mL) and washed with sat. aq. NaHCO₃ sol. (5 mL) and then water, dried over MgSO₄, filtered and concentrated under reduced pressure. The material was purified by flash chromatography using a gradient of 50-100% EtOAc in hexane to afford I-140 (21.0 mg, 41.8 μ mol, 60.2 %) as a white solid – mixture of two diastereomers. LCMS: rt= 1.78, 1.80, ESI (-) m/z= 547.4 [M-H]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 6.17 (d, *J* = 7.3 Hz, 1H), 6.11 (d, *J* = 7.6 Hz, 1H), 5.55 (t, *J* = 7.7 Hz, 2H), 5.06 (d, *J* = 5.7 Hz, 1H), 4.97 (d, *J* = 4.2 Hz, 1H), 4.64 – 4.44 (m, 3H), 4.40 (d, *J* = 6.8 Hz, 1H), 4.20 (ddd, *J* = 20.1, 8.4, 4.9 Hz, 3H), 4.14 – 4.00 (m, 2H), 3.88 (ddd, *J* = 13.6, 11.5, 4.9 Hz, 2H), 3.65 (d, *J* = 7.6 Hz, 1H), 3.63 – 3.41 (m, 3H), 3.41 – 3.20 (m, 6H), 3.02 – 2.83 (m, 3H), 2.68 (dd, *J* = 16.2, 8.7 Hz, 2H), 2.50 – 2.32 (m, 4H), 1.80 – 1.55 (m, 9H), 1.46 (d, *J* = 13.9 Hz, 1H), 1.09 (d, *J* = 14.5 Hz, 6H), 1.04 – 0.91 (m, 18H). ¹⁹F NMR (376 MHz, Acetone-*d*₆) δ -77.2 (d, *J* = 7.7 Hz), -77.3 (d, *J* = 7.1 Hz).

I-118

[00248] A solution of allocyathin B2 (20.0 mg, 1 Eq, 66.6 μ mol) in THF (2.20 mL) was cooled to -78 °C under a dry and inert atmosphere (argon). Isopropylmagnesium bromide solution (24.5 mg, 23.5 μ L, 2.5 Eq, 166 μ mol) in 2-Methyl-THF was added to the reaction. The reaction mixture was stirred at -78 °C for 20 min and then was warming slowly to rt over 12 hours. EtOAc (5 mL) was added, followed by sat. aq. NaHCO₃ sol. (5 mL), and the layers were separated. The aqueous phase was extracted with EtOAc (3x 5 mL). The organic layers were combined, dried over MgSO₄, filtered through a pad of silica and concentrated under reduced pressure. The material was purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (AmB 10 mM) to afford I-118 (7.0 mg, 20.3 μ mol, 31 %) as a white foam (mixture of two diastereomers). LCMS: rt= 2.13 min, ESI (+) m/z= 327.5 [M-H₂O+H], ESI (-) m/z= 343.2 [M-H]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 5.92 – 5.81 (m, 1H), 5.55 (dd, *J* = 10.1, 7.8 Hz, 1H), 5.35 (d, *J* = 6.5 Hz, 2H), 3.77 (dt, *J* = 10.7, 3.8 Hz, 2H), 3.73 – 3.51 (m, 4H), 3.42 (ddd, *J* = 10.6, 8.6, 6.8 Hz, 2H), 3.21 (dd, *J* = 10.7, 8.4 Hz, 2H), 3.01 – 2.85 (m, 3H), 2.80 – 2.67 (m, 0H), 2.57 – 2.45 (m, 4H), 2.44 – 2.26 (m, 7H), 2.24 – 2.11 (m, 2H), 2.03

– 1.93 (m, 5H), 1.86 – 1.66 (m, 4H), 1.62 (ddt, J = 15.0, 12.8, 4.5 Hz, 8H), 1.42 – 1.25 (m, 2H), 1.09 – 0.74 (m, 48H).

I-119

[00249] Following general procedure II-A, compound **I-119** was obtained as a white foam (16.0 mg, 32.6 μ mol, 70.5 %) - mixture of 2 diastereomers from erinacine A and isobutylmagnesium bromide. LCMS: rt = 1.89 and 1.91 min, ESI (+) m/z = 473.5 [M-H₂O+H], 341.5 [M-sugar]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 5.81 (dd, J = 29.1, 7.3 Hz, 1H), 5.49 (t, J = 7.3 Hz, 1H), 4.37 (dd, J = 6.9, 4.6 Hz, 1H), 4.29 – 4.00 (m, 1H), 3.89 (ddd, J = 12.1, 7.4, 4.9 Hz, 1H), 3.65 – 3.40 (m, 3H), 3.34 (td, J = 8.3, 2.0 Hz, 1H), 3.30 – 3.18 (m, 2H), 2.95 – 2.83 (m, 1H), 2.78 (s, 3H), 2.72 – 2.51 (m, 2H), 2.34 (td, J = 7.2, 3.3 Hz, 2H), 1.92 – 1.56 (m, 4H), 1.56 – 1.43 (m, 1H), 1.37 (ddd, J = 13.4, 8.1, 4.7 Hz, 1H), 1.10 (d, J = 7.1 Hz, 3H), 1.05 – 0.84 (m, 15H).

I-120

[00250] Following general procedure II-A, compound **I-120** was obtained as a white foam (8.0 mg, 16.3 μ mol, 35%) - mixture of 2 diastereoisomers from erinacine A and tert-butylmagnesium bromide. LCMS: rt = 1.99 and 2.00 min, ESI (+) m/z = 473.5 [M-H₂O+H], 341.5 [M-sugar]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 5.81 (d, J = 7.5 Hz, 1H), 5.74 (d, J = 6.8 Hz, 1H), 5.49 (dd, J = 7.2, 4.8 Hz, 2H), 4.42 (d, J = 6.9 Hz, 1H), 4.37 (d, J = 7.1 Hz, 1H), 4.20 (s, 2H), 3.88 (td, J = 11.8, 5.0 Hz, 2H), 3.82 – 3.73 (m, 2H), 3.64 – 3.56 (m, 2H), 3.56 – 3.44 (m, 3H), 3.39 – 3.21 (m, 7H), 2.93 – 2.83 (m, 2H), 2.62 – 2.49 (m, 2H), 2.39 – 2.30 (m, 5H), 2.00 – 1.87 (m, 1H), 1.73 (tdd, J = 11.9, 7.6, 4.5 Hz, 4H), 1.66 – 1.54 (m, 6H), 1.14 (s, 3H), 1.07 (s, 3H), 1.03 – 0.94 (m, 18H), 0.90 (d, J = 4.1 Hz, 12H).

I-121

[00251] Following general procedure II-A, compound **I-121** was obtained as a white foam (6.00 mg, 11.6 μ mol, 25.1 %) - mixture of 2 diastereomers from erinacine A and phenylmagnesium bromide. LCMS: rt = 1.97 and 1.99 min, ESI (+) m/z = 499.4 [M-H₂O+H], 367.4 [M-sugar]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 5.72 (dd, J = 13.4, 7.4 Hz, 2H), 5.47 (t, J = 6.8 Hz, 1H), 4.36 (dd, J = 7.0, 2.3 Hz, 2H), 4.17 (s, 3H), 3.90 (ddd, J = 14.4, 11.4, 5.0 Hz, 2H), 3.66 (dd, J = 12.3, 7.4 Hz, 2H), 3.52 (tdd, J = 7.5, 4.6, 2.1 Hz, 4H), 3.35 (ddd, J = 16.9, 8.0, 5.2 Hz, 2H), 3.29 – 3.16 (m, 3H), 2.96 – 2.83 (m, 2H), 2.70 – 2.50 (m, 3H), 2.41 – 2.28 (m, 3H), 1.84 – 1.47 (m, 11H), 1.36 – 1.12 (m, 4H), 1.08 (d, J = 11.7 Hz, 3H), 1.03 – 0.94 (m, 11H).

I-127

[00252] Following general procedure II-C, compound **I-127** was obtained as a clear yellow oil (15 mg, 31.0 μ mol, 23 %) from allocyathin B2 and n-boc-piperidine. LCMS: r_t = 2.44, ESI (+) m/z = 484.5 [M+H], 506.4 [M+Na]. ^1H NMR: desired compound- pure. ^1H NMR (Acetone- d_6 , 400 MHz): δ 9.42 (1H, s), 6.81 (1H, s), 5.72-5.90 (3H, m), 4.93-5.07 (5H, m), 4.74 (4H, s), 4.46 (2H, t, J = 15.1 Hz), 3.57 (1H, d, J = 16.6 Hz), 3.32 (3H, t, J = 7.4 Hz), 3.21 (4H, s), 2.84-2.91 (1H, m), 2.24-2.43 (10H, m), 1.62-1.76 (5H, m), 1.27-1.37 (2H, m), 0.96-1.04 (12H, m).

I-146

[00253] Following general procedure II-B, compound **I-146** was obtained as a white powder (16 mg, 27.9 μ mol, 80%) from erinacine A and benzenesulfonamide. LCMS: r_t = 1.83, ESI (-) m/z = 572.4 [M-H]. ^1H NMR (400 MHz, Acetone- d_6) δ 7.95 – 7.83 (m, 2H), 7.62 (dd, J = 10.2, 6.9 Hz, 3H), 6.46 (s, 1H), 5.72 (d, J = 7.5 Hz, 1H), 5.39 (d, J = 7.5 Hz, 1H), 4.42 (d, J = 6.6 Hz, 1H), 4.19 (s, 2H), 3.99 (dd, J = 11.6, 4.7 Hz, 1H), 3.69 – 3.50 (m, 3H), 3.47 (d, J = 7.1 Hz, 1H), 3.42 – 3.31 (m, 1H), 3.31 – 3.22 (m, 2H), 2.77 (s, 2H), 2.59 – 2.40 (m, 2H), 2.34 (dd, J = 9.3, 5.9 Hz, 2H), 1.68 (dq, J = 12.5, 6.2 Hz, 1H), 1.64 – 1.52 (m, 2H), 1.41 (d, J = 13.7 Hz, 1H), 1.04 – 0.88 (m, 12H).

I-141

[00254] Following general procedure II-B, compound **I-141** was obtained as a white powder (14 mg, 24.8 μ mol, 71%) from erinacine A and trifluoromethanesulfonamide. LCMS: r_t = 1.65, ESI (+) m/z = 583.4 [M+H₂O], ESI (-) m/z = 564.4 [M-H]. ^1H NMR (400 MHz, Acetone- d_6) δ 5.94 (d, J = 7.6 Hz, 1H), 5.50 (d, J = 7.6 Hz, 1H), 4.46 (d, J = 6.9 Hz, 1H), 4.20 (s, 1H), 3.92 (q, J = 5.1, 4.0 Hz, 3H), 3.63 (dd, J = 5.7, 2.3 Hz, 1H), 3.51 (td, J = 8.6, 4.9 Hz, 1H), 3.43 – 3.21 (m, 3H), 2.87 (d, J = 6.9 Hz, 1H), 2.73 (d, J = 6.1 Hz, 2H), 2.45 (s, 1H), 2.36 (dd, J = 9.2, 5.9 Hz, 2H), 1.80 – 1.54 (m, 4H), 1.41 (d, J = 13.9 Hz, 1H), 1.09 – 0.92 (m, 12H). ^{19}F NMR (376 MHz, Acetone- d_6) δ -78.4 (s, 3F).

I-124

[00255] Following general procedure II-B, compound **I-124** was obtained as a white powder (7 mg, 12.9 μ mol, 37%) from erinacine A and dimethylaminesulfonamide. LCMS: r_t = 1.75, ESI (+) m/z = 558.4 [M+H₂O], ESI (-) m/z = 539.4 [M-H]. ^1H NMR (400 MHz, Acetone- d_6) δ 6.01 (s, 1H), 5.88 (d, J = 7.6 Hz, 1H), 5.49 (d, J = 7.5 Hz, 1H), 4.45 (d, J = 6.6 Hz, 1H), 4.18 (d, J = 6.2 Hz, 2H), 4.05 (s, 1H), 3.98 (dd, J = 11.5, 4.8 Hz, 1H), 3.68 (d, J = 5.2 Hz, 2H), 3.59 (d, J = 6.9 Hz, 1H), 3.52 (d, J = 4.6 Hz, 0H), 3.36 (t, J = 8.2 Hz, 1H), 3.28 (dd, J = 11.6, 8.9 Hz, 2H), 2.87 (q, J =

6.9 Hz, 1H), 2.78 (d, $J = 1.3$ Hz, 6H), 2.75 – 2.62 (m, 2H), 2.44 – 2.31 (m, 2H), 1.76 – 1.55 (m, 4H), 1.44 (d, $J = 13.7$ Hz, 1H), 1.04 (s, 3H), 1.00 (d, $J = 6.9$ Hz, 3H), 0.98 – 0.94 (m, 6H).

I-122

Following general procedure II-B, compound **I-122** was obtained as a white powder (17 mg, 31 μ mol, 53%) from erinacine A and tert-butylsulfonamide. LCMS: $r_t = 1.80$, ESI (+) $m/z = 571.4$ [M+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 5.89 (dd, $J = 7.5, 1.3$ Hz, 1H), 5.75 (t, $J = 5.5$ Hz, 1H), 5.49 (d, $J = 7.5$ Hz, 1H), 4.45 (d, $J = 6.5$ Hz, 1H), 4.18 (s, 1H), 3.97 (dd, $J = 11.6, 4.7$ Hz, 1H), 3.81 (d, $J = 3.8$ Hz, 2H), 3.58 (d, $J = 7.1$ Hz, 1H), 3.55 – 3.45 (m, 1H), 3.36 (t, $J = 7.9$ Hz, 1H), 3.29 (td, $J = 9.0, 2.9$ Hz, 2H), 2.87 (p, $J = 6.9$ Hz, 1H), 2.79 – 2.71 (m, 2H), 2.64 (d, $J = 16.4$ Hz, 1H), 2.36 (dd, $J = 9.6, 5.4$ Hz, 2H), 1.77 – 1.55 (m, 4H), 1.44 (d, $J = 13.7$ Hz, 1H), 1.38 (s, 9H), 1.04 (s, 3H), 1.02 – 0.94 (m, 9H).

I-123

[00256] Following general procedure II-B, compound **I-123** was obtained as a white powder (14 mg, 24 μ mol, 42%) from erinacine A and pyridine-3-sulfonamide. LCMS: $r_t = 1.64$, ESI (+) $m/z = 575.3$ [M+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 9.02 (dd, $J = 2.3, 0.9$ Hz, 1H), 8.80 (dd, $J = 4.8, 1.6$ Hz, 1H), 8.22 (ddd, $J = 8.1, 2.4, 1.6$ Hz, 1H), 7.62 (ddd, $J = 8.0, 4.9, 0.9$ Hz, 1H), 6.76 (s, 1H), 5.73 (d, $J = 7.5$ Hz, 1H), 5.38 (d, $J = 7.5$ Hz, 1H), 4.42 (d, $J = 6.7$ Hz, 1H), 4.21 (s, 2H), 3.98 (dd, $J = 11.5, 4.7$ Hz, 1H), 3.66 (s, 2H), 3.62 – 3.44 (m, 2H), 3.43 – 3.20 (m, 3H), 2.63 – 2.41 (m, 3H), 2.34 (td, $J = 6.8, 3.1$ Hz, 2H), 1.82 – 1.52 (m, 4H), 1.42 (d, $J = 13.8$ Hz, 1H), 1.06 – 0.89 (m, 12H).

I-139

[00257] Following general procedure II-B, compound **I-139** was obtained as a white powder (10.0 mg, 19.9 μ mol, 43%) from erinacine A and morpholine. LCMS: $r_t = 1.78$, ESI (+) $m/z = 504.5$ [M+H]. ^1H NMR (Acetone- d_6 , 400 MHz): δ 5.76 (1H, d, $J = 7.4$ Hz), 5.45 (1H, d, $J = 7.5$ Hz), 4.44 (1H, d, $J = 6.4$ Hz), 4.21 (2H, s), 4.02 (1H, s), 3.89 (1H, d, $J = 11.7$ Hz), 3.60 (5H, s), 3.50 (2H, t, $J = 11.2$ Hz), 3.24-3.35 (3H, m), 2.74-2.85 (4H, m), 2.64 (1H, d, $J = 16.6$ Hz), 2.33 (7H, s), 1.58-1.67 (4H, m), 0.93-1.01 (12H, m).

I-135

[00258] Following general procedure II-B, compound **I-135** was obtained as a white powder (16 mg, 33 μ mol, 47%) from erinacine A and pyrrolidine. LCMS: $r_t = 1.84$, ESI (+) $m/z = 488.5$ [M+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 5.78 (d, $J = 7.4$ Hz, 1H), 5.47 (d, $J = 7.4$ Hz, 1H), 4.46

(d, $J = 6.1$ Hz, 1H), 3.92 (dd, $J = 11.6, 4.4$ Hz, 1H), 3.65 – 3.43 (m, 2H), 3.38 (t, $J = 7.4$ Hz, 1H), 3.34 – 3.23 (m, 2H), 3.12 (d, $J = 12.9$ Hz, 1H), 2.96 (d, $J = 12.9$ Hz, 1H), 2.87 (p, $J = 6.9$ Hz, 1H), 2.71 (d, $J = 4.3$ Hz, 2H), 2.43 (d, $J = 5.9$ Hz, 4H), 2.38 – 2.26 (m, 2H), 2.17 (ddd, $J = 18.5, 8.9, 4.1$ Hz, 1H), 1.76 – 1.50 (m, 9H), 1.05 (s, 3H), 1.03 – 0.90 (m, 9H).

I-133

[00259] To a solution of **I-115** (20 mg, 4.5 mL, 0.01 M, 1.0 eq, 45 μ mol) in DCM (5.0 mL) cooled to 0 °C, was added Dess-Martin periodinane (21 mg, 1.1 Eq, 49 μ mol). The reaction mixture was stirred at 0 °C for 1 h. LCMS displayed full conversion of the starting material. DCM (3 mL) was added, followed by sat. aq. NaHCO_3 sol. (4 mL). Layers were separated and the aqueous one was extracted with DCM (2x 3 mL). Organic layers were combined, dried over MgSO_4 , filtered through a pad of silica, and concentrated under reduced pressure. The material was purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (AmB 10 mM) to afford **I-133** (2.0 mg, 4.5 μ mol, 10 %) as a white foam. LCMS: $r_t = 1.66$, ESI (+) $m/z = 447.4$ [M+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 7.02 (d, $J = 7.9$ Hz, 1H), 5.75 (d, $J = 8.0$ Hz, 1H), 4.46 (d, $J = 5.6$ Hz, 1H), 4.36 – 4.13 (m, 2H), 3.88 (s, 1H), 3.78 (dd, $J = 11.8, 4.1$ Hz, 1H), 3.60 (d, $J = 7.3$ Hz, 1H), 3.42 (d, $J = 27.4$ Hz, 2H), 3.35 – 3.06 (m, 3H), 2.88 (p, $J = 6.8$ Hz, 1H), 2.78 (s, 2H), 2.40 (dd, $J = 9.1, 5.9$ Hz, 2H), 2.30 (d, $J = 1.3$ Hz, 3H), 1.82 – 1.58 (m, 4H), 1.51 (d, $J = 13.8$ Hz, 1H), 1.12 – 0.93 (m, 12H).

I-132

[00260] To a solution of **I-116** (22 mg, 4.6 mL, 0.01 molar, 1.0 eq, 46 μ mol) in DCM (5.00 mL) cooled to 0 °C, was added Dess-Martin periodinane (21.5 mg, 15.8 μ L, 1.1 eq, 51 μ mol). Reaction mixture was stirred at 0 °C for 1 hour. LCMS displayed full conversion of the starting material. DCM (3 mL) was added, followed by sat. aq. NaHCO_3 sol. (4 mL). Layers were separated and aqueous one was extracted with DCM (2x 3 mL). Organic layers were combined, dried over MgSO_4 , filtered through a pad of silica, and concentrated under reduced pressure. The material was purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0→100% acetonitrile and water (AmB 10 mM) to afford **I-132** (4.0 mg, 8.43 μ mol, 18%) as a white foam. LCMS: $r_t = 1.86$, ESI (+) $m/z = 475.5$ [M+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 7.04 (d, $J = 7.9$ Hz, 1H), 5.77 (d, $J = 7.9$ Hz, 1H), 4.46 (d, $J = 5.7$ Hz, 1H), 4.22 (s, 1H), 3.90 (s, 1H), 3.76 (dd, $J = 11.8, 4.0$ Hz, 1H), 3.60 (d, $J = 7.4$ Hz, 1H), 3.43 (dp, $J = 21.0, 7.0$ Hz, 3H), 3.30 (d, $J = 6.0$ Hz, 1H), 3.22 (dd, $J = 11.8, 7.3$ Hz, 1H), 3.18 – 3.01 (m, 1H), 2.89 (p, $J = 6.8$ Hz, 1H), 2.78 (s, 2H), 2.40 (dd, $J = 9.3, 5.9$ Hz, 2H), 2.34 (d, $J = 9.2$ Hz, 0H), 1.80 – 1.59 (m, 4H), 1.52 (d, $J = 14.0$ Hz, 1H), 1.12 – 0.95 (m, 18H).

I-134

[00261] To a solution of **I-117** (24.0 mg, 4.70 mL, 0.01 molar, 1.0 Eq, 47.0 μ mol) in DCM (5.00 mL) cooled to 0 °C, was added Dess-Martin periodinane (21.9 mg, 16.1 μ L, 1.1 Eq, 51.7 μ mol). Reaction mixture was allowed to stir at 0 °C for 1 hour. Because conversion of the starting material was not complete, Dess-Martin periodinane (21.9 mg, 16.1 μ L, 1.1 Eq, 51.7 μ mol) was added and reaction was stirred at r.t. for 2 additional hours. LCMS displayed full conversion of the starting material. DCM (3 mL) was added, followed by sat. aq. NaHCO₃ sol. (4 mL). Layers were separated and aqueous one was extracted with DCM (2x 3 mL). Organic layers were combined, dried over MgSO₄, filtered through a pad of silica, and concentrated under reduced pressure. The material was purified by column chromatography on silica gel using a gradient of 0-50% EtOAc in hexane to afford **I-134** (11.0 mg, 21 μ mol, 44 %, 95% Purity) as a white foam. LCMS: rt= 1.91, ESI (+) m/z= 509.4 [M+H]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.70 – 7.63 (m, 2H), 7.46 (t, *J* = 7.4 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 1H), 6.57 (d, *J* = 8.0 Hz, 1H), 5.71 (d, *J* = 8.0 Hz, 1H), 4.67 (t, *J* = 6.5 Hz, 1H), 4.50 (d, *J* = 5.9 Hz, 1H), 3.80 – 3.62 (m, 2H), 3.55 (t, *J* = 6.4 Hz, 2H), 3.46 – 3.14 (m, 5H), 2.92 – 2.83 (m, 2H), 2.55 (t, *J* = 13.3 Hz, 1H), 2.39 (t, *J* = 7.7 Hz, 2H), 1.06 – 0.90 (m, 12H).

I-147

[00262] To a solution of erinacine A (20.0 mg, 1 Eq, 46.2 μ mol) in THF (2.3 mL) cooled to -78 °C was added NaH (4.4 mg, 4.0 eq, 185 μ mol). The reaction mixture was stirred at -78 °C for 20 min and methyl iodide (32.8 mg, 15.0 μ L, 5.0 eq, 231 μ mol) was added. The reaction mixture was stirred at rt for 12 hours. TLC and LCMS analyses displayed full conversion of the starting material. EtOAc (6 mL) was added to the reaction mixture, followed by sat. aq. NaHCO₃ sol. (6 mL) and the layers were separated. The aqueous phase was extracted with EtOAc (2x 3 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The material was purified by column chromatography on silica gel using a gradient of 0→50% EtOAc in hexane to afford **I-147** (20 mg, 42 μ mol, 91%) as a yellowish foam. LCMS: rt= 2.22 min, ESI (+) m/z= 283.3 [M-sugar+H]; m/z= 475.4 [M+H]; m/z= 497.4 [M+Na]. ¹H NMR (Acetone-*d*₆, 400 MHz): δ 9.37 (1H, s), 6.75 (1H, dd, *J* = 8.0, 2.0 Hz), 5.89 (1H, d, *J* = 8.0 Hz), 4.30 (1H, d, *J* = 7.4 Hz), 3.81 (1H, dd, *J* = 11.0, 4.6 Hz), 3.63 (1H, d, *J* = 6.6 Hz), 3.48 (3H, s), 3.44 (3H, s), 3.38 (3H, s), 2.98-3.18 (4H, m), 2.84-2.91 (1H, m), 2.72 (1H, dd, *J* = 9.0, 7.4 Hz), 2.48-2.59 (2H, m), 2.41 (2H, dd, *J* = 9.2, 5.9 Hz), 1.61-1.77 (4H, m), 1.38 (1H, m), 0.97-1.04 (12H, m).

I-128

[00263] To a solution of sodium chlorite (59.1 mg, 10 Eq, 653 μmol) and NaH_2PO_4 (70.5 mg, 50 μL , 9.0 Eq, 588 μmol) in water (1.50 mL) was added a mixture of **I-147** (31.0 mg, 1 eq, 65 μmol) and delta-isoamylene (92 mg, 136 μL , 20 Eq, 1.31 mmol) in tBuOH (5.0 mL). The reaction mixture stirred at rt for 6 h. EtOAc (6 mL) was added, followed by sat. aq. NaHCO_3 sol. (6 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (2x 5 mL). The organic layers were combined, washed with brine (10 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The material was purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0 $\rightarrow$ 100% acetonitrile and water (AmB 10 mM) to afford **I-128** (10 mg, 20.4 μmol , 31%) as a white solid. LCMS: r_t = 1.40, ESI (-) m/z = 489.4 [M-H]. ^1H NMR (400 MHz, Acetone- d_6) δ 10.39 (s, 1H), 7.10 (d, J = 8.1 Hz, 1H), 5.71 (d, J = 8.1 Hz, 1H), 4.33 (d, J = 7.3 Hz, 1H), 3.86 (dd, J = 11.1, 4.8 Hz, 1H), 3.59 (d, J = 7.1 Hz, 1H), 3.51 (s, 3H), 3.47 (s, 3H), 3.39 (s, 3H), 3.20 – 2.99 (m, 5H), 2.93 – 2.86 (m, 1H), 2.48 – 2.35 (m, 3H), 1.80 – 1.58 (m, 4H), 1.44 (d, J = 13.8 Hz, 1H), 1.10 – 0.95 (m, 12H).

I-148

[00264] To a solution of erinacine A (80 mg, 1 eq, 185 μmol) and imidazole (113 mg, 110 μL , 9.0 eq, 1.66 mmol) in dry DMF (4.0 mL) cooled to 0 $^\circ\text{C}$ was added dropwise tert-Butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (391 mg, 340 μL , 8.0 eq, 1.48 mmol). The reaction mixture was stirred at 0 $^\circ\text{C}$ for 20 min and then at rt for 12 h. TLC and LCMS analysis displayed the full conversion of the starting material. Reaction mixture was concentrated under reduced pressure. The obtained residue was diluted in EtOAc (6 mL) and the resulting organic layer was rinsed with sat. aq. NaHCO_3 sol. (4 mL) and brine (4 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (4 g), 100% DCM, to afford the desired compound **I-148** (115 mg, 0.12 mmol, 64 %, 80% Purity) as a clear viscous oil. LCMS: r_t = 2.51, ESI (+) m/z = 331.3, unknown fragment. ^1H NMR (400 MHz, Acetone- d_6) δ 9.41 (s, 1H), 6.78 (dd, J = 8.1, 2.3 Hz, 1H), 5.89 (d, J = 8.2 Hz, 1H), 4.68 (s, 1H), 3.77 (dd, J = 11.9, 1.7 Hz, 1H), 3.69 – 3.53 (m, 3H), 3.45 (s, 1H), 3.33 – 3.17 (m, 2H), 2.90 (p, J = 6.8 Hz, 1H), 2.80 (d, J = 0.9 Hz, 1H), 2.79 – 2.74 (m, 1H), 2.59 – 2.35 (m, 4H), 1.84 – 1.58 (m, 4H), 1.46 – 1.36 (m, 1H), 1.04 (dd, J = 7.8, 5.5 Hz, 9H), 0.98 (d, J = 6.8 Hz, 3H), 0.94 – 0.85 (m, 27H), 0.14 – 0.05 (m, 18H).

I-131

[00265] Following general procedure II-C, compound **I-131** was obtained as a clear viscous oil (10 mg, 26 μmol , 39 %)- mixture of two diastereomers from allocyathin B2 and cyclohexene.

LCMS: t_r = 2.44, ESI (+) m/z = 381.5 [M+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 9.42 (d, J = 3.1 Hz, 1H), 6.81 (ddd, J = 7.6, 5.1, 1.9 Hz, 1H), 5.88 (d, J = 7.9 Hz, 1H), 5.83 – 5.59 (m, 2H), 5.41 (dq, J = 10.1, 2.5 Hz, 0H), 3.97 (s, 0H), 3.85 – 3.69 (m, 1H), 3.53 (dd, J = 15.5, 6.6 Hz, 1H), 3.40 (q, J = 7.0 Hz, 1H), 3.14 (ddd, J = 17.5, 6.7, 4.4 Hz, 1H), 2.89 (p, J = 6.9 Hz, 1H), 2.79 – 2.74 (m, 1H), 2.55 – 2.36 (m, 4H), 2.01 – 1.82 (m, 2H), 1.80 – 1.58 (m, 6H), 1.56 – 1.34 (m, 2H), 1.08 – 1.01 (m, 6H), 1.01 – 0.95 (m, 6H).

I-142

[00266] To a solution of Erinacine A (30.0 mg, 1 Eq, 69.4 μmol) in pyridine (0.50 g, 0.50 mL, 91 Eq, 6.3 mmol) was added acetic anhydride (142 mg, 131 μL , 20 Eq, 1.39 mmol) at r.t. The reaction mixture was allowed to stir at this temperature for 12 hour. LCMS and TLC analyses displayed the full conversion of the starting material. The reaction mixture was concentrated under reduced pressure and the resulting material was purified by flash chromatography using a gradient 0-50% of EtOAc in hexane to afford **I-142** (36.0 mg, 64.4 μmol , 92.9 %) as a white solid. LCMS: t_r = 2.07, ESI (+) m/z = 559.8 [M+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 9.39 (s, 1H), 6.78 (dd, J = 8.0, 2.0 Hz, 1H), 5.88 (d, J = 8.0 Hz, 1H), 5.10 (t, J = 8.5 Hz, 1H), 4.93 – 4.64 (m, 3H), 3.89 (dd, J = 11.9, 5.0 Hz, 1H), 3.68 (d, J = 6.6 Hz, 1H), 3.40 (dd, J = 11.9, 8.4 Hz, 1H), 3.21 (dd, J = 17.6, 6.6 Hz, 1H), 2.88 (p, J = 6.9 Hz, 1H), 2.78 (s, 3H), 2.53 (d, J = 17.5 Hz, 1H), 2.40 (ddd, J = 18.6, 11.4, 5.4 Hz, 4H), 1.99 (d, J = 1.7 Hz, 6H), 1.94 (s, 3H), 1.79 – 1.59 (m, 5H), 1.37 (dt, J = 13.8, 3.5 Hz, 1H), 1.09 – 1.01 (m, 6H), 1.00 – 0.92 (m, 6H).

I-138

[00267] To a solution of erinacine A (20 mg, 1 Eq, 46.2 μmol) in a mixture of MeOH (0.30 mL), DCM (0.30 mL) and trimethyl orthoformate (TMOF) (0.30 mL) at r.t., under a dry and argon atmosphere, was added 7-Amino-4-(trifluoromethyl)chromen-2-one (11.7 mg, 8.27 μL , 1.1 Eq, 50.9 μmol) followed by acetic acid (11 mg, 0.011 mL, 4.1 Eq, 0.19 mmol). The reaction mixture was allowed to stir at 70 °C for 12 hours. More than 60% of the ErA material was consumed according to LCMS. Sodiumtetrahydroborate (2.62 mg, 2.45 μL , 1.5 Eq, 69.4 μmol) was added to the reaction mixture cooled at r.t. and the resulting mixture was allowed to stir at this temperature for 2 hours. EtOAc (5 mL) was added, followed by sat. aq. NaHCO_3 sol. (5 ml). Layers were separated and the aqueous layer was extracted with EtOAc (2x 3 mL). Organic layers were combined, dried over Na_2SO_4 , filtered and then concentrated under reduced pressure. The material was pre-purified by column chromatography on silica gel (4 g) using a gradient of 0-60% EtOAc in Hexane to afford a cleaner product (13 mg). The compound was finally purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-

100% acetonitrile and water (AmB 10 mM) to afford **I-138** (3.00 mg, 4.65 μ mol, 10.0 %) as a yellow solid. LCMS: t_r = 2.04, ESI (+) m/z = 668.6 [M+Na], ESI (-) m/z = 494.4 [M-sugar-H₂O-H]. λ_{max} = 385 nm

I-136

[00268] To a solution of allocyathin B2 (25 mg, 1 eq, 83.2 μ mol) in a mixture of MeOH (0.30 mL)/DCM (0.30 mL)/trimethyl orthoformate (TMOF) (0.30 mL) at rt, under a dry and argon atmosphere, was added 7-amino-4-(trifluoromethyl)chromen-2-one (21 mg, 14.9 μ L, 1.1 eq, 92 μ mol) followed by acetic acid (11 mg, 0.011 mL, 2.3 Eq, 0.19 mmol). The reaction mixture was stirred at 70 °C for 12 hours. NaBH₄ (4.7 mg, 4.4 μ L, 1.5 eq, 125 μ mol) was added to the reaction mixture cooled at rt and the resulting mixture was stirred at this temperature for 2 hours. EtOAc (5 mL) was added, followed by sat. aq. NaHCO₃ sol. (5 mL). Layers were separated and the aqueous layer was extracted with EtOAc (2x 3 mL). Organic layers were combined, dried over Na₂SO₄, filtered and then concentrated under reduced pressure. The material was pre-purified by column chromatography on silica gel (4 g) using a gradient of 0-50% EtOAc in hexane to afford a cleaner product (11 mg). The compound was finally purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (AmB 10 mM) to afford **I-136** (3 mg, 5.84 μ mol, 7%) as a yellow solid. LCMS: t_r = 2.26, ESI (+) m/z = 285.3 [M-H₂O-coum.+H], ESI (-) m/z = 512.4 [M-H]. λ_{max} = 386 nm. ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.44 (dq, J = 9.0, 2.1 Hz, 1H), 6.80 (dd, J = 9.0, 2.4 Hz, 1H), 6.57 (d, J = 2.4 Hz, 1H), 6.38 (s, 1H), 5.98 (dt, J = 7.9, 1.6 Hz, 1H), 5.56 (d, J = 7.8 Hz, 1H), 3.94 (d, J = 4.7 Hz, 2H), 3.61 (ddd, J = 8.4, 6.3, 1.9 Hz, 1H), 3.00 (d, J = 8.7 Hz, 1H), 2.92 – 2.83 (m, 1H), 2.78 (s, 2H), 2.67 – 2.60 (m, 2H), 2.35 (dd, J = 9.2, 5.8 Hz, 3H), 1.77 – 1.53 (m, 4H), 1.38 – 1.27 (m, 2H), 1.03 – 0.91 (m, 12H).

I-137

[00269] To a solution of erinacine A (20.0 mg, 1 eq, 46 μ mol) in a mixture of MeOH (0.30 mL)/DCM (0.30 mL)/trimethyl orthoformate (TMOF) (0.30 mL) at rt, under a dry and argon atmosphere, was added 3-amino pyrazole (3.8 mg, 2.9 μ L, 1 eq, 46.2 μ mol) followed by acetic acid (11 mg, 0.011 mL, 4.1 Eq, 0.19 mmol). The reaction mixture was stirred at 20 °C for 12 hours. NaBH₄ (2.62 mg, 2.45 μ L, 1.5 Eq, 69.4 μ mol) was added to the reaction mixture cooled at r.t., followed by MeOH (0.30 mL) and the resulting mixture was allowed to stir at this temperature for 2 hours. The reaction mixture was concentrated under reduced pressure. EtOAc (5 mL) was added to the obtained material, followed by sat. aq. NaHCO₃ sol. (5 mL). Layers were separated and the aqueous layer was extracted with EtOAc (2x 3 mL). Organic layers were combined, dried

over Na₂SO₄, filtered and then concentrated under reduced pressure. The material was purified by column chromatography on silica gel (4 g) using a gradient of 0-15% MeOH in DCM to afford a cleaner product (12 mg). The compound was purified further by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (AmB 10 mM) to afford **I-137** (2.0 mg, 3.8 µmol, 8%, 95% Purity) as a white foam. LCMS: rt= 1.59, ESI (+) m/z= 500.4 [M+H], ESI (-) m/z= 498.4 [M-H]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.35 (d, *J* = 2.2 Hz, 1H), 5.85 (d, *J* = 7.4 Hz, 1H), 5.59 – 5.40 (m, 2H), 4.73 (s, 1H), 4.45 (s, 1H), 4.00 (dd, *J* = 11.6, 4.6 Hz, 1H), 3.78 (s, 2H), 3.54 (td, *J* = 8.0, 3.0 Hz, 2H), 3.45 – 3.25 (m, 3H), 2.66 (t, *J* = 15.9 Hz, 2H), 2.34 (dd, *J* = 9.8, 5.2 Hz, 2H), 1.77 – 1.46 (m, 6H), 1.10 – 0.91 (m, 12H).

I-129

[00270] To a solution of allocyathin B2 (20.0 mg, 1 Eq, 66.6 µmol) in THF (0.50 mL) cooled to -78 °C was added sodium hydride (3.99 mg, 3.3 µL, 60% Wt, 1.5 Eq, 99.9 µmol) in one portion. The reaction mixture was stirred at this temperature for 20 min and methyl sulfate (16.8 mg, 12.6 µL, 2 Eq, 133 µmol) was added. The reaction mixture was allowed to slowly warm up to r.t. and to stir for 12 hours. EtOAc (3 mL) was added, followed by sat. aq. NaHCO₃ sol. (4 mL), and the layers were separated. The aqueous phase was extracted with EtOAc (2x 2 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The material was purified by column chromatography on silica gel using a gradient of 0-20% EtOAc in hexane to afford **I-129** (9.00 mg, 28.6 µmol, 43.0 %) as a yellow oil. LCMS: rt= 2.17, ESI (+) m/z= 315.5 [M+OH]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 9.42 (s, 1H), 6.80 (dd, *J* = 7.9, 2.1 Hz, 1H), 5.87 (d, *J* = 7.9 Hz, 1H), 3.25 (d, *J* = 6.6 Hz, 1H), 3.18 (s, 4H), 2.88 (p, *J* = 6.9 Hz, 1H), 2.49 – 2.34 (m, 5H), 1.80 – 1.57 (m, 5H), 1.37 (ddd, *J* = 13.9, 4.4, 2.8 Hz, 1H), 1.08 – 1.01 (m, 6H), 1.01 – 0.94 (m, 6H).

I-166

[00271] To a solution of allocyathin B2 (20.0 mg, 1 Eq, 66.6 µmol) in THF (0.50 mL) cooled to 0 °C was added sodium hydride (5.33 mg, 4.4 µL, 60% Wt, 2 Eq, 133 µmol) in one portion. The reaction mixture was stirred at this temperature for 1 hour and diethyl sulfate (30.8 mg, 26.1 µL, 3 Eq, 200 µmol) was added. The reaction mixture was allowed to slowly warm up to r.t. and to stir for 12 hours. EtOAc (3 mL) was added, followed by sat. aq. NaHCO₃ sol. (4 mL), and the layers were separated. The aqueous phase was extracted with EtOAc (2x 2 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The material was purified by column chromatography on silica gel using a gradient of 0-20% EtOAc in hexane to afford **I-166** (2.70 mg, 8.22 µmol, 12.3 %) as a yellow oil. LCMS: rt= 2.19, ESI (+) m/z=

285.3 [M-EtOH+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 5.99 – 5.86 (m, 1H), 5.56 (d, J = 7.8 Hz, 1H), 3.90 (d, J = 1.4 Hz, 2H), 3.63 – 3.52 (m, 1H), 3.44 (qd, J = 7.0, 4.9 Hz, 2H), 2.88 (p, J = 6.9 Hz, 1H), 2.79 – 2.71 (m, 2H), 2.56 (d, J = 4.6 Hz, 2H), 2.36 (dd, J = 9.0, 6.0 Hz, 3H), 1.78 – 1.53 (m, 4H), 1.39 – 1.24 (m, 2H), 1.15 (t, J = 7.0 Hz, 3H), 1.07 – 0.92 (m, 12H).

I-143

[00272] *I-142* was diluted in neat in diethylaminosulfurtrifluoride (0.62 g, 0.50 mL, 1.1e+2 Eq, 3.9 mmol) (plastic vial) at r.t. The reaction mixture was allowed to stir at 40 °C for 6 hours. The reaction mixture was diluted in EtOAc (4 mL) and cooled at 0 °C. Sat. aq. NaHCO_3 sol. (4 mL) was slowly added, and the resulting mixture was stirred at 0 °C for 15 min. The layers were separated, and the aqueous layer was extracted with EtOAc (2x 3 mL). Organic layers were combined, dried over MgSO_4 , filtered and concentrated under reduced pressure. The resulting material was purified by flash chromatography using a gradient 0-25% of EtOAc in hexane to afford ***I-143*** (7.0 mg, 12.1 μmol , 34%) as a white foam. LCMS: rt = 2.20, ESI (+) m/z = 561.4 [M-F+H]. ^1H NMR (Acetone- d_6 , 400 MHz): δ 6.34-6.06 (2H, m), 5.59 (1H, d, J = 7.6 Hz), 5.16 (1H, t, J = 8.8 Hz), 4.85-4.91 (2H, m), 4.77 (1H, d, J = 7.1 Hz), 4.09 (1H, dd, J = 11.8, 5.2 Hz), 3.63 (1H, d, J = 6.6 Hz), 3.50 (1H, dd, J = 11.8, 9.0 Hz), 2.38 (2H, t, J = 7.5 Hz), 2.16-2.22 (1H, m), 1.99 (3H, s), 1.97 (3H), 1.96 (3H, s), 1.58-1.75 (4H, m), 1.41-1.45 (1H, m), 1.29 (2H, s), 0.94-1.04 (12H, m). ^{19}F NMR (Acetone- d_6 , 376 MHz): δ F -113.5, -113.7, -114.3, -114.5, -115.1, -115.3, -116.0, -116.1.

I-144

[00273] To a solution of ***I-143*** (13.0 mg, 1 eq, 22.4 μmol) in MeOH (0.50 mL) cooled at 0 °C was added sodium methoxide (4.67 mg, 5.00 μL , 3.86 Eq, 86.4 μmol). The reaction mixture was allowed to stir at r.t. for 1 hour. Full conversion of the starting material was observed by LCMS. The reaction mixture was diluted in EtOAc (3 mL). sat. aq. NaHCO_3 sol. (3 mL) was slowly added. The layers were separated, and the aqueous layer was extracted with EtOAc (2x 2 mL). Organic layers were combined, dried over MgSO_4 , filtered and concentrated under reduced pressure. The resulting material was purified by flash chromatography using a gradient 0-100% of EtOAc in hexane to afford ***I-144*** (4.0 mg, 8.8 μmol , 39%) as a white foam. LCMS: rt = 1.82, ESI (+) m/z = 436 [M-F+H]. ^1H NMR (Acetone- d_6 , 400 MHz): δ 6.03-6.32 (2H, m), 5.58 (1H, d, J = 7.7 Hz), 4.46 (1H, d, J = 6.1 Hz), 3.90 (1H, dd, J = 11.7, 4.4 Hz), 3.61 (1H, d, J = 6.5 Hz), 3.45-3.50 (1H, m), 3.37 (1H, t, J = 7.5 Hz), 3.23-3.31 (2H, m), 2.73-2.76 (2H, m), 2.28-2.39 (3H, m), 1.58-1.74 (5H, m), 1.52 (1H, d, J = 14.0 Hz), 1.23-1.30 (2H, m), 0.94-1.04 (12H, m). ^{19}F NMR (Acetone- d_6 , 376 MHz): δ F -113.0, -113.1, -113.8, -113.9, -114.6, -114.8, -115.4, -115.5.

I-145

[00274] To a solution of **Allocyathin B2** (25.0 mg, 1 eq, 83 μ mol) and trimethyl(trifluoromethyl)silane (29.6 mg, 30.7 μ L, 2.5 eq, 208 μ mol) in anh. THF (3.5 mL) cooled to 0 °C, was added cesium fluoride (31.6 mg, 7.61 μ L, 2.5 eq, 208 μ mol). The reaction mixture was allowed to stir at 0 °C for 20 min and then to warm up to r.t. and be stirred for 12 hour. Full consumption of the starting material was observed by TLC and LCMS. Tetrabutylammonium fluoride (54.4 mg, 208 μ L, 1.00 molar, 2.5 Eq, 208 μ mol) was added at r.t. and the reaction mixture was allowed to stir at r.t. for 1 hour. Desired material was observed by LCMS. EtOAc (6 mL) was added followed by sat. aq. NaHCO₃ sol. (6 mL), and the layers were separated. The aqueous layer was extracted with EtOAc (2x 5 mL). The organic layers were combined, washed with brine (10 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The material was purified by flash chromatography using a gradient of 0-50% EtOAc in hexane to afford **I-145** (4.00 mg, 11 μ mol, 13 %, 98% Purity) as a yellowish solid. LCMS: *rt*= 2.01, ESI (-) *m/z*= 369.4 [M-H]. ¹H NMR (Acetone-*d*₆, 400 MHz): δ _H 6.18 (1H, d, *J* = 7.8 Hz), 6.12 (2H, d, *J* = 7.7 Hz), 5.59 (3H, dd, *J* = 7.7, 5.3 Hz), 5.39 (1H, d, *J* = 4.9 Hz), 5.30 (1H, s), 4.56 (3H, d, *J* = 7.9 Hz), 3.61 (3H, t, *J* = 6.8 Hz), 3.06 (1H, t, *J* = 8.5 Hz), 2.66-2.67 (2H, m), 2.57 (2H, d, *J* = 17.0 Hz), 2.24-2.39 (9H, m), 2.08 (1H, s), 1.57-1.74 (12H, m), 1.42 (4H, t, *J* = 13.3 Hz), 1.28 (2H, s), 0.96-1.02 (40H, m). ¹⁹F NMR (Acetone-*d*₆, 376 MHz): δ _F -76.9, -76.9, -77.2, -77.2.

I-110

[00275] Following general procedure I-B compound I-110 was obtained as a white solid (29 mg, 19%) from compound II-2. ¹H NMR (700 MHz, Chloroform-*d*) δ 7.37 – 7.34 (m, 2H), 7.18 – 7.15 (m, 2H), 7.15 – 7.12 (m, 1H), 6.09 (dd, *J* = 8.4, 2.6 Hz, 1H), 5.42 (d, *J* = 8.3 Hz, 1H), 3.43 (s, 1H), 3.37 (s, 4H), 2.79 – 2.57 (m, 3H), 2.54 (d, *J* = 18.1 Hz, 1H), 2.44 – 2.25 (m, 4H), 1.86 – 1.76 (m, 1H), 1.68 – 1.59 (m, 1H), 1.56 (q, *J* = 2.7, 1.9 Hz, 1H), 1.33 – 1.15 (m, 4H), 0.96 – 0.92 (m, 3H), 0.92 – 0.85 (m, 6H), 0.77 (s, 3H).

I-113

[00276] I-110 (10 mg, 0.03 mmol) was added to a oven-dried round bottom flask followed by DMAP (0.6 mg, 0.2 eq, 0.005 mmol) and pyridine (9.9 mg, 10 μ L, 5 eq). The contents were dissolved in DCM (0.08 mL) and secured under argon. Benzoyl chloride (17 mg, 14 μ L, 5 eq) was added dropwise to the mixture at room temperature. The mixture was stirred at rt for 18 h at which point TLC indicated complete consumption of I-110. To the reaction was added saturated aqueous NaHCO₃, which was extracted with EtOAc three times. The collected organic fractions

were washed with brine three times, dried with Na₂SO₄, concentrated under reduced pressure and the crude oil subjected to purification *via* preparatory TLC (30% EtOAc:hexanes, 1000 µm 20x20 silica plate). I-113 was obtained as a white solid (5 mg, 43%). ¹H NMR (700 MHz, Chloroform-*d*) δ 7.93 (dt, *J* = 8.2, 1.3 Hz, 2H), 7.59 – 7.52 (m, 1H), 7.41 (ddd, *J* = 8.7, 7.5, 1.3 Hz, 2H), 7.03 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.00 – 6.91 (m, 3H), 6.07 (dd, *J* = 8.4, 2.1 Hz, 1H), 5.51 (d, *J* = 8.4 Hz, 1H), 4.99 (dd, *J* = 6.4, 1.6 Hz, 1H), 3.29 (d, *J* = 1.1 Hz, 3H), 2.92 (dd, *J* = 18.8, 6.3 Hz, 1H), 2.78 – 2.65 (m, 2H), 2.33 (dd, *J* = 9.0, 6.1 Hz, 2H), 2.00 (td, *J* = 13.8, 4.7 Hz, 1H), 1.65 (dt, *J* = 12.4, 6.1 Hz, 2H), 1.61 – 1.52 (m, 3H), 1.50 (ddd, *J* = 13.1, 4.8, 3.1 Hz, 1H), 1.35 – 1.26 (m, 4H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.93 (s, 3H), 0.92 (d, *J* = 7.0 Hz, 3H), 0.87 (s, 3H).

I-112

[00277] Compound II-1 (8 mg, 0.026 mmol, 1 eq) was added to a reaction vial, which was secure under argon. II-1 was then dissolved in DCM (0.09 mL) and 1-piperidinecarbonyl chloride (4 mg, 3.5 µL, 1.05 eq) followed by addition of triethylamine (3 mg, 4 µL, 1.05 eq) to the vial. The vial was secured under argon again, and the mixture was stirred at rt for 48 h. The reaction was quenched with saturated aqueous NaHCO₃ and the water layer extracted three times with DCM. The combined organic fractions were washed with brine and then collected and dried over Na₂SO₄. The crude collection was evaporated under reduced pressure, and the crude residue purified by preparatory TLC (30 % EtOAc:hex, 20x20 cm 1000 µm silica plate). I-112 was collected as a white solid (1.8 mg, 17%). ¹H NMR (700 MHz, Chloroform-*d*) δ 6.03 (ddt, *J* = 8.2, 2.8, 1.3 Hz, 1H), 5.57 (d, *J* = 8.0 Hz, 1H), 4.63 – 4.50 (m, 2H), 3.57 (ddd, *J* = 11.5, 6.0, 1.5 Hz, 1H), 3.44 (t, *J* = 5.5 Hz, 4H), 2.84 (p, *J* = 6.9 Hz, 1H), 2.70 (d, *J* = 17.5 Hz, 1H), 2.59 (dd, *J* = 17.5, 6.0 Hz, 1H), 2.47 (dt, *J* = 13.9, 9.3 Hz, 1H), 2.40 – 2.30 (m, 2H), 2.22 (t, *J* = 7.7 Hz, 1H), 1.96 (d, *J* = 11.5 Hz, 1H), 1.75 – 1.68 (m, 1H), 1.60 (ddq, *J* = 17.8, 9.7, 3.6 Hz, 7H), 1.38 – 1.26 (m, 2H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.96 – 0.91 (m, 9H).

I-111

[00278] I-110 (20 mg, 0.05 mmol, 1 eq) and DMP (25 mg, 1.2 eq) were added to a round bottom flask and was secured under argon. DCM (2.4 mL) was added via syringe and the reaction stirred at room temperature until TLC indicated complete consumption of I-110 (2.5 h). The mixture was quenched with aqueous Na₂CO₃ and extracted three times with DCM. The collected organic fractions were dried with Na₂SO₄, filtered, concentrated under reduced pressure and purified *via* preparatory TLC (30% EtOAc:hexanes, 20x20 cm 1000 µm silica plate). I-111 was collected as a yellow solid (11 mg, 54%). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.30 (td, *J* = 7.5, 6.9, 1.3 Hz, 2H), 7.23 – 7.16 (m, 1H), 7.12 – 7.04 (m, 2H), 6.11 (ddd, *J* = 6.1, 2.2, 0.8 Hz, 1H),

5.63 (dd, $J = 6.1, 1.0$ Hz, 1H), 3.41 (d, $J = 0.8$ Hz, 1H), 3.39 (s, 3H), 3.24 (ddd, $J = 11.1, 2.2, 1.1$ Hz, 1H), 2.67 (hept, $J = 6.9$ Hz, 1H), 2.33 (dd, $J = 9.2, 5.6$ Hz, 2H), 1.92 – 1.81 (m, 1H), 1.75 – 1.63 (m, 2H), 1.63 – 1.47 (m, 3H), 1.40 – 1.21 (m, 3H), 1.01 – 0.88 (m, 12H).

I-69

[00279] Compound II-1 (6.5 mg) was added to a reaction vial and purged with argon. To the vial was added DCM (0.1 mL), benzoyl chloride (3 mg, 2.5 μ L, 1.01 eq) and triethylamine (2.1 mg, 3 μ L, 1.01 eq). The vial was sealed and stirred at rt for 20 h at which point TLC indicated complete consumption of starting material. The mixture was diluted with DCM, washed with saturated aqueous NaHCO_3 , then brine, and the collected organic fraction was dried with Na_2SO_4 . The crude residue was purified by preparatory TLC (20% EtOAc, 20x20 cm 1000 μ m silica plate) to yield **I-69** as a colourless oil (4.1 mg, 48%). ^1H NMR (700 MHz, Chloroform- d) δ 8.12 – 8.02 (m, 2H), 7.60 – 7.52 (m, 1H), 7.45 (t, $J = 7.7$ Hz, 2H), 6.21 – 6.06 (m, 1H), 5.60 (d, $J = 8.1$ Hz, 1H), 4.90 – 4.73 (m, 2H), 3.61 (dd, $J = 11.0, 5.9$ Hz, 1H), 2.90 – 2.75 (m, 2H), 2.69 (dd, $J = 17.6, 6.0$ Hz, 1H), 2.49 (dt, $J = 13.9, 9.3$ Hz, 1H), 2.40 – 2.30 (m, 2H), 1.99 (d, $J = 11.7$ Hz, 1H), 1.87 – 1.66 (m, 1H), 1.66 – 1.55 (m, 4H), 1.38 – 1.19 (m, 3H), 1.00 (d, $J = 6.9$ Hz, 3H), 0.97 – 0.92 (m, 9H).

I-49

[00280] Allocyathin B2 (10 mg, 0.03 mmol, 1 eq), cyclopropylamine (2.1 mg, 2.5 μ L, 1.1 eq) were dissolved in MeOH (0.11 mL) and AcOH (3.5 μ L). The mixture was stirred at rt for 30 minutes and then followed by the addition of NaCNBH_3 (6.3 mg, 3 eq). The reaction was stirred at rt until TLC indicated completed consumption of starting material (3.5 h). The reaction was diluted with saturated ammonium chloride, the methanol removed *in vacuo* and the whole mixture diluted with EtOAc. The organic fraction was washed with brine, then dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude residue was purified by preparatory TLC (30% EtOAc, 20x20 cm 1000 μ m silica plate) to yield **I-49** as a colourless oil 1.3 mg. ^1H NMR (700 MHz, Chloroform- d) δ 5.93 – 5.86 (m, 1H), 5.54 (d, $J = 8.0$ Hz, 1H), 3.52 (d, $J = 5.7$ Hz, 1H), 3.21 – 3.01 (m, 2H), 2.83 (p, $J = 6.8$ Hz, 1H), 2.73 – 2.63 (m, 1H), 2.62 – 2.55 (m, 1H), 2.54 – 2.38 (m, 1H), 2.36 – 2.28 (m, 3H), 2.22 (t, $J = 7.7$ Hz, 2H), 1.74 – 1.52 (m, 6H), 1.00 (d, $J = 6.9$ Hz, 3H), 0.98 – 0.91 (m, 6H), 0.90 (s, 3H).

I-149

[00281] Allocyathin B2 (16 mg, 0.05 mmol, 1 eq) and benzene sulfonamide (9 mg, 1.1 eq) were added to a round bottom flask. The flask was secured under argon and THF (0.13 mL) was

added. $\text{Ti}(\text{OEt})_4$ (23 mg, 21 μL) was added to the mixture with stirring at room temperature and the whole reaction was then added to a 60 °C oil bath and heated for 22 h. At which point, TLC indicated complete consumption of starting material. Brine was added to the reaction until the Ti salts precipitated out, the crude mixture was filtered rinsing with EtOAc. The collected organic fraction was dried with Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude residue was purified by reverse phase C-18 chromatography (4 g cartridge, 10%→100% MeCN) yielding I-149 as a yellow solid (21 mg, 95%). LCMS: t_r = 4.49 min, ESI (+) m/z = 440.2269 [M+H]. ^1H NMR (700 MHz, Chloroform- d) δ 8.58 (s, 1H), 7.98 – 7.91 (m, 2H), 7.60 (t, J = 7.5 Hz, 1H), 7.57 – 7.47 (m, 2H), 6.83 (dd, J = 8.6, 2.4 Hz, 1H), 5.92 (d, J = 8.5 Hz, 1H), 5.30 (s, 1H), 3.68 (ddd, J = 9.4, 5.9, 1.7 Hz, 1H), 3.25 (dd, J = 18.4, 5.9 Hz, 1H), 2.80 (p, J = 6.8 Hz, 1H), 2.66 (dt, J = 18.5, 2.1 Hz, 1H), 2.52 (td, J = 13.3, 5.8 Hz, 1H), 2.45 – 2.36 (m, 2H), 1.79 – 1.59 (m, 5H), 1.37 – 1.30 (m, 1H), 1.04 (d, J = 6.9 Hz, 3H), 0.98 (s, 3H), 0.97 – 0.94 (m, 6H).

I-97

[00282] To a flask containing I-149 (13 mg, 0.03 mmol, 1 eq) was added MeOH (0.3 mL) followed by NaBH_4 (3.5 mg, 3 eq). The reaction was stirred at rt for 15 minutes, at which point TLC indicated complete consumption of I-149. The reaction was concentrated, dissolved in DCM, washed with 5% HCl, brine, and the organic fractions collected and dried over Na_2SO_4 . The crude product was I-149 in sufficient purity collected as a white solid (11 mg, 80%). LCMS: t_r = 4.39 min, ESI (+) m/z = 442.2402 [M+H]

I-125

[00283] I-125 was obtained following General Procedure II-B from erinacine A and tert-butylsulfonamide as a white foam (26 mg). LCMS: t_r = 4.49 min, ESI (+) m/z = 422.2726 [M+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 5.95 (dt, J = 7.8, 1.1 Hz, 1H), 5.87 (t, J = 6.1 Hz, 1H), 5.55 (d, J = 7.8 Hz, 1H), 3.87 – 3.71 (m, 2H), 3.58 (dt, J = 8.4, 4.0 Hz, 1H), 2.87 (p, J = 6.8 Hz, 2H), 2.64 (d, J = 4.1 Hz, 2H), 2.44 – 2.29 (m, 3H), 1.78 – 1.55 (m, 4H), 1.37 (s, 9H), 1.33 – 1.24 (m, 2H), 1.01 (d, J = 6.9 Hz, 3H), 0.99 – 0.92 (m, 9H).

I-126

[00284] I-126 was obtained following General Procedure II-B from erinacine A and dimethylaminesulfonamide as a white foam (12 mg). LCMS: t_r = 4.14 min, ESI (+) m/z = 409.2531 [M+H]. ^1H NMR (400 MHz, Acetone- d_6) δ 6.17 – 6.05 (m, 1H), 5.98 – 5.88 (m, 1H), 5.54 (d, J = 7.8 Hz, 1H), 3.68 (d, J = 6.1 Hz, 2H), 3.58 (ddd, J = 8.3, 5.2, 2.7 Hz, 1H), 2.98 – 2.83 (m, 2H),

2.77 (s, 6H), 2.67 – 2.62 (m, 2H), 2.43 – 2.31 (m, 3H), 1.76 – 1.54 (m, 4H), 1.31 (dt, $J = 13.8$, 3.7 Hz, 1H), 1.01 (d, $J = 6.9$ Hz, 3H), 0.98 – 0.92 (m, 9H).

I-33

[00285] Erinacine A (21 mg, 0.05 mmol, 1 eq) and respective aniline (46 mg, 3 eq) were added to a round bottom flask. The components were dissolved in DMF:MeOH (0.1:0.2 mL) and the reaction heated at 70 °C for 2 h with stirring. At which point, NaCNBH₃ (9.5 mg, 3 eq) was added to the reaction, heating with stirring at 70 °C for 4 h. The heat was turned off and the mixture stirred at rt for 12 h. The MeOH was removed under reduced pressure and the crude residue added directly to C-18 column (12 g, 10→100% MeCN) for purification, yielding I-33 as yellow flakes (4 mg, 11%). LCMS: $rt = 3.92$ min, ESI (+) $m/z = 723.4017$ [M+H]. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.21 (s, 1H), 7.81 – 7.68 (m, 2H), 7.64 – 7.49 (m, 4H), 6.91 (t, $J = 6.0$ Hz, 1H), 6.63 (d, $J = 8.9$ Hz, 2H), 5.67 (d, $J = 7.4$ Hz, 1H), 5.36 (d, $J = 7.3$ Hz, 1H), 4.95 (d, $J = 4.9$ Hz, 1H), 4.92 – 4.78 (m, 2H), 4.19 (d, $J = 7.6$ Hz, 1H), 3.81 – 3.74 (m, 2H), 3.70 (dd, $J = 11.2$, 5.2 Hz, 1H), 3.46 (d, $J = 6.9$ Hz, 1H), 3.17 (d, $J = 5.2$ Hz, 1H), 3.14 – 3.03 (m, 2H), 3.03 – 2.92 (m, 1H), 2.83 (t, $J = 2.6$ Hz, 1H), 2.80 – 2.70 (m, 1H), 2.35 – 2.14 (m, 6H), 1.78 (q, $J = 7.2$ Hz, 2H), 1.71 – 1.62 (m, 1H), 1.62 – 1.50 (m, 4H), 1.43 (d, $J = 13.7$ Hz, 1H), 1.00 – 0.88 (m, 12H).

I-150

[00286] To a solution of **Allocyathin B2** (200 mg, 1 eq, 67 μ mol) in dry toluene (6.0 mL) and 4Å MS under a dry and argon atmosphere was added p-toluenesulfonic acid monohydrate (12.7 mg, 10.2 μ L, 0.1 eq, 66.6 μ mol) and neopentyl glycol (100 mg, 94.3 μ L, 1.44 eq, 960 μ mol). The reaction mixture was stirred at 120 °C for 12 hours. EtOAc (8 mL) was added to the reaction mixture, followed by sat. aq. NaHCO₃ sol. (8 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2x 4 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The material was purified by column chromatography on silica gel using a gradient of 0-10% EtOAc in hexane to afford **I-150** (124 mg, 321 μ mol, 48.2 %) as a clear yellow oil. LCMS: $rt = 2.26$, ESI (+) $m/z = 387.4$ [M+H]. ¹H NMR (Acetone-*d*₆, 400 MHz): δ_H 6.06 (1H, d, $J = 7.9$ Hz), 5.57 (1H, d, $J = 7.9$ Hz), 4.77 (1H, s), 3.49-3.61 (5H, m), 2.82-2.89 (1H, m), 2.59-2.73 (2H, m), 2.36 (3H, dd, $J = 9.0$, 6.0 Hz), 1.56-1.72 (4H, m), 1.28-1.34 (1H, m), 1.18 (3H, s), 0.94-1.00 (12H, m), 0.73 (3H, s).

I-151

[00287] To a solution of **I-150** (30.0 mg, 1 eq, 77.6 μ mol) in DCM (2.0 mL) was added Dess-Martin periodinane (49.4 mg, 36.1 μ L, 1.5 Eq, 116 μ mol). The reaction mixture was stirred

at r.t. for 2 hours. Sat. aq. NaHCO_3 sol. (3 mL) was added to the reaction mixture, followed by DCM (3 mL), and the layers were separated. The aqueous phase was extracted with DCM (2x 3 mL). The organic layers were combined, dried over MgSO_4 , filtered through a pad of silica and concentrated under reduced pressure. The material **I-151** (23.0 mg, 59.8 μmol , 77.1 %) was obtained as a pure clear oil according to ^1H NMR. LCMS: $\text{rt}= 2.27$, ESI (+) $m/z= 385.6$ $[\text{M}+\text{H}]$. ^1H NMR (Acetone- d_6 , 400 MHz): δ_{H} 6.27 (1H, d, $J = 5.9$ Hz), 5.87 (1H, d, $J = 5.9$ Hz), 4.87 (1H, s), 3.60-3.65 (2H, m), 3.54 (2H, d, $J = 11.0$ Hz), 3.27-3.36 (2H, m), 2.89-2.96 (1H, m), 2.39 (2H, dd, $J = 9.2, 5.6$ Hz), 1.53-1.77 (4H, m), 1.28-1.34 (1H, m), 1.19 (3H, s), 1.10 (3H, s), 0.99-1.05 (10H, m), 0.74 (3H, s).

I-152

[00288] Following general procedure II-A, compound **I-152** was obtained as a white foam (14.7 mg, 29.7 μmol , 67 %) - mixture of 2 diastereomers. LCMS: $\text{rt}= 1.73$ min, ESI (+) $m/z= 457.4$ $[\text{M}-\text{H}_2\text{O}+\text{H}]$, 325.3 $[\text{M}-\text{sugar}]$. ^1H NMR (Acetone- d_6 , 400 MHz): 5.85 (1H, d, $J = 7.3$ Hz), 5.75 (1H, d, $J = 7.2$ Hz), 5.48 (2H, t, $J = 8.3$ Hz), 4.42 (1H, d, $J = 6.5$ Hz), 4.37 (1H, d, $J = 6.7$ Hz), 3.84 (2H, ddd, $J = 15.6, 11.5, 4.8$ Hz), 3.49-3.57 (6H, m), 3.14-3.36 (8H, m), 2.83-2.89 (3H, m), 2.74 (5H, s), 2.32-2.36 (5H, m), 1.58-1.74 (12H, m), 1.09 (8H, d, $J = 12.5$ Hz), 0.95-1.00 (22H, m), 0.35-0.48 (6H, m), 0.27 (1H, s), 0.18 (1H, d, $J = 6.9$ Hz)

I-153

[00289] Following general procedure II-A, compound **I-153** was obtained as a white foam (10.0 mg, 20.47 μmol , 44%) - mixture of 2 diastereomers. LCMS: $\text{rt}= 1.83$ and 1.85 min, ESI (+) $m/z= 471.6$ $[\text{M}-\text{H}_2\text{O}+\text{H}]$, 339.6 $[\text{M}-\text{sugar}]$. ^1H NMR (Acetone- d_6 , 400 MHz): 5.81 (1H, d, $J = 7.2$ Hz), 5.72 (1H, d, $J = 7.1$ Hz), 5.46 (2H, dd, $J = 8.7, 7.2$ Hz), 4.34 (2H, dd, $J = 12.1, 6.9$ Hz), 4.14-4.26 (1H, m), 3.86-3.97 (5H, m), 3.44-3.55 (4H, m), 3.22-3.40 (8H, m), 2.80-2.85 (4H, m), 2.51-2.72 (5H, m), 2.32-2.36 (5H, m), 1.54-1.97 (23H, m), 1.08 (7H, d, $J = 6.6$ Hz), 0.95-1.00 (21H, m).

I-154

[00290] To a solution of crude Erinacine H (70.0 mg, 1 eq, 123 μmol) in DCM (2.0 mL) cooled at 0 °C was added Oxalyl chloride (15.6 mg, 10.8 μL , 1 eq, 123 μmol) followed by a drop of DMF (5.0 μL). The reaction mixture was allowed to stir at this temperature for 1 hour. MeOH (0.70 mL) was added and the reaction was stirred at r.t. for 1 hour. EtOAc (5.0 mL) was added to the reaction mixture, followed by sat. aq. NaHCO_3 (4 mL), and the layers were separated. The aqueous phase was extracted with EtOAc (3x 3 mL). The organic layers were combined, dried

over MgSO_4 , filtered through a pad of silica and concentrated under reduced pressure. The material was pre-purified by flash column chromatography (silica gel), using a gradient of 0-10% MeOH in EtOAc. The obtained material was then purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (Ammonium Bicarbonate 10 mM) to afford **I-154** (9.00 mg, 19.5 μmol , 16 %) as a white powder. LCMS: $\text{rt}= 1.75$, ESI (+) $m/z= 463.4$ [M-H]. ^1H NMR (400 MHz, Acetone- d_6) δ 7.07 (dd, $J = 8.2$, 1.7 Hz, 1H), 5.70 (d, $J = 8.2$ Hz, 1H), 4.50 (d, $J = 5.7$ Hz, 1H), 4.23 (s, 1H), 3.83 (dd, $J = 11.7$, 4.1 Hz, 1H), 3.70 (s, 3H), 3.61 (dd, $J = 6.9$, 1.3 Hz, 1H), 3.47 (td, $J = 7.2$, 4.1 Hz, 1H), 3.39 (t, $J = 7.0$ Hz, 1H), 3.35 – 3.10 (m, 3H), 2.93 – 2.73 (m, 4H), 2.40 (dd, $J = 9.1$, 5.9 Hz, 2H), 1.80 – 1.55 (m, 4H), 1.46 (d, $J = 13.8$ Hz, 1H), 1.09 – 0.88 (m, 12H).

I-155

[00291] To a solution of methyl **I-154** (38.0 mg, 1 eq, 82.1 μmol) in THF (1.50 mL) cooled at -78°C was added bromomethylmagnesium (3M solution, 58.8 mg, 164 μL , 6 eq, 493 μmol). The reaction mixture was allowed to stir at this temperature for 1 hour and then to slowly warm up to r.t. and be stirred for 12 hours. EtOAc (2 mL) was added to the reaction mixture followed by a 1M HCl sol. (1 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2x 2 mL). Organic layers were combined, dried over MgSO_4 , filtered and concentrated under reduced pressure. The material was pre-purified by flash column chromatography (silica gel), using a gradient of 0-10% MeOH in EtOAc. The obtained material was then purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (ammonium bicarbonate 10 mM). Material may be unstable. LCMS: $\text{rt}= 1.68$, ESI (-) $m/z= 445.4$ [M-H $_2\text{O}$ +H]. ^1H NMR (400 MHz, Acetone- d_6) δ 5.94 (d, $J = 7.1$ Hz, 1H), 5.49 (d, $J = 7.2$ Hz, 1H), 4.32 (d, $J = 7.2$ Hz, 1H), 4.22 (s, 2H), 3.83 (dd, $J = 11.3$, 5.2 Hz, 1H), 3.59 – 3.39 (m, 2H), 3.32 (t, $J = 8.5$ Hz, 1H), 3.27 – 3.09 (m, 2H), 2.90 (d, $J = 6.9$ Hz, 0H), 2.82 (s, 2H), 2.74 (d, $J = 15.0$ Hz, 1H), 2.63 (dd, $J = 15.2$, 8.7 Hz, 1H), 2.39 – 2.28 (m, 2H), 1.99 – 1.86 (m, 1H), 1.85 – 1.68 (m, 2H), 1.68 – 1.53 (m, 3H), 1.36 (s, 3H), 1.30 (s, 3H), 1.14 (s, 3H), 1.04 – 0.88 (m, 9H).

I-156

[00292] To a solution of **I-124** (30 mg, 1 eq, 55 μmol) in dry DMF (0.50 mL) was added cesium carbonate (27 mg, 6.7 μL , 1.5 eq, 83 μmol) followed by methyl iodide (39 mg, 18 μL , 5 eq, 277 μmol). The reaction mixture was stirred at 20°C for 12 hours. EtOAc (4 mL), followed by the addition of sat. aq. NaHCO_3 sol. (4 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2x 2 mL). The organic layers were combined, dried over MgSO_4 ,

filtered and concentrated under reduced pressure. The material was pre-purified by flash column chromatography using 100% EtOAc. The obtained material was purified by reverse phase chromatography (ACCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (AmB 10 mM) to afford **I-156** (3.00 mg, 5.41 μ mol, 10%) as a white powder. LCMS: r_t = 1.82, ESI (+) m/z = 576.9 [M+Na]. ^1H NMR (Acetone- d_6 , 400 MHz): 5.84 (1H, d, J = 7.6 Hz), 5.49 (1H, d, J = 7.6 Hz), 4.41 (1H, d, J = 6.5 Hz), 4.14 (1H, br s), 3.92 (1H, dd, J = 11.6, 4.6 Hz), 3.72-3.84 (2H, m), 3.55 (1H, d, J = 7.1 Hz), 3.45-3.50 (1H, m), 3.35 (1H, t, J = 7.9 Hz), 3.23-3.28 (2H, m), 2.84-2.89 (1H, m), 2.70-2.71 (4H, m), 2.60 (1H, d, J = 16.5 Hz), 2.34 (3H, t, J = 7.5 Hz), 1.55-1.73 (4H, m), 1.46 (1H, d, J = 13.6 Hz), 0.93-1.02 (12H, m).

I-157

[00293] To a solution of **I-151** (202.0 mg, 1 eq, 522 μ mol) in THF (3.00 mL) cooled to r.t. was added sodium hydride (52.3 mg, 44 μ L, 60% Wt, 2.5 eq, 1.31 mmol) in one portion. The reaction mixture was stirred at this temperature for 30 min and diethylsulfate (242 mg, 205 μ L, 3 eq, 1.57 mmol) was added. The reaction mixture was allowed to stir at r.t. for 12 hours. EtOAc (8 mL) was added, followed by sat. aq. sol. NaHCO_3 (5 mL). The layers were separated, and the aqueous one was extracted with (3x 3 mL). Organic layers were combined, dried over NaSO_4 , filtered and concentrated under reduced pressure. The material was purified by flash chromatography on silica gel, using a gradient of 0-10% EtOAc in Hexane to afford **I-157** (164 mg, 0.38 mmol, 73%, 97% Purity) as a clear viscous oil. LCMS: r_t = 2.55, ESI (+) m/z = 415.4 [M+Na]. ^1H -NMR (400 MHz, acetone- d_6) δ 5.90 (d, J = 7.1 Hz, 1H), 5.46 (d, J = 7.3 Hz, 1H), 4.77 (s, 1H), 3.70 (dt, J = 16.2, 7.0 Hz, 1H), 3.60-3.49 (m, 4H), 3.29 (dt, J = 16.2, 7.0 Hz, 1H), 3.17 (d, J = 7.1 Hz, 1H), 2.86-2.76 (m, 1H), 2.64 (dd, J = 16.4, 7.2 Hz, 1H), 2.53 (d, J = 16.3 Hz, 1H), 2.34-2.30 (m, 2H), 2.13-2.06 (m, 1H), 1.71-1.53 (m, 4H), 1.48-1.45 (m, 1H), 1.18 (s, 3H), 1.07 (t, J = 7.0 Hz, 3H), 0.98-0.92 (m, 12H), 0.71 (s, 3H).

I-158

[00294] **I-157** (109.0 mg, 1 eq, 263 μ mol) was diluted in HCl 10% MeOH (2.0 mL) at r.t. The reaction mixture was stirred at 20 $^\circ\text{C}$ for 1 hour. EtOAc (8 mL) was added, followed by sat. aq. sol. NaHCO_3 (5 mL). The layers were separated, and the aqueous one was extracted with (3x 2 mL). Organic layers were combined, dried over NaSO_4 , filtered and concentrated under reduced pressure. The material was purified by flash chromatography on silica gel, using a gradient of 0-10% EtOAc in Hexane to afford **I-158** (32.0 mg, 97 μ mol, 37%) as a clear pale-yellow viscous oil. LCMS: r_t = 2.28, ESI (+) m/z = 329.3 [M+H]. ^1H -NMR (400 MHz, acetone- d_6) δ 9.38 (s, 1H), 6.77 (d, J = 7.8 Hz, 1H), 5.84 (dd, J = 7.9, 2.6 Hz, 1H), 3.47-3.39 (m, 1H), 3.32-3.20 (m, 2H), 3.10 (dd,

$J = 17.4, 6.2$ Hz, 1H), 2.89-2.76 (m, 2H), 2.41-2.35 (m, 4H), 2.05 (t, $J = 2.6$ Hz, 1H), 1.74-1.58 (m, 4H), 1.35 (dd, $J = 13.6, 3.1$ Hz, 1H), 1.04-0.93 (m, 16H).

I-130

[00295] To a solution of **I-158** (30.0 mg, 1 eq, 91 μ mol) in EtOH (1.00 mL) cooled to 0 °C was added sodium borohydride (6.91 mg, 6.46 μ L, 2 Eq, 183 μ mol) in one portion. The reaction mixture was stirred at r.t. for 1 hour. EtOAc (4 mL) was added, followed by sat. aq. sol. NaHCO₃ (3 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3x 2 mL). Organic layers were combined, dried over NaSO₄, filtered and concentrated under reduced pressure. The material was pre-purified on silica gel chromatography using a gradient of 0-20% EtOAc in Hexane. The obtained material was purified by reverse phase chromatography (ACCCQ-Prep system, C18, 20x150 mm), using a gradient of 0-100% acetonitrile and water (AmB 10 mM) to afford **I-130** (3.2 mg, 9.68 μ mol, 11%) as a colorless clear oil. LCMS: $r_t = 2.17$, ESI (+) $m/z = 313.5$ [M-H₂O+H]. ¹H NMR (400 MHz, Acetone-*d*₆) δ 5.82 (dd, $J = 7.1, 1.4$ Hz, 1H), 5.47 (d, $J = 7.3$ Hz, 1H), 4.06 (d, $J = 5.4$ Hz, 2H), 3.80 (t, $J = 5.8$ Hz, 1H), 3.74 – 3.58 (m, 1H), 3.32 (dq, $J = 9.2, 7.0$ Hz, 1H), 3.20 (dd, $J = 7.6, 1.1$ Hz, 1H), 2.94 – 2.77 (m, 2H), 2.59 (dd, $J = 16.2, 7.6$ Hz, 1H), 2.39 – 2.28 (m, 2H), 2.16 – 2.07 (m, 1H), 1.71 (ddd, $J = 12.0, 6.8, 4.9$ Hz, 1H), 1.65 – 1.56 (m, 3H), 1.52 – 1.43 (m, 1H), 1.11 (t, $J = 7.0$ Hz, 3H), 1.06 – 0.87 (m, 12H).

I-159

[00296] **I-159** was synthesized following General Procedure II-D. Erinacine A (20 mg, 0.046 mmol), aniline (13 mg, 0.14 mmol), MeOH (0.23 mL), AcOH (1 drop), NaCNBH₃ (8.7 mg, 0.14 mmol). **I-159** was isolated as a white solid (13 mg, 75%). LCMS: $r_t = 3.914$ min, ESI (+) $m/z = 510.3204$ [M+H] ¹H NMR (400 MHz, Acetonitrile-*d*₃) δ 7.20 – 7.02 (m, 1H), 6.69 – 6.53 (m, 3H), 5.78 (dt, $J = 7.6, 1.3$ Hz, 1H), 5.46 (d, $J = 7.8$ Hz, 1H), 4.70 (t, $J = 5.9$ Hz, 1H), 4.38 (d, $J = 6.6$ Hz, 1H), 3.88 (dd, $J = 11.6, 4.7$ Hz, 1H), 3.73 (d, $J = 5.7$ Hz, 1H), 3.53 (dd, $J = 7.0, 1.5$ Hz, 1H), 3.46 (td, $J = 8.4, 4.7$ Hz, 1H), 3.35 (s, 2H), 3.31 – 3.12 (m, 4H), 2.79 (p, $J = 6.9$ Hz, 1H), 2.70 – 2.53 (m, 2H), 2.38 – 2.21 (m, 3H), 1.74 – 1.52 (m, 5H), 1.41 (d, $J = 13.7$ Hz, 1H), 1.02 – 0.89 (m, 12H).

I-160

[00297] **I-160** was synthesized following General Procedure II-D. Erinacine A (20 mg, 0.046 mmol), 4-fluoroaniline (15.6 mg, 0.14 mmol), MeOH (0.23 mL), AcOH (1 drop), NaCNBH₃ (8.7 mg, 0.14 mmol). **I-160** was isolated as a white solid (11 mg, 46%). LCMS: $r_t = 3.934$ min, ESI (+) $m/z = 528.3142$ [M+H] ¹H NMR (400 MHz, Acetonitrile-*d*₃) δ 6.95 – 6.80 (m, 2H), 6.62 – 6.46

(m, 2H), 5.78 (dq, $J = 7.6, 1.4$ Hz, 1H), 5.46 (d, $J = 7.6$ Hz, 1H), 4.65 (s, 1H), 4.37 (d, $J = 6.6$ Hz, 1H), 3.86 (dd, $J = 11.6, 4.7$ Hz, 1H), 3.69 (s, 2H), 3.52 (dd, $J = 6.9, 1.6$ Hz, 1H), 3.46 (td, $J = 8.3, 4.7$ Hz, 1H), 3.42 – 3.32 (m, 2H), 3.32 – 3.14 (m, 4H), 2.79 (p, $J = 6.9$ Hz, 1H), 2.69 – 2.51 (m, 2H), 2.38 – 2.20 (m, 3H), 1.75 – 1.52 (m, 4H), 1.40 (d, $J = 13.8$ Hz, 1H), 1.01 – 0.90 (m, 12H). ^{19}F NMR (377 MHz, Acetonitrile- d_3) δ -131.17.

I-161

[00298] I-161 was synthesized following General Procedure II-D. Erinacine A (20 mg, 0.046 mmol), *N*-methylaniline (15 mg, 0.14 mmol), MeOH (0.23 mL), AcOH (1 drop), NaCNBH₃ (8.7 mg, 0.14 mmol). I-161 was isolated as a white solid (9 mg, 38%). LCMS: t_r =4.354 min, ESI (+) m/z = 524.3354 [M+H]. ^1H NMR (400 MHz, Acetonitrile- d_3) δ 7.22 – 7.11 (m, 2H), 6.76 – 6.68 (m, 2H), 6.62 (tt, $J = 7.3, 1.1$ Hz, 1H), 5.55 (dt, $J = 7.5, 1.8$ Hz, 1H), 5.47 – 5.40 (m, 1H), 4.35 (d, $J = 6.8$ Hz, 1H), 3.92 (d, $J = 1.8$ Hz, 2H), 3.84 (dd, $J = 11.5, 4.8$ Hz, 1H), 3.53 (dd, $J = 6.4, 2.0$ Hz, 1H), 3.45 (td, $J = 8.6, 4.9$ Hz, 1H), 3.36 (d, $J = 19.8$ Hz, 2H), 3.31 – 3.14 (m, 4H), 2.94 (s, 3H), 2.78 (p, $J = 6.9$ Hz, 1H), 2.62 – 2.45 (m, 2H), 2.39 – 2.20 (m, 3H), 1.75 – 1.51 (m, 4H), 1.41 (d, $J = 13.7$ Hz, 1H), 1.03 – 0.90 (m, 12H).

I-162

[00299] I-162 was synthesized following General Procedure II-D. Erinacine A (40 mg, 0.092 mmol), 4-aminoacetophenone (37 mg, 0.28 mmol), MeOH (0.50 mL), AcOH (3 drops), NaCNBH₃ (17.6 mg, 0.28 mmol). I-162 was isolated as a yellow solid (23 mg, 45%). LCMS: t_r =3.581 min, ESI (+) m/z = 552.3313 [M+H]. ^1H NMR (400 MHz, Acetonitrile- d_3) δ 7.84 – 7.69 (m, 2H), 6.72 – 6.52 (m, 2H), 5.77 (d, $J = 7.6$ Hz, 1H), 5.45 (d, $J = 7.5$ Hz, 2H), 4.37 (d, $J = 6.8$ Hz, 1H), 3.87 (dd, $J = 11.5, 4.8$ Hz, 1H), 3.81 (d, $J = 5.8$ Hz, 2H), 3.54 (dd, $J = 6.8, 1.5$ Hz, 1H), 3.46 (s, 1H), 3.42 – 3.31 (m, 2H), 3.31 – 3.12 (m, 4H), 2.86 – 2.72 (m, 1H), 2.70 – 2.52 (m, 2H), 2.42 (s, 3H), 2.33 (td, $J = 6.9, 3.1$ Hz, 2H), 1.74 – 1.52 (m, 4H), 1.44 – 1.25 (m, 3H), 1.03 – 0.92 (m, 12H).

I-163

[00300] I-163 was synthesized following General Procedure II-D. Allocyathin B2 (26 mg, 0.086 mmol), 3-aminopyrazole (22 mg, 0.26 mmol), MeOH (0.43 mL), AcOH (1 drop), NaCNBH₃ (16 mg, 0.26 mmol). I-163 was isolated as a yellow solid (12 mg, 38%). LCMS: t_r =1.817 min, ESI (+) m/z = 366.2550 [M+H-2H]. ^1H NMR (700 MHz, Acetonitrile- d_3) δ 10.70 (s, 2H), 7.05 (d, $J = 6.3$ Hz, 2H), 5.70 (d, $J = 7.9$ Hz, 1H), 5.47 (d, $J = 7.9$ Hz, 1H), 4.24 (s, 1H), 3.83 (d, $J = 42.5$ Hz, 4H), 3.46 (p, $J = 3.8$ Hz, 1H), 3.33 – 3.13 (m, 1H), 2.80 (p, $J = 6.8$ Hz, 1H), 2.64 (s, 3H), 2.36 – 2.28

(m, 2H), 1.67 (ddd, $J = 12.0, 6.4, 5.2$ Hz, 1H), 1.65 – 1.49 (m, 3H), 1.23 (ddt, $J = 17.4, 14.0, 3.7$ Hz, 1H), 0.97 (d, $J = 6.9$ Hz, 3H), 0.95 – 0.91 (m, 6H), 0.88 (s, 3H).

I-164

[00301] I-164 was synthesized following General Procedure I-B from Allocyathin B2 carboxylic acid II-2 (35 mg, 0.11 mmol), *N*-methylaniline (15 mg, 0.14 mmol), HATU (53 mg, 0.14 mmol), DIPEA (28 mg, 0.22 mmol) and DMF (1.1 mL). I-164 was isolated as a white solid (10 mg, 27%). LCMS: $rt=4.357$ min, ESI (+) $m/z = 406.2745$ [M+H]. ^1H NMR (400 MHz, Acetonitrile- d_3) δ 7.41 – 7.28 (m, 2H), 7.27 – 7.12 (m, 3H), 5.92 (dd, $J = 8.1, 2.1$ Hz, 1H), 5.36 (d, $J = 8.1$ Hz, 1H), 3.42 (ddd, $J = 8.2, 6.2, 1.7$ Hz, 1H), 3.29 (s, 3H), 2.72 – 2.51 (m, 2H), 2.43 (dt, $J = 18.0, 2.0$ Hz, 1H), 2.35 – 2.24 (m, 3H), 2.24 – 2.16 (m, 1H), 1.70 – 1.49 (m, 4H), 1.25 – 1.16 (m, 1H), 0.96 – 0.82 (m, 9H), 0.79 – 0.69 (m, 3H).

I-6

[00302] I-6 was synthesized following General Procedure I-B from Allocyathin B2 carboxylic acid II-2 (25 mg, 0.079 mmol), dimethylamine-HCl (19.6 mg, 0.24 mmol, 3 eq), HATU (45.6 mg, 0.12 mmol, 1.5 eq), DIPEA (41.4 mg, 0.32 mmol, 4 eq) and DMF (0.79 mL). I-6 was isolated as a beige solid (17 mg, 63%). LCMS: $rt=3.5908$ min, ESI (+) $m/z = 344.2598$ [M+H]. ^1H NMR (700 MHz, Acetonitrile- d_3) δ 5.94 (dd, $J = 7.9, 2.2$ Hz, 1H), 5.60 (d, $J = 7.8$ Hz, 1H), 3.60 (ddd, $J = 7.8, 6.3, 1.5$ Hz, 1H), 2.96 (s, 6H), 2.84 (q, $J = 6.9$ Hz, 1H), 2.74 – 2.66 (m, 2H), 2.63 (dd, $J = 17.4, 6.3$ Hz, 1H), 2.37 (dd, $J = 9.1, 6.0$ Hz, 2H), 2.32 (td, $J = 13.5, 5.0$ Hz, 1H), 1.74 – 1.57 (m, 4H), 1.30 (dt, $J = 13.9, 3.8$ Hz, 1H), 1.01 (d, $J = 6.9$ Hz, 3H), 0.96 (d, $J = 10.3$ Hz, 9H).

I-165

[00303] I-165 was synthesized following General Procedure I-B from Allocyathin B2 carboxylic acid II-2 (20 mg, 0.063 mmol), methylamine hydrochloride (13 mg, 0.19 mmol, 3 eq), HATU (36 mg, 0.094 mmol, 1.5 eq), DIPEA (32.6 mg, 0.25 mmol, 4.0 eq), and DMF (0.6 mL). I-165 was isolated as a white powder (4 mg, 19%). LCMS: $rt=3.2094$ min, ESI (+) $m/z = 330.2438$ [M+H]. ^1H NMR (700 MHz, Acetonitrile- d_3) δ 6.62 (dd, $J = 8.1, 2.2$ Hz, 1H), 6.55 – 6.42 (m, 1H), 5.66 (d, $J = 8.1$ Hz, 1H), 3.58 (ddd, $J = 8.3, 6.5, 1.5$ Hz, 1H), 2.91 – 2.80 (m, 2H), 2.73 (d, $J = 4.7$ Hz, 3H), 2.67 (dt, $J = 17.4, 1.9$ Hz, 1H), 2.47 (d, $J = 8.4$ Hz, 1H), 2.37 (dd, $J = 9.1, 6.0$ Hz, 2H), 2.31 (td, $J = 13.6, 4.9$ Hz, 1H), 1.74 – 1.58 (m, 4H), 1.33 – 1.28 (m, 1H), 1.01 (d, $J = 7.0$ Hz, 3H), 0.98 – 0.93 (m, 9H).

I-167

[00304] Following General Procedure I-B, Erinacine H (14 mg), dimethylamine hydrochloride (3.1 mg), HATU (14.1 mg), DIPEA (8 mg) and DCM (0.31 mL) afforded **I-167** as a white solid (4.3 mg, 31%). ¹H NMR (700 MHz, Chloroform-*d*) δ 5.99 (dd, *J* = 7.9, 1.7 Hz, 1H), 5.56 (d, *J* = 8.0 Hz, 1H), 4.60 (d, *J* = 5.5 Hz, 1H), 4.14 (dd, *J* = 12.1, 4.1 Hz, 1H), 3.75 – 3.62 (m, 3H), 3.60 (t, *J* = 7.0 Hz, 1H), 3.49 (d, *J* = 5.7 Hz, 1H), 3.40 (dd, *J* = 12.2, 7.2 Hz, 2H), 3.03 (s, 6H), 2.96 – 2.89 (m, 1H), 2.88 – 2.85 (m, 2H), 2.85 – 2.74 (m, 1H), 2.40 – 2.31 (m, 2H), 2.26 (td, *J* = 12.7, 6.1 Hz, 1H), 1.71 (dt, *J* = 12.2, 5.6 Hz, 1H), 1.68 – 1.53 (m, 4H), 1.35 – 1.20 (m, 2H), 1.00 – 0.95 (m, 9H), 0.94 (d, *J* = 6.8 Hz, 3H).

Results:

Purification of Erinacine A from lion's mane mycelium extract.

[00305] Erinacine A (ErA) was detected in the *Hericium erinaceus* mycelial extract by LC-MS-ESI analysis (positive mode) and at absorption wavelength 340 nm (Figure 2A and 2B). Peaks corresponding to mass of the ErA aglycon (*m/z* 301.2177), [M+Na]⁺ *m/z* 455.2424 and [2M+Na]⁺ *m/z* 887.4951 were obtained (Figure 2C). The ErA concentration in the mycelia was determined to be 10 mg of ErA per gram of mycelia. To obtain bulk quantities of ErA to be used in *in vitro* biological assays and semi-synthesis, its purification protocol was optimized, as described in experimental procedures above, and its spectroscopic parameters were confirmed by NMR analysis [H. Kawagishi, 1994].

Testing the neurotrophic potential of Erinacine A using neurite outgrowth assay.

[00306] The ability of ErA to stimulate nerve growth factor (NGF) production in human cells was quantified using the neurite outgrowth assay. Immortalized human astrocytoma cells like U87 are known to produce and secrete NGF and BDNF protein when stimulated by neurotrophin inducing compounds [A. Zabłocka, 2015]. Rat pheochromocytoma (PC12) cells further provide a well characterized model for neuronal differentiation and neurite outgrowth studies. PC12 cells respond to NGF by activating the receptor tyrosine kinase, TrkA, in response to which they cease to proliferate, extend multiple neurites, and acquire properties of sympathetic neurons [N. Inagaki, 1995]. To study the potential of ErA for inducing neurite outgrowth in PC12 cells, these were exposed to conditioned supernatants from ErA stimulated U87 cells and imaged after 24 h and 48 h. As a positive control, U87 cells were also exposed to J147, a novel drug shown to have neurotrophic potential and proposed for treatment of Alzheimer's disease (AD) [M. Prior, 2013].

Supernatants harvested from J147, and ErA treated cells displayed the presence of neurite bearing PC12 cells unlike the cells treated with DMSO or untreated PC12 cells (Figure 3)

In vitro toxicity testing of Erinacine A

[00307] The cytotoxicity of ErA against normal human cells was evaluated by using Peripheral Blood mononuclear cells (PBMCs). PBMCs are isolated from the peripheral blood and comprise of multitude of immune cells like lymphocytes, monocytes, and dendritic cells. They are widely used as a model in drug discovery and toxicity screening [J. Pourahmad, 2015]. The viability of PBMCs exposed to ErA (0-60 μ M) was assessed using the PrestoBlue reagent after 24 h. The assay measures cell viability and cytotoxicity by quantifying the reduction of resazurin dye by living cells, producing a fluorescent signal proportional to the number of viable cells. ErA exhibited significant loss of cell viability at 30 μ M and upwards (Figure 4).

Testing the in vitro BBB permeability of Erinacine A

[00308] Upon establishing the neurotrophic potential of ErA by the neurite outgrowth assay, the PAMPA-BBB method was employed to study its ability to cross the blood-brain barrier *in vitro* [L. Di, 2003]. This assay is commonly used to predict the BBB penetration of central nervous system associated drugs. It utilises a donor compartment and an acceptor compartment, separated by a filter supporting a liquid artificial membrane made of different phospholipid mixtures. The compound being tested is placed in the donor compartment and allowed to permeate through the artificial membrane between the donor and acceptor compartments. As shown in Figure 5, ErA exhibited a remarkable *in vitro* BBB permeability, thus establishing its potential for the treatment of CNS related disorders. This observation was found to be in accordance with a recent study which showed that ErA can penetrate the BBB of rats *in vivo* by the means of passive diffusion [P. C. Tsai, 2021].

Characterizing the biological activity of ErA aglycon

[00309] As mentioned previously the ErA aglycon was obtained as shown in Scheme 1 and was characterized for its neurotrophic potential, cytotoxicity, and *in vitro* BBB permeability. As shown in Figure 6A, the aglycon showed approximately 80% cell viability at 30 μ M concentration, unlike the parent molecule which displayed only 40% viability at the same concentration (Figure 4). The ErA aglycon also exhibited neurite outgrowth activity on PC12 cells same as ErA (Figure 6B). Interestingly, however in the BBB-PAMPA assay, KKEB004 seemed to lose the ability to penetrate the phospholipids layered onto the membrane. Therefore a semi-

synthesis approach to obtain ErA analogs that display no cellular toxicity, have neurotrophic activity, and have restored the BBB permeability was explored.

Characterizing ErA analogs (compounds of the application) for their toxicity on primary cells

[00310] As mentioned previously in experimental procedures, analogs of the (i) ErA aglycon and at the (ii) aldehyde functional group of ErA were obtained as shown in Schemes 2-6 (exemplary compounds of the application).

[00311] Further the synthetic compounds were examined for their *in vitro* cytotoxicity against PBMCs in comparison to ErA. As shown in Figures 7 and 8, PBMCs were exposed to varying concentrations of analogs at concentrations 1, 3, 10 and 30 μ M for 24 h and percentage viability was assessed using the PrestoBlue reagent. Interestingly, compounds I-108, I-34, II-2, I-3, I-52 displayed >90% viability at 30 μ M, unlike ErA. Also, analogs I-39, I-4, I-6, I-53 displayed 70-80% viability at concentration 30 μ M in the PrestoBlue assay (Table 3).

Table 3: Summary of the cytotoxicity analysis of exemplary compounds of the application against Peripheral Blood Mononuclear Cells (PBMCs)

	Compounds of the Application
Compounds that displayed >90% viability at 30 μ M	I-108, I-34, II-2, I-3, I-52
Compounds that displayed 70-80% viability at 30 μ M	(ErA Aglycon, I-39, I-4, I-6, I-53

Characterizing exemplary compounds of the application for in vitro BBB permeability

5 [00312] Exemplary compounds of the application that displayed no or less cytotoxicity than the parent compound (ErA) were obtained. These analogs were assessed for their potential to cross the BBB in the BBB-PAMPA assay. As shown in Figure 9, analog II-2 exhibited a higher permeability than ErA while I-4 maintained a similar permeability. However, all the other analogs displayed lower ability to penetrate the BBB *in vitro*. Also, compounds I-52 and I-53 could not be tested in the assay
10 since they precipitated immediately in PBS, while making the required 500 μ M dilution for measurement.

[00313] Since II-2 showed no associated cytotoxicity in primary cells and a remarkable BBB permeability, its neurotrophic potential on PC12 cells was examined. Encouragingly, II-2 at concentration 0.3 μ M exhibited neurite outgrowth potential similar to ErA (Figure 10).

15 *Conclusion*

In this study, a semi-synthesis approach was employed to understand the Structure Activity Relationship (SAR) of ErA analogs, in order to improve the therapeutic index of ErA, i.e., reduce cellular toxicity, improve its neurotrophic potential, enhance/maintain BBB permeability as well as to improve its solubility and pharmacokinetic parameters.

20 *Example 3*

EXPERIMENTAL PROCEDURES:

In vitro neurite outgrowth assay in mouse hippocampal and primary rat sensory neurons

[00314] The HT22 cells were seeded at density of 20,000 cells/ml in 384 well plates with an optical bottom in high glucose DMEM medium and allowed to adhere at 37°C in the presence of 5% CO₂. After 24 hrs, cells were moved to serum free DMEM and treated with ErA and NGF (100 ng/ml). The final concentration of DMSO in the treatments did not exceed 0.5%. Plates were incubated for 5 48 hours, after which the cells were fixed with ice-cold methanol for twenty minutes and rinsed two times with 1X PBS. To avoid nonspecific binding, wells were blocked with PBS containing Triton X-100 (0.2 %) and 1 % Bovine Serum Albumin (BSA). Subsequently, the cells were incubated with primary antibody against beta-tubulin (E7, DSHB) at dilution factor (DF) of 1 in 13.7 and left overnight at 4°C on a shaker. Following incubation, wells were washed thrice with 1X PBS at room temperature, 10 with the incubation of ten minutes each between subsequent washes. Fluorescein isothiocyanate (FITC) labelled secondary antibody (ThermoFisher Scientific) at DF of 1 in 200. Nuclei were counterstained with 5 µg/ml Hoechst 33342 (Thermo Fisher Scientific) for 30 minutes in dark and thereafter washed thrice with 1X PBS. At the last step, 50 µl of PBS was added to the wells and images were captured using the BioTek Cytation 5 Cell Imaging Multimode Reader (Agilent 15 Technologies). All treatments were performed in triplicates and images were captured at 20X magnification.

[00315] For the neurite outgrowth assay in rat sensory neurons, experiments were conducted by Neuron Experts SAS, France. Briefly, dorsal root ganglia (DRG) neurons were isolated from embryonic day 15 female rats and cultured in DMEM-F-12 (PanBiotech) supplemented with 1% N-2 20 (Gibco), L-Glutamine, Phosphatidyl serine (2 %) and NGF (5 ng/ml). Cells were seeded at density of 20,000 cells/well in 96 well-plates (precoated with poly-D-lysine; Greiner) and incubated at 37°C in the presence of 5% CO₂. Half of the medium was changed every two days with fresh medium. After 4 days of culture, cells were treated with ErA (0.003 µM – 10 µM) and NGF (50 ng/ml) and DMSO (0.1 %). All treatments were performed in six replicates. After 3 days of treatment, cells were fixed 25 with 5 % acetic acid and 95 % ethanol for 5 minutes at 20°C. The cells were then permeabilized and non-specific sites were blocked with 1X PBS containing 0.1 % saponin (Sigma). Subsequently, the cells were incubated with monoclonal mouse anti-beta-tubulin antibody (1/2000, Sigma Aldrich T8660) in PBS overnight at 4°C. Alexa Fluor 568 labelled goat anti-mouse secondary antibody (1/400, Molecular probe) in PBS with 1 % fetal calf serum (FCS) was added for 1 hour at RT. Nuclei were 30 labelled with Hoechst 33342 (Sigma) in the same solution. Images were captured using the InCell Analyzer™ 2200 (GE Healthcare) at 20X magnification and analysis was performed using the Developer software (GE Healthcare).

In vitro migration assay

[00316] Primary dermal fibroblasts from normal human adult (ATCC) were cultured in low serum fibroblast growth medium 2 (PromoCell) at 37°C in presence of 5% CO₂ until confluent. For the *in vitro* migration assay, 2-well cell culture inserts (Ibidi) were used and placed in each well of the 24-well plate with the help of sterile forceps. 70 µl of fibroblast cells was added on either side of the insert at cell density of 1.5 X 10⁵ cells per ml. Plates were incubated at 37°C until a confluent monolayer was formed after which the inserts were carefully removed from each well. The wells were gently washed twice with fibroblast basal media (PromoCell) to remove any cellular debris, and 400 µl of fresh basal medium was added to each well. ErA was added at various concentrations (0.01 µM – 1 µM). DMSO (0.5%) treated wells were used as the untreated control. Plate was incubated at 37°C in the presence of 5% CO₂ and was monitored and imaged at regular intervals under a microscope to assess the extent of cell migration (EVOS M7000). All treatments were done in triplicates and images were captured at 4X magnification.

In vitro tube formation assay

[00317] The tube formation assay was performed using Human Umbilical Vein Endothelial Cells (HUVEC) from PromoCell as described by Arnaoutova *et al.* with some modifications [Arnaoutova I, 2010]. Cells were grown in low serum endothelial cell growth medium (PromoCell) at 37°C in presence of 5% CO₂ until they reached confluence. To study the angiogenic potential of ErA on endothelial cells, growth factor containing supernatant from ErA treated fibroblast cells was added onto HUVEC cells as described below. Primary dermal fibroblast cells were grown at 37°C in presence of 5% CO₂ until confluent. Fibroblasts were then trypsinized and seeded in 96-well plates at a density of 1.5 X 10⁵ cells per ml (200 µl). After overnight incubation, the wells were washed twice with fibroblast basal medium and treated with ErA at various concentrations (and 0.5 % DMSO) in triplicates. 30 minutes prior to the tube formation assay with HUVEC cells, black 96-well plates with optical bottom (Nunc) were pre-coated with 70 µl of reduced growth factor basement membrane matrix, Geltrex (ThermoFisher Scientific) and incubated at 37°C. Following 24 hrs of incubation of fibroblasts with ErA, 150 µl supernatants from each well were removed carefully and HUVEC cells at density of 1.5 X 10⁵ cells per ml were resuspended into it. 120 µl of the HUVECs resuspended in supernatant were then gradually added on top of the Matrigel coated wells. Plates were incubated at 37°C in presence of 5% CO₂ to allow the cells to organize into tubular structures, mimicking *in vivo* angiogenesis. After 16 hrs of incubation, tubes were stained with Calcein AM (6 µM) and labeled cells

were observed and photographed using the EVOS M7000 fluorescent microscope at 20X magnification.

Cell proliferation assay

[00318] Proliferation assay for fibroblasts and endothelial cells was performed using the Presto Blue viability dye (ThermoFisher Scientific) as per the manufacturer's instructions. 200 µl of each cell type was seeded at density 2.5×10^4 cells/ml in four 96-well white plates with optical bottom (Nunc) and allowed to adhere overnight. Each plate pertained to the individual time points of 6 h, 12 h, 24 h and 48 h at which the extent of proliferation was evaluated. ErA was added to all plates at concentration of 0.01 µM – 1 µM in triplicates. Control wells were treated with 0.5% DMSO. Following incubation with ErA at the required end point, Presto Blue reagent (20 µl) was added to the wells and incubated with for 2 h in the dark. Metabolically active cells reduced the dye, resulting in a change in color and fluorescence intensity, which was proportional to the number of viable cells. The fluorescence signal was measured using a microplate reader at excitation/emission of 560/590 nm. A no cell media blank and control wells containing untreated cells were included for baseline measurements.

Trans well migration assay

[00319] To investigate the migration of HUVECs in response to ErA treatment, the transwell migration assay was employed which used Hoechst staining to identify the migrated cells. Briefly, 150 µl of HUVECs (1.5×10^5 cells per ml) resuspended in serum free DMEM were added to sterile 1.5 ml Eppendorf tubes and treated with ErA (0.01- 10 µM) in triplicates. Plates containing 6.5 mm Transwell with 8.0 µm pore polycarbonate membrane inserts (Corning) were used for the assay. 100 µl of each cell suspension containing exemplary compounds of the application was carefully plated onto the filter membrane in the transwell insert and incubated for 10 minutes at 37 °C and 5% CO₂ to facilitate the settling down of cells. 600 µl of DMEM supplemented with 10 % FBS was later added into the bottom of the lower chamber, without moving the trans well insert and without generating bubbles. After 24 h of incubation, non-migratory cells in the upper chamber were removed carefully with a cotton tip applicator, and the migrated cells adhering to the lower surface of the membrane were fixed with 4 % formaldehyde. These cells were then stained with Hoechst dye, which selectively binds to DNA, allowing to visualize and quantify the migrated cell nuclei under fluorescence microscopy. The number of Hoechst-stained nuclei in predefined fields of view was counted and used as a measure of migrated cells.

In vivo biopsy punch model for wound healing

[00320] For the *in vivo* wound healing studies with ErA, six to eight weeks old female diabetic mice, each weighing 40-45 g, were obtained from the Jackson Laboratories (BKS.Cg-Dock7m +/- Leprdb/J ; JAX stock #000642). Animals were acclimatized for one week before the experiment. Mice were anesthetized with 4% isofluorane and eye lubricant was then applied. Their nails were trimmed, and blood glucose was measured using a OneTouch Verio Reflect (the tail of a mouse was pricked with a lancet to withdraw blood). The dorsal surface of the mouse was then shaved, and the area was treated with an ethanol wipe. To cause the punch, mice were rotated on their side, and their skin was pinched along their midline. The skin was then folded over a metal surface, and a 6 mm biopsy punch was created with disposable device, yielding two wound areas per mice. Immediately after, 10 µL of treatment was applied to each wound (either solvent control 80% butylene glycol, 0.5 mg ErA or 1 mg ErA). Mice recovered on a heating pad until they were alert and singly caged. Treatment was applied every 24 hours and wound healing progress was assessed by taking images of the injured area every day. Mice were sacrificed on day 8 and the wound tissues were collected for histology. All animal procedures were conducted in accordance with the guidelines of the Institute Animal Ethics committee (AUP NO. 20-12-43)

Histological analysis and Immunohistochemistry

[00321] Tissues were collected from the wound sites using a biopsy punch on day 8 of the experiment. Samples were fixed in 10% formalin for 48 hours. They were then rinsed once with 1X PBS, immersed in 70 % ethanol, and further processed at the MIRC histology Core Facility at McMaster Immunology Research Centre. Tissues were embedded in paraffin, and 3-µm-thick sections were cut and proceeded for Hematoxylin and Eosin (H&E) staining and *Masson's trichrome staining*. Expression of key biomarkers was evaluated by immunohistochemistry using antibodies against CD31, fibronectin, Ki67 and IL-1b. Images were captured at 4X magnification on the EVOS M7000 microscope and at 10X magnification on the EVOS™ XL Core Imaging System.

RESULTS AND DISCUSSION*Studying the neurotrophic potential of ErA on hippocampal neurons by neurite outgrowth assay.*

[00322] Neurite outgrowth plays a role in the establishment of neuronal circuits during development and in the formation of functional neural networks [Reese D, 1998]. The dynamic extension of neurites enables neurons to establish connections with target cells, forming synapses and facilitating communication within the nervous system [Batool S, 2019]. Neurotrophic factors, like

NGF and BDNF, have long been recognized for their roles in promoting neurite outgrowth and synaptic plasticity [Castrén E, 2017, Bathina S, 2015]. Neurotrophin based treatments to counteract the progression of neurodegenerative diseases extend back to at least twenty five years [Alfonsetti M, 2023]. However, recently there has been a growing interest in identifying small molecules with neurotrophic properties as potential alternatives to protein-based neurotrophic factors. These molecules offer advantages such as improved bioavailability, better BBB permeability, ease of administration, and enhanced drug-like properties which makes them attractive candidates for therapeutic development [Schiavone S, 2018, Kazim SF, 2016].

[00323] HT22 neurons, derived from the murine hippocampus, provide a reliable platform to study neurite outgrowth [Liu Z, 2021]. These cells exhibit neuronal characteristics, including the expression of specific markers such as β -III tubulin and MAP2, while offering the practical advantages of a stable cell line. ErA was purified from erinacine A enriched *H. erinaceus* mycelium and its neurotrophic potential on hippocampal neurons was evaluated by visualising tubulin morphology by immunofluorescence. Following exposure to ErA for 48 hours in serum free media, a significant increase in neurite lengths was observed at 0.1 nM concentration, in comparison to the DMSO treated cells (Figure 11), thus indicating its neurotrophic potential in the CNS.

Studying the neurotrophic potential of ErA on sensory neurons by neurite outgrowth assay

[00324] Cutaneous wounds are often associated with anatomical and/or functional impairment of peripheral sensory neurons at the site of injury [Matsuda H, 1998]. NGF has been shown to be produced at the wound site and is speculated to play a role in the regeneration of injured neurons. Supplementation with NGF and BDNF has been reported to enhance the survival and axonal regrowth of injured neurons following spinal cord injury [Harvey AR, 2015]. Also, *in vitro* studies using fruiting body extracts of *H. erinaceus* have indicated their regenerative potential on peripheral neurons following laser microdissection [Üstün R, 2019]. It should be noted that erinacines are not extracted from the fruiting bodies but rather the mycelium of *H. erinaceus*. However, the neurotrophic activity of purified ErA was explored on sensory neurons isolated from embryonic day 15 rats. Primary sensory neurons were cultured for four days and further treated with varying concentrations of ErA (0.003 μ M-10 μ M) for a period of three days after which the plates were visualized and examined for neurite outgrowth. As shown in Figure 12A and B, treatment with ErA at 0.01 μ M exhibited a significant increase in neurite outgrowth, at par with the positive control, NGF. Moreover, ErA exhibited a neuroprotective effect on the survival of sensory neurons in comparison to the DMSO

treated wells (Figure 13). This observation thus establishes the remarkable neurotrophic and neuroprotective potential of ErA on peripheral sensory neurons.

Studying the effect of ErA on fibroblast migration and proliferation

[00325] Fibroblasts are the main cellular components of dermis layer of the skin and fibroblast migration is known to play a role during the inflammatory phase of the wound healing process [Tracy LE, 2016]. Encouraged by the prominent activity of ErA on sensory neurons, it was decided to explore its wound healing potential by studying its effects on the migration of fibroblast cells *in vitro*. Primary human dermal fibroblast cells were seeded in 24-well plates using 2-well silicone inserts, such that, a cell-free gap of 70 μm is created after cell attachment, in which the cellular migration can be visualized. Cells were treated with varying concentrations of ErA (0.01 μM – 1 μM) and plates visualised at regular intervals until the gap was observed to be filled. The assay was carried out in serum free media to make sure that the effects observed, are strictly due to cellular migration and not related to cell proliferation. Remarkably, ErA exhibited a dose-dependent response causing accelerated migration of fibroblasts, thus filling the wound gap completely in 24 hours at 1 μM , in comparison to DMSO (Figure 14).

[00326] Since small molecules that induce the secretion of growth factors secretion, can also have the potential to induce cellular proliferation, a PrestoBlue assay cell viability assay that allows the monitoring of changes in cellular proliferation with time was conducted [Xu M, 2015]. Fibroblast cells were seeded in 96-well plates and treated with varying ErA concentrations for 6 h, 12 h, 24 h and 48 h in serum containing media. At the end of each time interval, Prestoblue dye was added to the treated wells and cell viability (or cell proliferation) was assessed by measuring the fluorescence. We observed similar levels of viability in ErA treated cells and DMSO treated cells, thus concluding that ErA did not induce fibroblast proliferation *in vitro* (Figure 15).

Studying the effect of ErA on angiogenesis by endothelial cells

[00327] Angiogenesis, i.e. the process by which new blood vessels are formed from existing ones is a component of the wound healing process as it ensures an adequate blood supply to the injured area [Johnson KE, 2014]. Accelerating blood vessel formation enhances nutrient and oxygen delivery, needed for efficient tissue repair. Endothelial cells form the inner lining of blood vessels and actively participate in creating new capillaries in and around the wound site. The endothelial cell tube formation assay is a valuable tool to assess the potential of therapeutic agents to promote angiogenesis at the site of injury [Arnaoutova I, 2010]. It involves culturing endothelial cells on a gel

matrix and allows to examine the formation of capillary-like structures resembling blood vessels, upon compound treatment. Primary endothelial cells derived from human umbilical veins (HUVECs) were used to study tube formation in response to treatment with ErA.

[00328] Furthermore, the crosstalk between HUVECs and other cell types involved in wound healing, such as fibroblasts and immune cells, is important for a coordinated healing response. Hence, the angiogenic potential of ErA on endothelial cells was studied by employing an indirect assay, where the supernatants obtained from ErA treated fibroblast cells was added to HUVEC cells and the extent of tube formation was studied in comparison to control. The rationale behind first exposing fibroblast cells to ErA for 24 hrs and exposing HUVECs to compound treated supernatants was to mimic the wound environment more closely. Treatment with ErA would induce the secretion of neurotrophic factors in fibroblasts, which would then signal the endothelial cells to promote vascularisation and wound repair. Interestingly, ErA induced the occurrence of tube-like structures *in vitro* in HUVEC cells, in comparison to DMSO treated cells, thus demonstrating its ability to promote new blood vessel formation during angiogenesis (Figure 16). The tube formation phenotype could also be attributed to the secretion of growth factors like vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which are regulated by NGF, and stimulate angiogenesis [Lamalice L, 2007].

[00329] Further, the effect of ErA on the cellular proliferation of HUVEC cells was assessed by employing the Presto Blue assay, as described previously for fibroblasts. Treatment with Erinacine A did not induce HUVEC cell proliferation and similar levels of viability were observed in both compound and DMSO treated cells (Figure 17).

[00330] The migration of endothelial cells constitutes an aspect of vascularisation and tissue repair following injury [Lamalice L, 2007]. This dynamic process is governed by chemotactic, haptotactic, and mechanotactic stimuli. Endothelial cell chemotaxis is induced by growth factors, such as VEGF and bFGF, while haptotaxis involves the migration of ECs in response to an extracellular matrix (ECM) gradient. Trans-well chamber migration assay was used to assess the effect of ErA on endothelial cells migration. As shown in Figure 18 A-B, ErA exhibited increased HUVEC cell migration at 10 μ M, which was relatively high in comparison to the concentrations which promote endothelial cells tube formation. This indicates the involvement of different mechanisms or growth factors induced by ErA in the two processes, leading to a robust induction of pro-angiogenic stimuli without promoting endothelial cell migration.

ErA accelerated wound closure in diabetic mice in vivo.

[00331] The effect of ErA in a mouse model of diabetic wound healing (db/db) was also evaluated. These mice possess a mutation in their leptin receptor leading to type 2 diabetes, with severe obesity and hyperglycemia. They are commonly used for wound healing research since they exhibit a delayed wound healing phenotype and exhibit wound closure primarily by epidermal migration rather than wound contraction [Sullivan SR, 2004]. Excisional wounds were made on the dorsal area of mice and were treated topically with vehicle or ErA at regular intervals, until wound closure was observed. Wounds treated with ErA (at 0.5 and 1 mg doses) for 8 days, exhibited accelerated wound closure in comparison to the control group (Figure 19).

[00332] Further, the histological analysis of wound tissues by hematoxylin and eosin staining also displayed enhanced re-epithelialisation with ErA treatment than the solvent treated wounds (Figure 20).

[00333] The tissue sections were also stained with Masson's trichrome which is commonly employed to visualise collagen fibers and evaluate the phase of wound healing. It stains collagen fibres blue, while muscle fibres are stained red. Treatment with ErA at 1 mg dose displayed higher collagen density in the wound area, in contrast to control untreated wounds (Figure 21).

[00334] Further, immunohistochemical staining in the mice tissues was used, to examine the levels of biochemical markers associated with different stages of wound healing. This method involves the use of antibodies that selectively bind to the target proteins. The bound antibody can then be detected by the immune-peroxidase technique, that uses diaminobenzidine as the chromogen for visualization. Interleukin-1 beta (IL-1 β) is an inflammation marker associated with wound healing. It is a pro-inflammatory cytokine known to play a role in the early stages of the inflammatory response following tissue injury, by recruiting immune cells to the wound site [Mirza RE, 2013]. Untreated wounds from diabetic mice expressed higher levels of IL-1 β , while treatment with ErA showed lower levels of the pro-inflammatory cytokine, thus indicating, the concurrent process of tissue repair and remodelling (Figure 22).

[00335] Further, to explore the effect of ErA on cell proliferation in wound tissues, the expression of Ki67, a commonly used marker for the proliferative phase of wound healing was studied [Mistry K, 2021]. As shown in Figure 22, the expression of Ki67 was significantly induced in ErA-treated wounds compared with the control mice (indicated by arrows). As mentioned previously, effective wound healing also involves the establishment of vasculature in newly formed tissue. A significant augmentation in blood vessel density is commonly observed during the proliferative phase of healing due to angiogenesis [Johnson KE, 2014]. The effect of ErA on angiogenesis *in vivo* was

examined by employing antibody against the protein CD31, also known as platelet endothelial cell adhesion molecule-1 (PECAM-1), which is expressed on the surface of endothelial cells. ErA treated tissues expressed higher levels of CD31, indicating the occurrence of angiogenesis *in vivo*, which was in accordance with the results we obtained *in vitro*.

5 [00336] Fibronectin is a high-molecular weight glycoprotein, that plays a role in the wound healing process, specifically contributing to extracellular matrix (ECM) formation and reepithelialization [Lenselink EA. 2015, Patten J, 2021]. In the later phases of wound healing, fibroblasts and endothelial cells migrate to the wound site and contribute to the deposition of cellular fibronectin, a factor in the formation of granulation tissue. Treatment with ErA expressed higher levels
10 of fibronectin in the wound tissues, thus indicating the ongoing process of repair and regeneration (Figures 22).

Example 4

Experimental methods

Isolation and culture of rat hippocampal and cortical neurons

15 [00337] Rat hippocampal and cortical neurons were cultured as described by Callizot et al., with minor modifications for cortical cultures. Pregnant Wistar rats (Janvier Labs, France) were euthanized at 17 days of gestation (hippocampal cultures) or 15 days of gestation (cortical cultures) using deep CO₂ anesthesia followed by cervical dislocation. Fetuses were immediately placed in ice-cold L15 Leibovitz medium supplemented with 2% penicillin-streptomycin solution (10,000 U/mL
20 penicillin, 10 mg/mL streptomycin) and 1% bovine serum albumin (BSA).

[00338] The hippocampus or cortex was dissected and enzymatically dissociated in 0.05% trypsin-EDTA at 37°C for 20 minutes. Dissociation was halted by adding Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose, 0.5 mg/mL DNase I (grade II), and 10% fetal calf serum (FCS). Tissues were further dissociated mechanically via three passages through a 10-mL pipette. Cells
25 were centrifuged at 515 × g for 10 minutes at 4°C, the supernatant was removed, and the pellet was resuspended in Neurobasal medium containing 2% B27 supplement, 2 mmol/L L-glutamine, 2% penicillin-streptomycin solution, and 10 ng/mL brain-derived neurotrophic factor (BDNF).

[00339] Viable cells were counted using the trypan blue exclusion test in a Neubauer chamber and seeded into 96-well plates precoated with poly-L-lysine. Hippocampal neurons were seeded at
30 20,000 cells per well, while cortical neurons were seeded at 25,000 cells per well. Cultures were maintained at 37°C in a humidified incubator with 95% air and 5% CO₂. For cortical neurons, the

medium was changed every two days. To avoid edge effects, only 60 wells per plate were used, with outermost rows and columns excluded, and empty wells filled with water.

Treatment of primary neurons with ErA and its analogs

[00340] After seeding cells into 96-well plates, test compounds were applied on the same day and added directly to the cultures up to a final concentration of 0.1%. Plates were incubated for 3 days *in vitro* (DIV 3) followed by immunostaining and microscopy. Each test condition was assessed in six replicate wells (n=6) per 96-well plate. Brain-derived neurotrophic factor (BDNF) at 50 ng/mL served as the positive control, while DMSO alone (vehicle) was used as the untreated control.

[00341] The effect of ErA was tested for neuroprotective and neuritogenic effects in both rat hippocampal and cortical neurons by immunostaining and fluorescence microscopy. For screening of semi-synthetic ErA analogs, primary cultures of cortical neurons were treated with test compounds at concentrations 1, 10, 100 and 1000 nM for 72 hours and images were captured and analysed as described below.

Immunostaining for analysis of neurite outgrowth

[00342] Seventy-two hours post-treatment, the cell culture supernatant was removed, and the cells were washed with phosphate-buffered saline (PBS). Cortical neurons were fixed for 5 minutes at -20°C using a cold ethanol (95%) and acetic acid (5%) solution. After fixation, cells were washed twice with PBS and permeabilized. Non-specific binding sites were blocked with PBS containing 0.1% saponin and 1% fetal calf serum (FCS) for 15 minutes at room temperature (RT). Cells were then incubated for 2 hours at RT with a mouse monoclonal antibody against microtubule-associated protein 2 (MAP-2) diluted 1:400 in PBS containing 1% FCS and 0.1% saponin. This antibody selectively labels neurons and neurites, facilitating the assessment of neuronal survival and neurite networks. A goat anti-mouse IgG secondary antibody conjugated to Alexa Fluor 488 (1:400 dilution in PBS with 1% FCS and 0.1% saponin) was used to visualize MAP-2, with a 1-hour incubation at RT. Cell nuclei were counterstained with Hoechst dye (1:1000 dilution, Sigma-Aldrich).

[00343] For each condition, 30 images per well were automatically captured at 20× magnification using either the ImageXpress® system (Molecular Devices) or the Operetta CLS™ system (PerkinElmer). All images were acquired using identical parameters, and subsequent analyses were conducted using MetaXpress® (Molecular Devices) or Harmony™ (PerkinElmer) software.

[00344] The following readouts were quantified:

- Number of neurons (number of MAP-2 positive neurons)
- Total neurite network (lengths of MAP-2 positive neurite in μm)
- Analysis of branch points/roots (number of neurite branches) and extremities

Results

5 *Evaluation of Neuroprotective and Neuritogenic Activity of Erinacine A in Primary Rat Hippocampal Neurons*

[00345] Primary rat hippocampal neurons, owing to their critical role in memory and learning, serve as a relevant *in vitro* model for evaluating potential therapeutics targeting neurological disorders. To determine the neuroprotective and neuritogenic effects of *Erinacine A* (ErA), primary
10 hippocampal neurons were treated for 72 hours at concentrations of 10,000, 5000, 1000, 500, 100, 10, 1, and 0.1 nM. Brain-derived neurotrophic factor (BDNF) at 50 ng/mL was used as a positive control, while 0.1% DMSO served as the vehicle control.

[00346] The neuroprotective or cytotoxic effects of the compounds were assessed by measuring the number of neurons after 72 hours, as an increase in neuron count reflects enhanced
15 neuronal survival in an ex vivo environment. The neuritogenic activity of the compounds was evaluated by immunostaining for MAP-2 by analyzing total neurite network length (μm), the number of neurite branching points, and the number of neurite extremities, which together provide insights into the stimulation of neurite outgrowth and the development of neuronal networks. Representative image of the neuron and the phenotypes measured in microscopic analysis are shown in Figure 23A.

20 [00347] As shown in Figure 23B, treatment with ErA at 100 nM exhibited a significant neuroprotective effect on the survival of hippocampal neurons in comparison to the DMSO treated wells, which was even higher than the positive control BDNF. At 100 nM, ErA significantly enhanced the neurite network and neurite extremities, surpassing the effects observed with BDNF. Moreover, ErA at 10 nM significantly increased the number of roots or branching points on the neuronal cell
25 bodies. This observation thus establishes the remarkable neurotrophic and neuroprotective potential of ErA in neurons derived from the Central Nervous System (CNS).

Evaluation of Neuroprotective and Neuritogenic Activity of Erinacine A and its analogs in Primary Rat Cortical Neurons

[00348] Cortical neurons play a key role in sensory processing, motor control, and cognitive
30 functions, making them highly relevant for studying neurological disorders. They are relatively easy

to culture and maintain *in vitro*, and their ability to form extensive networks makes them ideal for evaluating neuroprotective and neuritogenic effects of compounds. Therefore, the effect of ErA and its semi-synthetic analogs was tested on cortical neurons treated with 1, 10, 100 and 1000 nM of the compounds for 72 h in culture. The number of neurons that survived the treatment duration, the neurite network, roots and extremities were measured and evaluated in comparison to BDNF.

[00349] Table 1 shows the minimum compound concentrations that are needed to show neuroprotective effects and neuritogenic phenotype in cortical neurons. 1000 nM of ErA showed significantly higher number of MAP-2 positive neurons implying neuroprotective effects at this concentration and neuritogenic effects were observed at concentration of 10 nM. As shown in Table 4, a variety of semi-synthetic analogs of ErA were synthesized with a wide range of neuroprotective and neuritogenic effects. For example, I-116 shows both neuroprotection and increased neuritogenesis at 1 nM; while I-123 seemed to have lost both activities observed in the parent compound.

Table 4: Summary of the minimum compound concentration at which neuroprotection and neuritogenesis was observed in rat cortical neurons.

Compound ID	Minimum Neuroprotective Concentration	Minimum Neuritogenesis Concentration
Erinacine A	+	+++
II-2	+	+
I-8	+	++
I-5	+	+
I-41	+	+
I-69	+	+++
Alloxyathin B2	+	+
I-113	++	++
I-97	++	+++
I-167	+	++++
II-1	+	++
I-33	++++	++++
I-107	+	++++
I-115	+++	+++
I-116	++++	++++
I-117	+++	++
I-114	++	+++
I-149	+	+
I-148	++++	++++
I-147	+	++
I-131	+	+++

I-146	+	+++
I-127	+++	+++
I-141	+	+
I-124	++++	++++
I-133	+	+
I-132	+	+
I-134	++	++
I-128	++	+++
I-125	+	+
I-126	+++	+++
I-122	++++	++++
I-123	+	+
I-139	+	+
I-118	++++	++++
I-119	+	+++
I-120	+	++
I-121	+	+
I-135	++++	++++
I-138	+++	++
I-136	++++	+++
I-142	++++	++++
I-137	++++	++++
I-129	+	++++
I-166	++++	++++
I-143	++	++
I-144	++++	++++
I-145	++++	++++
I-159	+++	+
I-160	+	+
I-161	+	+
I-162	+	++
I-140	+	++++
I-152	+	++
I-156	+	+
I-153	++++	++++

Legend: +($\geq 1 \mu\text{M}$); ++(100 nM); +++(10 nM); ++++(1 nM)

FULL CITATIONS FOR DOCUMENTS REFERRED TO IN THE APPLICATION

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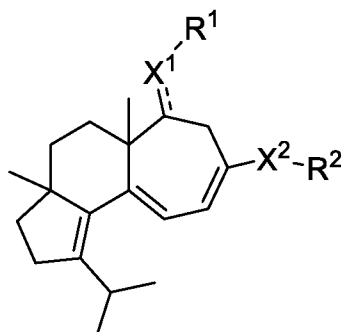
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5 Methods 71.

CLAIMS:

1. A compound of Formula I, or a pharmaceutically acceptable salt and/or solvate thereof:



I

wherein:

---- is a single or double bond;

when ---- is a double bond, X^1 is selected from O and NR^3 and R^1 is absent;

when ---- is a single bond, X^1 is selected from O, $OC(O)$ and NR^3 and R^1 is selected from H, $SO_2NR^4R^5$, $C_{1-10}alkyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{6-10}aryl$, $C_{5-10}heteroaryl$, $C_{1-10}alkylenearyl$, $C_{1-10}alkyleneheteroaryl$, $C_{1-10}alkyleneC_{3-8}cycloalkyl$ and $C_{1-10}alkyleneC_{3-8}heterocycloalkyl$, the latter nine groups being optionally substituted with one or more of OH, halo, $OC_{1-6}alkyl$, $OC(O)C_{1-6}alkyl$, $C(O)OC_{1-6}alkyl$, $OSi(CH_3)_2(C_{1-6}alkyl)$ and $C_{1-6}alkyl$;

X^2 is selected from a direct bond, $C(O)$, $C(O)Y^1$, $C(NR^{11})$, Y^1SO_2 , $C_{1-6}alkyleneY^1$, $C_{1-6}alkyleneY^1C(O)$, $C_{1-6}alkyleneY^1-SO_2$, $C_{1-6}alkyleneY^1-SO_2Y^2$ and $C_{1-6}alkyleneY^1C(O)Y^2$;

R^2 is selected from H, $C_{1-10}alkyl$, $C_{2-10}alkenyl$, $C_{2-10}alkynyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{6-10}aryl$, $C_{6-10}heteroaryl$, $C_{1-10}alkylenearyl$, $C_{1-10}alkyleneheteroaryl$, $C_{1-10}alkyleneC_{3-8}cycloalkyl$, $C_{1-10}alkyleneC_{3-8}heterocycloalkyl$ and $phenylC(O)phenylY^3C(O)R^6$, the latter twelve groups being optionally substituted with one or more of OH, $=O$, $C_{1-6}alkyl$, $OC_{1-6}alkyl$, NR^7R^8 , $NR^9C(O)OC_{1-6}alkyl$, and halo;

Y^1 , Y^2 and Y^3 are independently selected from NR^{10} and O;

R^3 , R^4 , R^5 , R^7 , R^8 , and R^9 are independently selected from H and $C_{1-6}alkyl$;

R^6 is selected from $C_{1-10}alkyl$, $C_{2-10}alkenyl$ and $C_{2-10}alkynyl$,

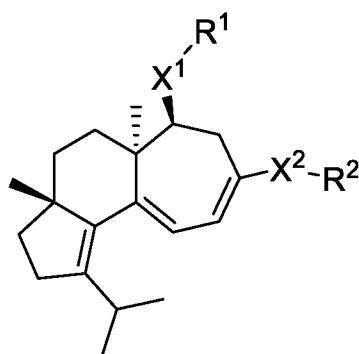
R^{10} is selected from H and C_{1-6} alkyl;

R^{11} is selected from H, C_{1-6} alkyl, C_{6-10} aryl and SO_2C_{6-10} aryl; and

wherein all available hydrogen atoms are optionally and independently replaced with a fluorine or chlorine atom,

5 provided that when X^1-R^1 is OH, then X^2-R^2 is not CH_2OH , $C(O)OH$ or $C(O)H$.

2. The compound of claim 1, wherein --- is a single bond, the compound has the following relative stereochemistry:



I.

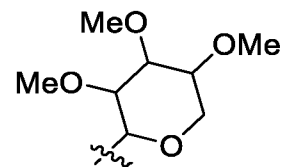
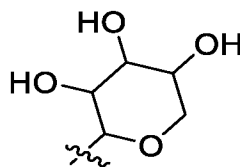
10 3. The compound of claim 1 or 2, wherein --- is a single bond X^1 is selected from O, $OC(O)$, NH and NC_{1-6} alkyl.

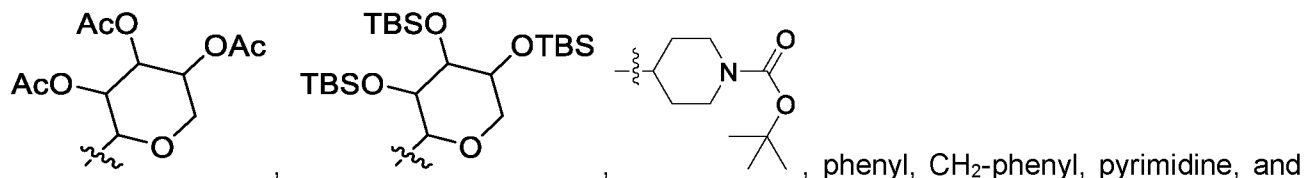
4. The compound of any one of claims 1 to 3, wherein R^1 is selected from H, $SO_2NR^4R^5$, C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{3-10} heterocycloalkyl, C_{6-10} aryl, C_{5-10} heteroaryl, C_{1-6} alkylenearyl, C_{1-6} alkyleneheteroaryl, C_{1-6} alkylene C_{3-8} cycloalkyl and C_{1-6} alkylene C_{3-8} heterocycloalkyl, the latter nine groups being optionally substituted with one to three of OH, halo, OC_{1-6} alkyl, $OC(O)C_{1-6}$ alkyl, $C(O)OC_{1-6}$ alkyl, $OSi(CH_3)_2(C_{1-6}$ alkyl) and C_{1-6} alkyl.

5. The compound of claim 4, wherein R^1 is selected from H, SO_2NH_2 , CH_3 , CH_2CH_3 , CH_2CH_2OH ,

cyclopropane,

cyclohexene,





wherein the phenyl is optionally substituted with one or two of CH_3 .

6. The compound of any one of claims 1 to 5, wherein X^2 is selected from a direct bond, $\text{C}(\text{O})$, $\text{C}(\text{O})\text{Y}^1$, $\text{C}(\text{NR}^{11})$, Y^1SO_2 , $\text{C}_{1-4}\text{alkyleneY}^1$, $\text{C}_{1-4}\text{alkyleneY}^1\text{C}(\text{O})$, $\text{C}_{1-4}\text{alkyleneY}^1\text{SO}_2$, $\text{C}_{1-4}\text{alkyleneY}^1\text{SO}_2\text{Y}^2$ and $\text{C}_{1-4}\text{alkyleneY}^1\text{C}(\text{O})\text{Y}^2$, wherein Y^1 and Y^2 are independently selected from NR^{10} and O .

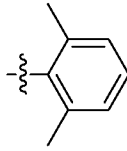
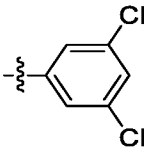
7. The compound of claim 6, wherein X^2 is selected from a direct bond, $\text{C}(\text{O})$, $\text{C}(\text{O})\text{O}$, $\text{C}(\text{O})\text{NH}$, $\text{C}(\text{O})\text{NCH}_3$, $\text{C}(\text{O})\text{NH}$, $\text{C}(\text{NH})$, $\text{C}(\text{NSO}_2\text{Ph})$, $\text{C}(\text{NPh})$, NHSO_2 , CH_2NH , CH_2O , CH_2NCH_3 , $\text{CH}_2\text{OC}(\text{O})$, $\text{CH}_2\text{NHC}(\text{O})$, CH_2NHSO_2 , $\text{CHNHSO}_2\text{NCH}_3$, $\text{CH}_2\text{N}(\text{CH}_3)\text{SO}_2\text{NCH}_3$, $\text{CH}_2\text{OSO}_2\text{NH}$, $\text{CH}_2\text{OSO}_2\text{NCH}_3$, $\text{CH}_2\text{OC}(\text{O})\text{NH}$, CH_2OSO_2 , $\text{CH}_2\text{OC}(\text{O})\text{O}$ and $\text{CH}_2\text{NHC}(\text{O})\text{NH}$ wherein all available hydrogen atoms are optionally and independently replaced with a fluorine.

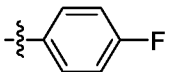
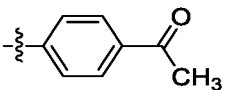
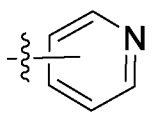
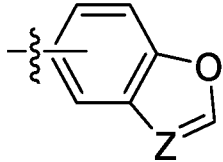
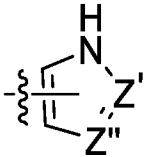
8. The compound of any one of claims 1 to 7, wherein R^2 is selected from H , $\text{C}_{1-10}\text{alkyl}$, $\text{C}_{2-5}\text{alkenyl}$, $\text{C}_{2-5}\text{alkynyl}$, $\text{C}_{3-8}\text{cycloalkyl}$, $\text{C}_{3-8}\text{heterocycloalkyl}$, $\text{C}_{6-10}\text{aryl}$, $\text{C}_{6-10}\text{heteroaryl}$, $\text{C}_{1-5}\text{alkylenearyl}$, $\text{C}_{1-5}\text{alkyleneheteroaryl}$, $\text{C}_{1-5}\text{alkyleneC}_{3-8}\text{cycloalkyl}$, $\text{C}_{1-5}\text{alkyleneC}_{3-8}\text{heterocycloalkyl}$ and $\text{phenylC}(\text{O})\text{phenylNR}^{10}\text{C}(\text{O})\text{R}^6$, the latter twelve groups being optionally substituted with one to three of OH , $=\text{O}$, $\text{C}_{1-4}\text{alkyl}$, $\text{OC}_{1-4}\text{alkyl}$, NR^7R^8 , $\text{NR}^9\text{C}(\text{O})\text{OC}_{1-6}\text{alkyl}$, and halo, wherein all available hydrogen atoms are optionally and independently replaced with a fluorine.

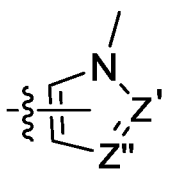
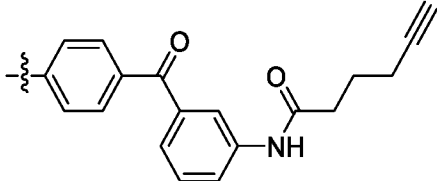
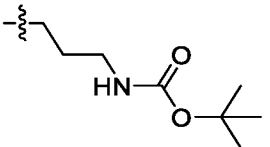
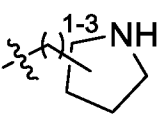
9. The compound of claim 8, wherein R^2 is selected from H , $\text{C}_{1-8}\text{alkyl}$, $\text{C}_{2-5}\text{alkynyl}$, $\text{C}_{3-8}\text{cycloalkyl}$, $\text{C}_{3-8}\text{heterocycloalkyl}$, phenyl, $\text{C}_{6-10}\text{heteroaryl}$, $\text{C}_{1-2}\text{alkylenephenyl}$, $\text{C}_{1-2}\text{alkyleneC}_{3-8}\text{cycloalkyl}$, $\text{C}_{1-4}\text{alkyleneC}_{3-6}\text{heterocycloalkyl}$ and $\text{phenylC}(\text{O})\text{phenylNHC}(\text{O})\text{C}_{1-6}\text{alkynyl}$, the latter ten groups being optionally substituted with one to three of OH , $=\text{O}$, CH_3 , OCH_3 , NH_2 , $\text{N}(\text{CH}_3)_2$, NHCH_3 , $\text{NHC}(\text{O})\text{OC}(\text{CH}_3)_3$, fluoro or chloro, wherein all available hydrogen atoms are optionally and independently replaced with a fluorine.

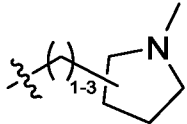
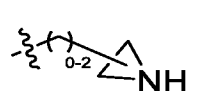
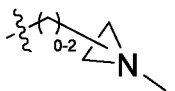
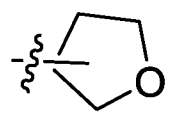
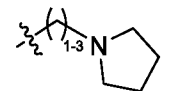
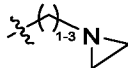
10. The compound of claim 9, wherein R^2 is selected from H , CH_2OH , $\text{CH}(\text{OH})\text{CH}_3$, $\text{CH}(\text{OH})\text{CF}_3$, $\text{CH}(\text{OH})\text{CH}(\text{CH}_3)_2$, $\text{CH}(\text{OH})\text{CH}_2\text{CH}(\text{CH}_3)_2$, $\text{CH}(\text{OH})\text{C}(\text{CH}_3)_3$, $\text{C}(\text{OH})(\text{CH}_3)_2$, CH_3 , CF_3 , CHF_2 , $\text{CH}(\text{CH}_3)_2$, CH_2CH_3 , $\text{C}(\text{CH}_3)_3$, $\text{CH}(\text{CH}_3)\text{NH}_2$, CH_2NH_2 , $\text{CH}_2\text{N}(\text{CH}_3)_2$, $(\text{CH}_2)_2\text{NH}_2$, $(\text{CH}_2)_2\text{NHCH}_3$, $(\text{CH}_2)_2\text{OH}$, $\text{CH}_2\text{C}\equiv\text{CH}$, $(\text{CH}_2)_3\text{NH}_2$, $(\text{CH}_2)_3\text{N}(\text{CH}_3)_2$, cyclopropyl, cyclobutyl, cyclohexyl,

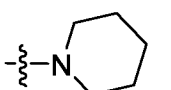
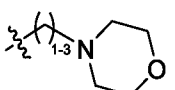
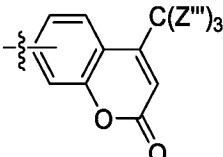
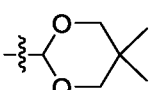
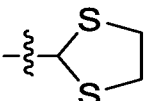
CH(OH)cyclopropyl, CH(OH)cyclobutyl, CH(OH)-cyclohexyl, phenyl, CH(OH)phenyl, o-methylphenyl,

m-methylphenyl, p-methylphenyl, p-chlorophenyl, p-methoxyphenyl, , ,

, , CH₂phenyl, , , ,

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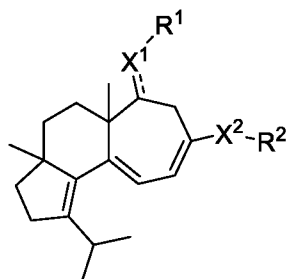
, , ,  and ,

wherein Z is selected from CH and N, wherein at least one of Z' and Z'' is CH and the other one is N, and wherein Z''' is selected from F and H.

11. The compound of any one of claims 1 to 10, wherein the compound of Formula I is selected from the compounds listed in Table 2, or a pharmaceutically acceptable salt and/or solvate thereof.

12. A pharmaceutical composition comprising one or more compounds of any one of claims 1 to 11, or a pharmaceutically acceptable salt and/or solvate thereof, and a pharmaceutically acceptable carrier.

13. A method of treating a disease, disorder or condition that benefits from induction of biosynthesis of nerve growth factor (NGF) and/or brain-derived neurotrophic factor (BDNF), the method comprising administering a therapeutically effective amount of one or more compounds of Formula II to a subject in need thereof:



II

wherein:

---- is a single or double bond;

5 when ---- is a double bond, X^1 is selected from O and NR^3 and R^1 is absent;

when ---- is a single bond, X^1 is selected from O, $OC(O)$ and NR^3 and R^1 is selected from H, $SO_2NR^4R^5$, $C_{1-10}alkyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{6-10}aryl$, $C_{5-10}heteroaryl$, $C_{1-10}alkylenearyl$, $C_{1-10}alkyleneheteroaryl$, $C_{1-10}alkyleneC_{3-8}cycloalkyl$ and $C_{1-10}alkyleneC_{3-8}heterocycloalkyl$, the latter nine groups being optionally substituted with one or more of OH, halo, $OC_{1-6}alkyl$, $OC(O)C_{1-6}alkyl$, $C(O)OC_{1-6}alkyl$, $OSi(CH_3)_2C_{1-6}alkyl$ and $C_{1-6}alkyl$;

10 X^2 is selected from a direct bond, $C(O)$, $C(O)Y^1$, $C(NR^{11})$, Y^1SO_2 , $C_{1-6}alkyleneY^1$, $C_{1-6}alkyleneY^1C(O)$, $C_{1-6}alkyleneY^1SO_2$, $C_{1-6}alkyleneY^1SO_2Y^2$ and $C_{1-6}alkyleneY^1C(O)Y^2$;

R^2 is selected from H, $C_{1-10}alkyl$, $C_{2-10}alkenyl$, $C_{2-10}alkynyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{6-10}aryl$, $C_{6-10}heteroaryl$, $C_{1-10}alkylenearyl$, $C_{1-10}alkyleneheteroaryl$, $C_{1-10}alkyleneC_{3-8}cycloalkyl$, $C_{1-10}alkyleneC_{3-8}heterocycloalkyl$ and $phenylC(O)phenylY^3C(O)R^6$, the latter twelve groups being optionally substituted with one or more of OH, =O, $C_{1-6}alkyl$, $OC_{1-6}alkyl$, NR^7R^8 , $NR^9C(O)OC_{1-6}alkyl$, and halo;

15 Y^1 , Y^2 and Y^3 are independently selected from NR^{10} and O;

R^3 , R^4 , R^5 , R^7 , R^8 , R^9 and R^{10} are independently selected from H and $C_{1-6}alkyl$;

20 R^6 is selected from $C_{1-10}alkyl$, $C_{2-10}alkenyl$ and $C_{2-10}alkynyl$;

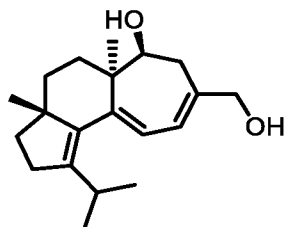
R^{10} is selected from H and $C_{1-6}alkyl$;

R^{11} is selected from H, $C_{1-6}alkyl$, $C_{6-10}aryl$ and $SO_2C_{6-10}aryl$; and

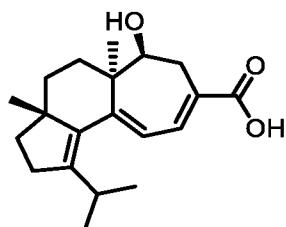
wherein all available hydrogen atoms are optionally and independently replaced with a fluorine.

14. The method of claim 13, wherein the compound of Formula II is a compound of Formula I as defined in any one of claims 1 to 13.

15. The method of claim 13, wherein the compound of Formula II is selected from:

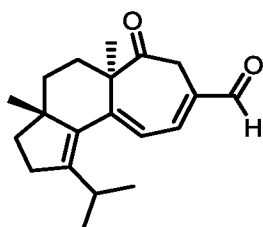


II-1;



; and

II-2



II-3,

or a pharmaceutically acceptable salt and/or solvate thereof.

16. The method of any one of claims 13 to 15, wherein the disease, disorder or condition that benefits from induction of biosynthesis of nerve growth factor (NGF) and/or brain-derived neurotrophic factor (BDNF) is a central nervous system (CNS) or a peripheral nervous system (PNS) disease, disorder or condition.

17. The method of claim 16, wherein the CNS disease, disorder or condition is selected from neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's

disease, amyotrophic lateral sclerosis (ALS, also known as motor neurone disease), ataxia-telangiectasia, progressive bulbar palsy, progressive muscular atrophy, dementia with Lewy bodies, multiple system atrophy, spinocerebellar ataxia type 1 (SCA 1), a retinal degenerative disease, an age-related neurodegenerative disorder, and spinal cord injury.

5 18. The method of claim 16, wherein the PNS disease, disorder or condition is selected from acute motor axonal neuropathy, Charcot-Marie-Tooth disease types 1A, 1B and 1X, Guillain-Barré syndrome, Lambert-Eaton syndrome, diabetic neuropathy, chemotherapy induced peripheral neuropathy, cisplatin neuropathy, familial amyloid neuropathy, diphtheritic neuropathy, neuropathy with IgM1 anti-myelin-associated glycoprotein, pyridoxine neuropathy, Refsum's disease, and
10 neuropathy associated with Leprosy and Botulism.

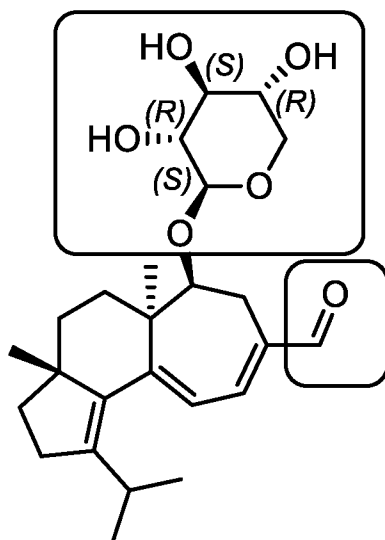
19. The method of any one of claims 13 to 18, further comprising administering a therapeutically effective amount of ErA, or a pharmaceutically acceptable salt and/or solvate thereof.

20. A method of promoting or improving wound healing, the method comprising administering to a subject in need thereof, a therapeutically effective amount of ErA, or a pharmaceutically acceptable
15 salt and/or solvate thereof, and/or one or more compounds of Formula II as defined in any one of claims 13 to 15, or a pharmaceutically acceptable salt and/or solvate thereof.

21. The method of claim 19, wherein the wound healing comprises healing acute or chronic wounds.

22. The method of claim 20, wherein the chronic wounds comprise diabetic ulcers, pressure
20 ulcers, or burns.

FIGURE 1



Erinacine A (ErA)

FIGURE 2

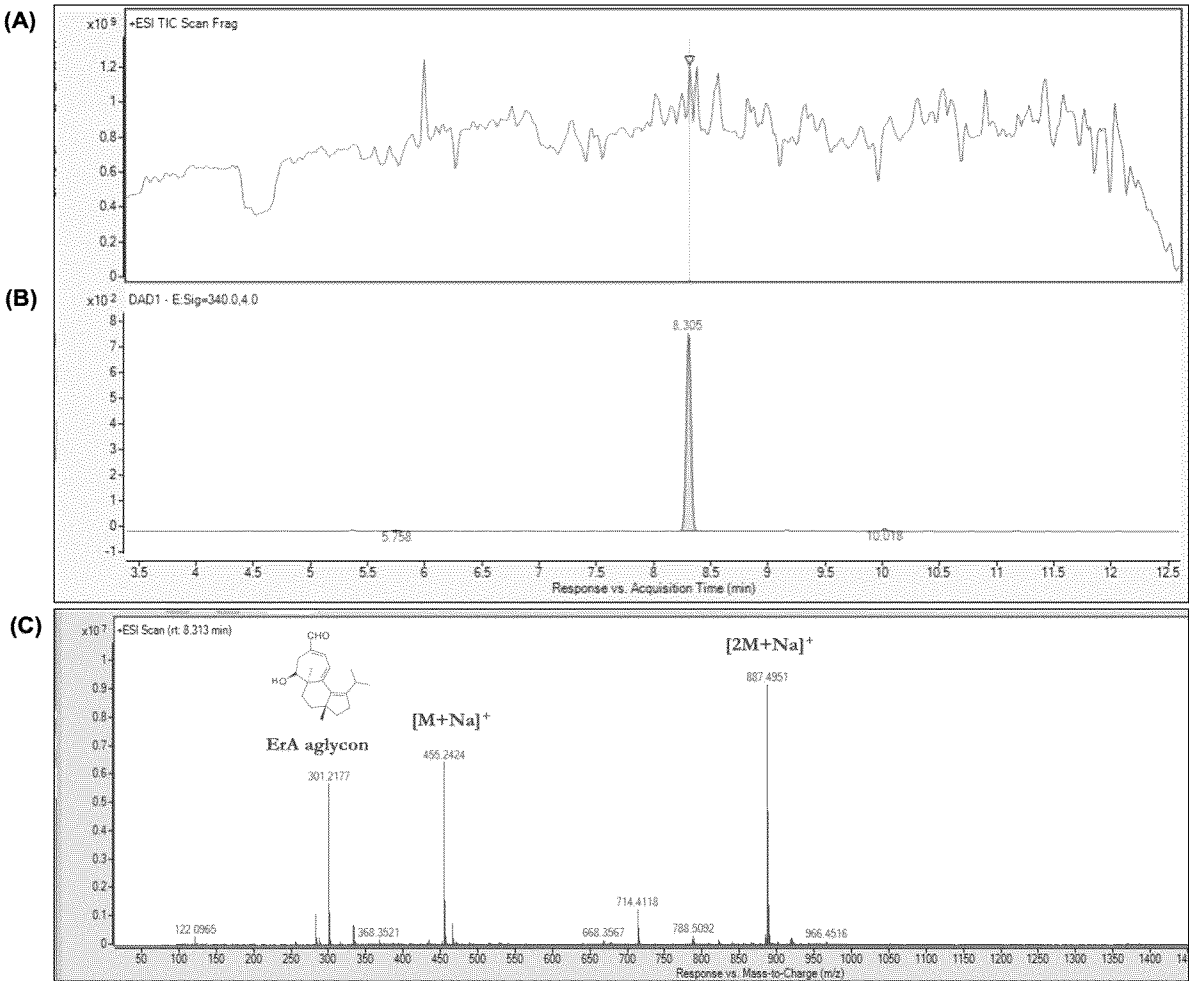


FIGURE 3

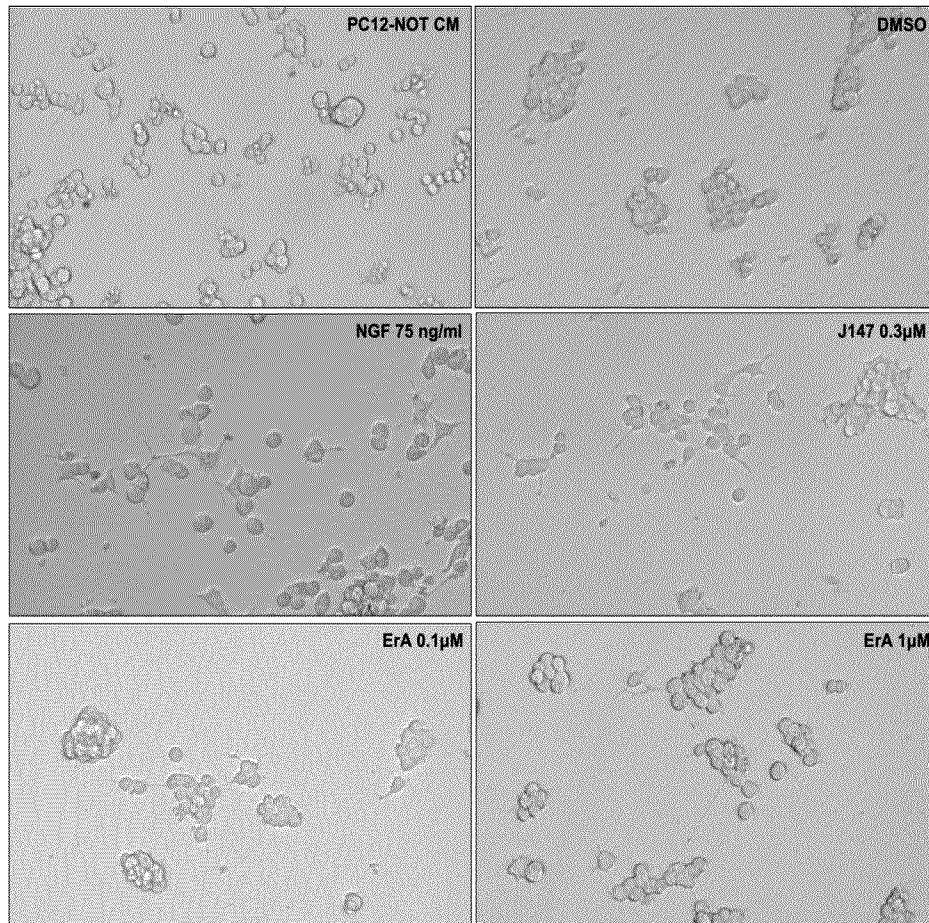


FIGURE 4

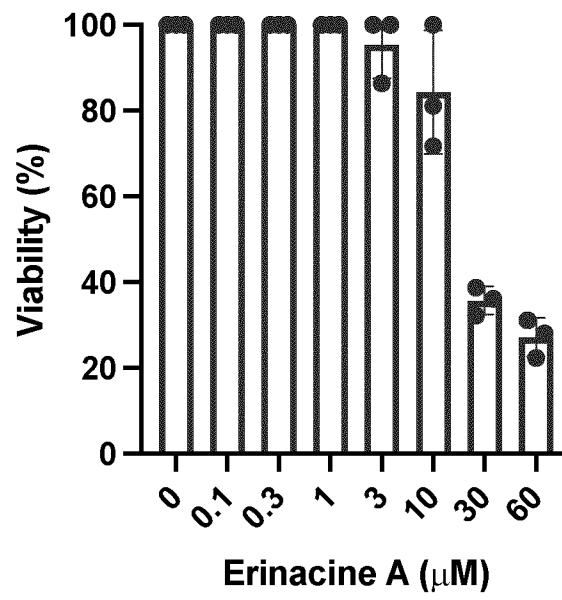


FIGURE 5

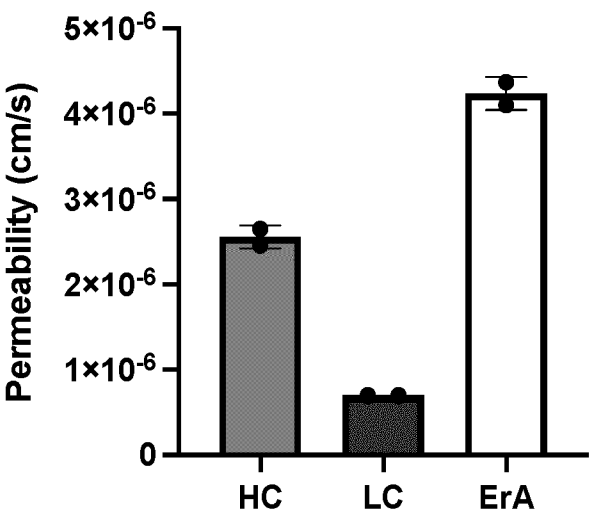
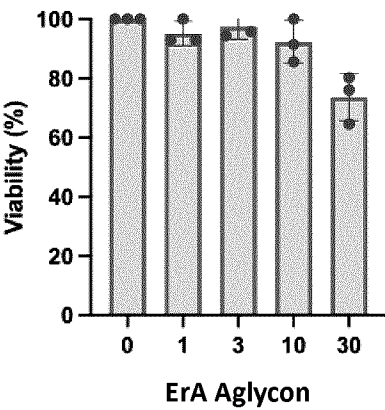
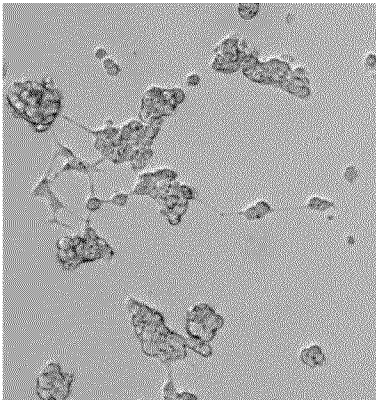


FIGURE 6

A.



B.



C.

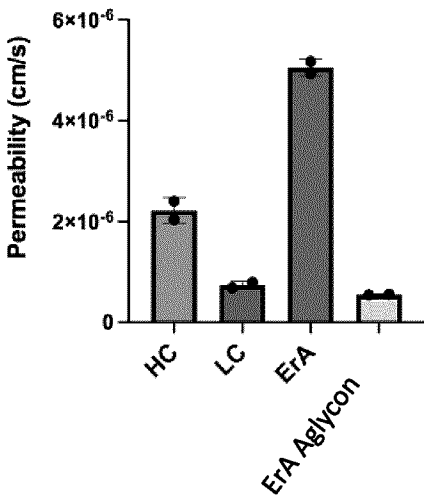


FIGURE 7

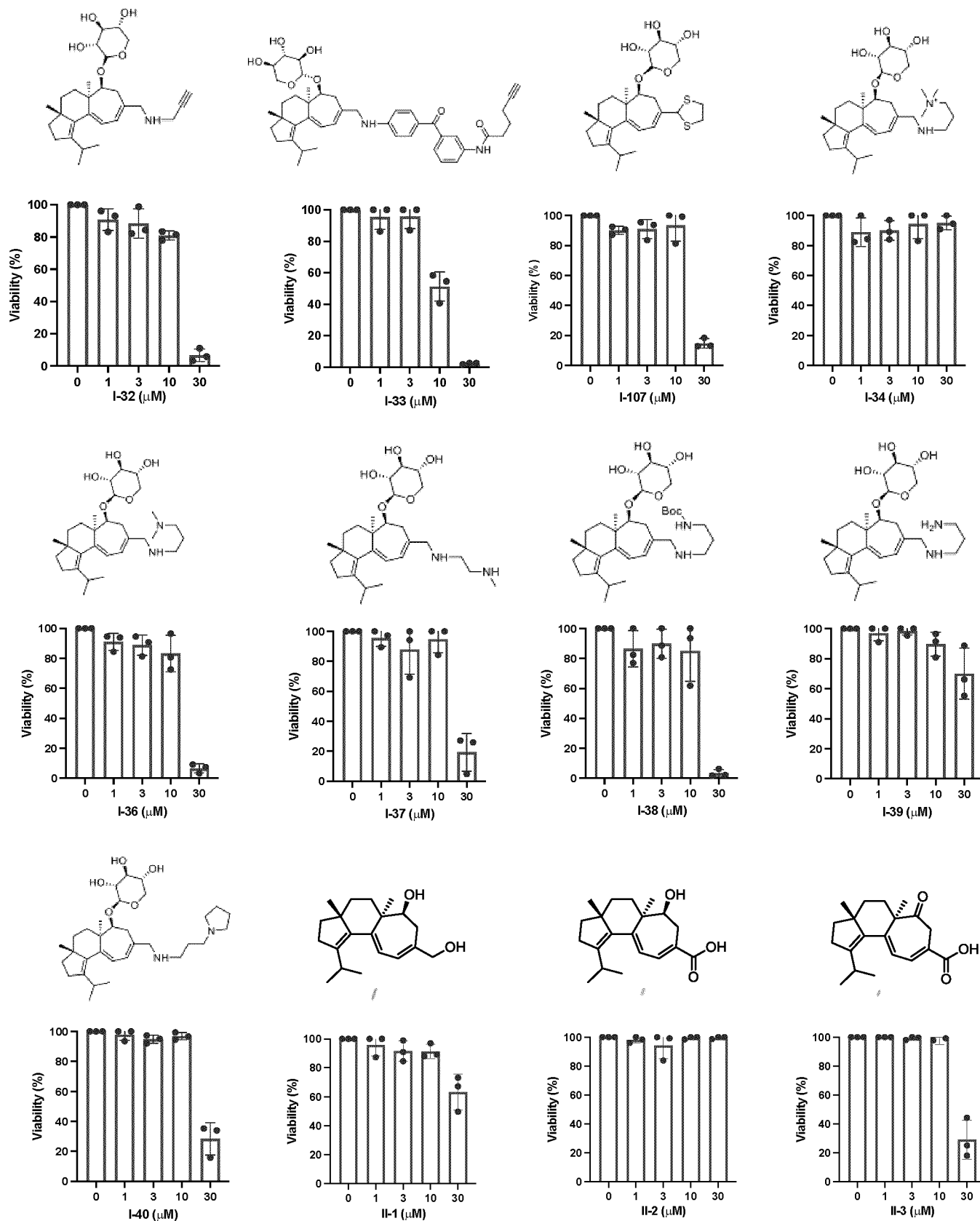


FIGURE 8

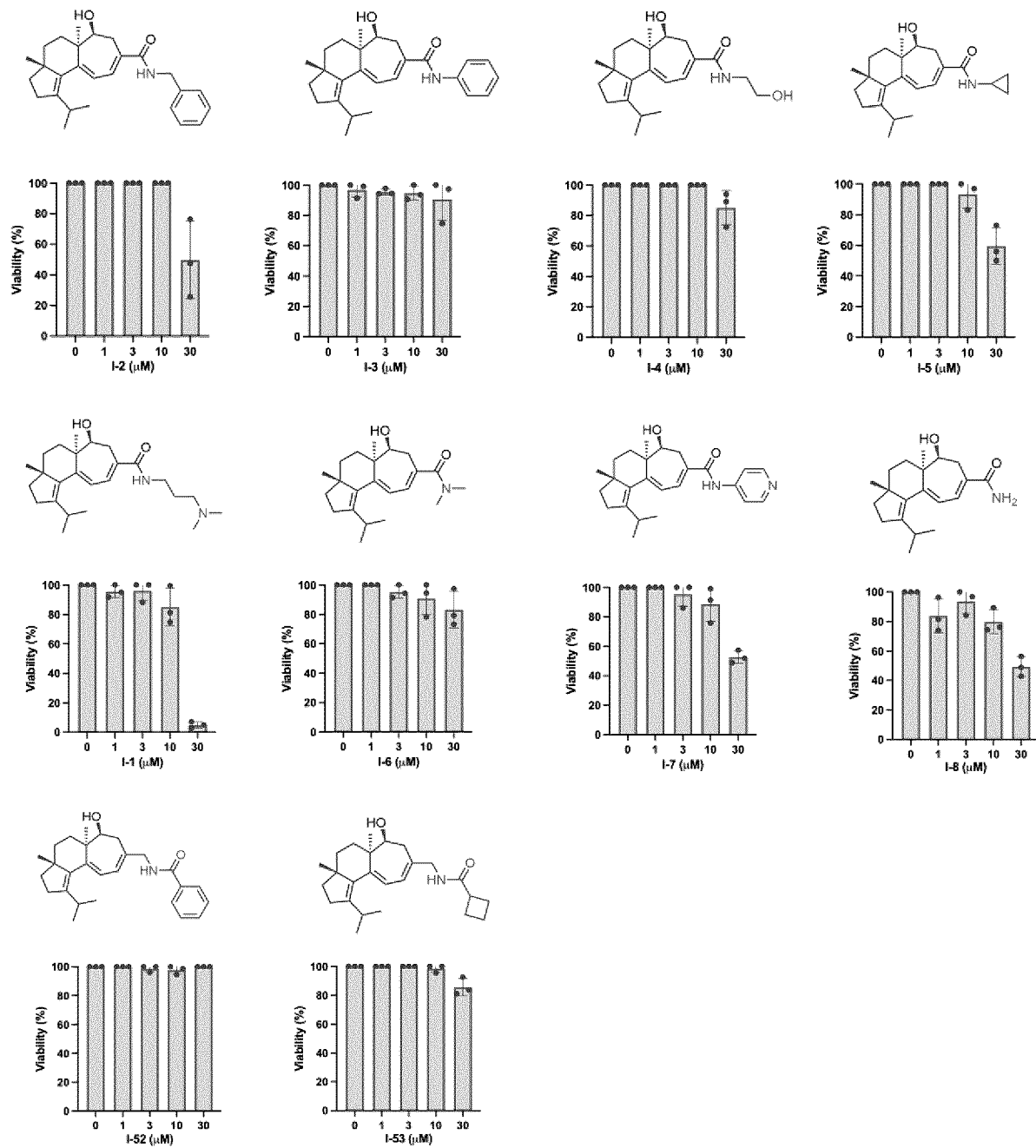


FIGURE 9

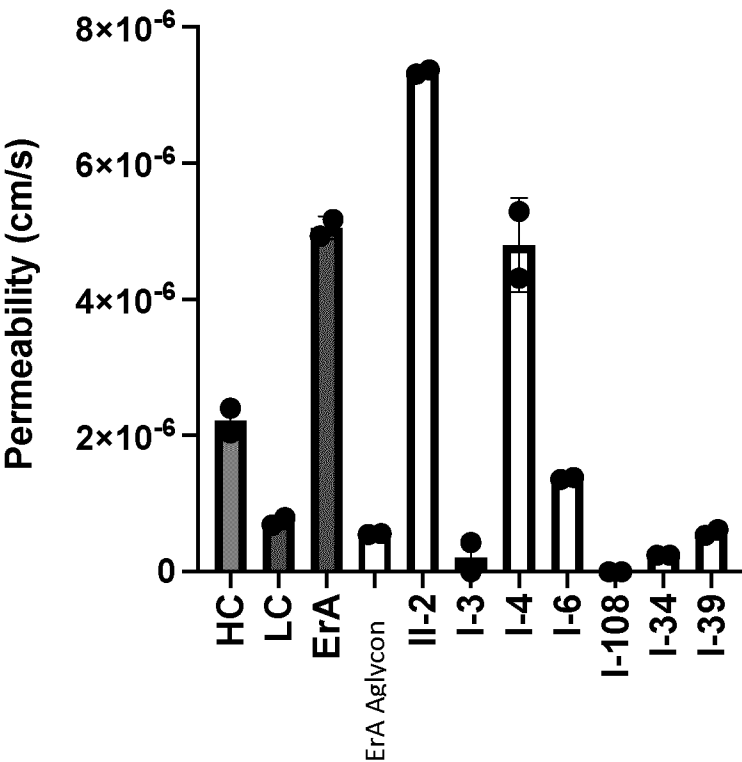


FIGURE 10

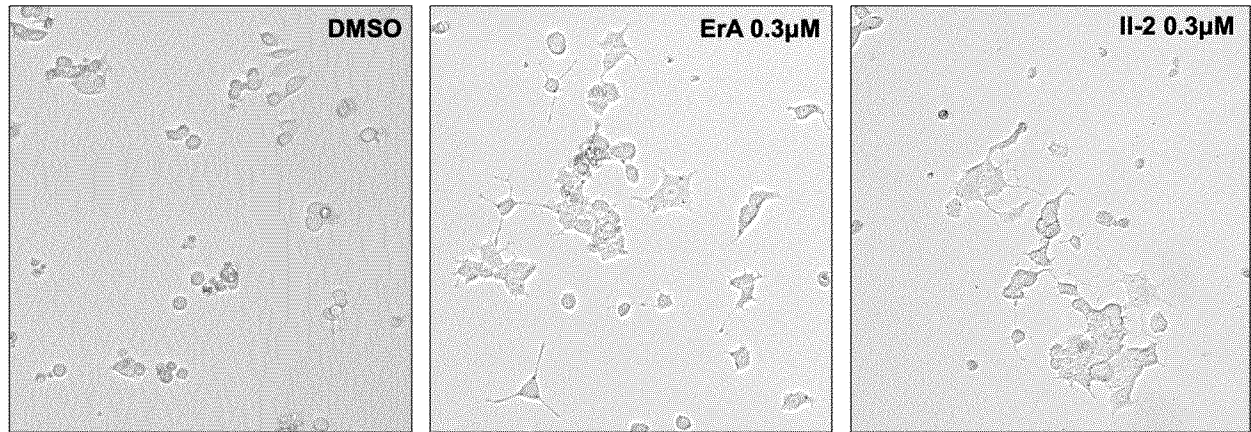


FIGURE 11

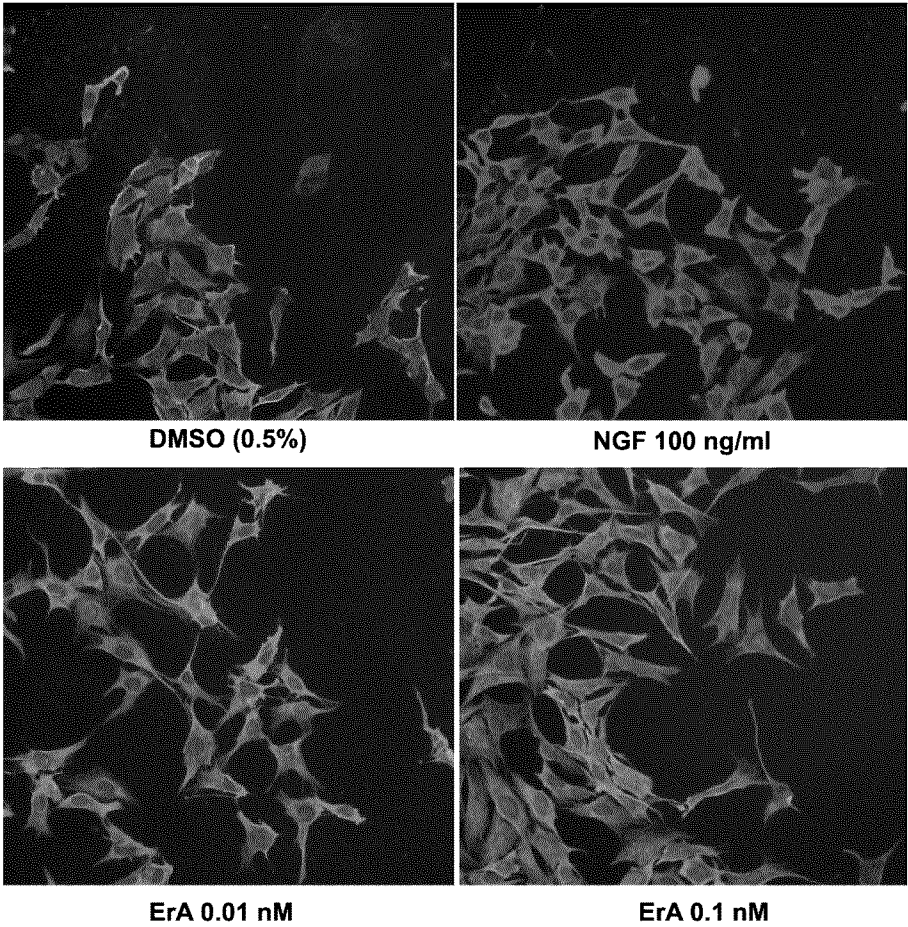


FIGURE 12

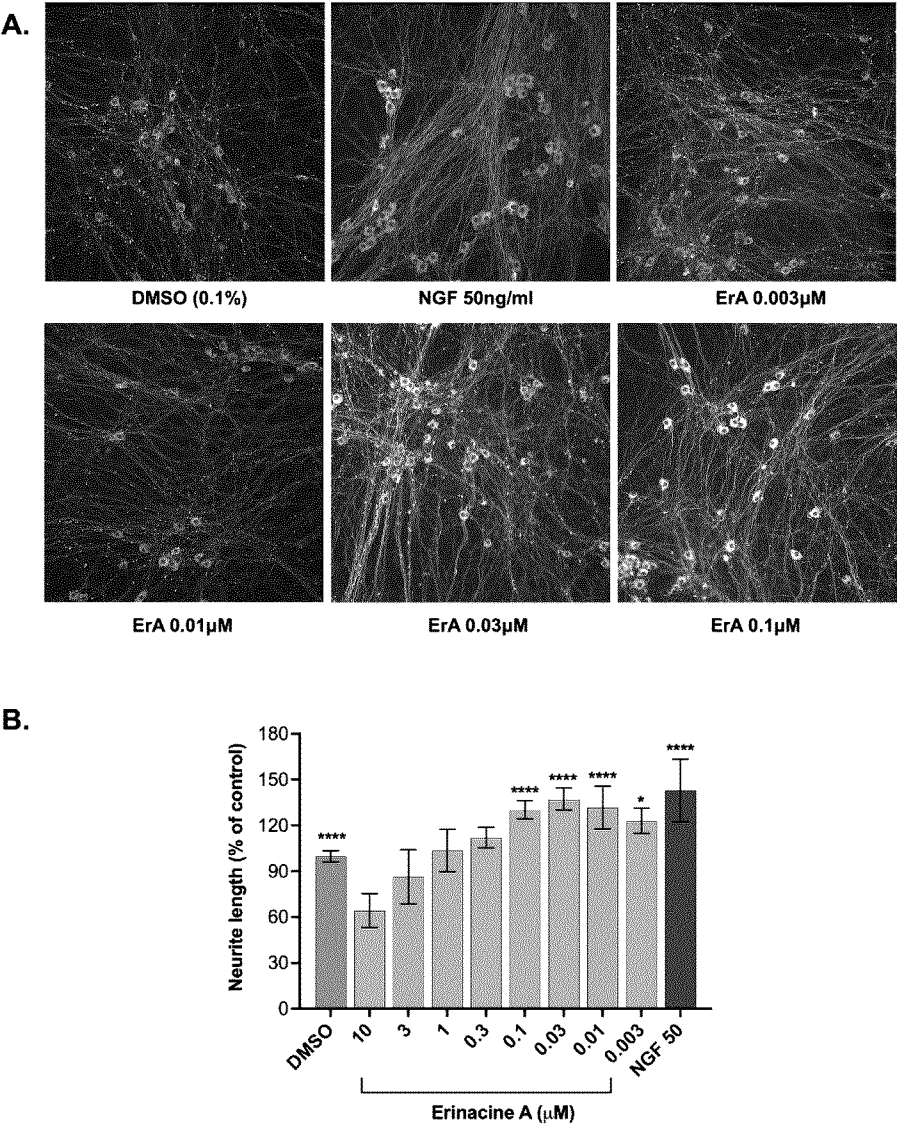


FIGURE 13

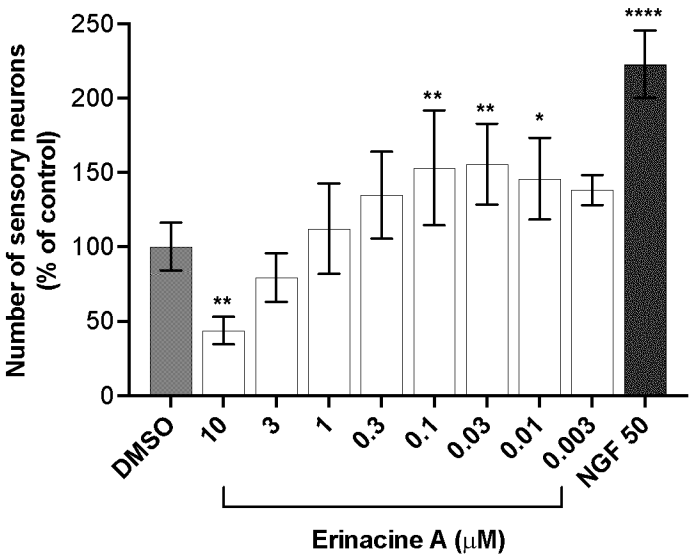


FIGURE 14

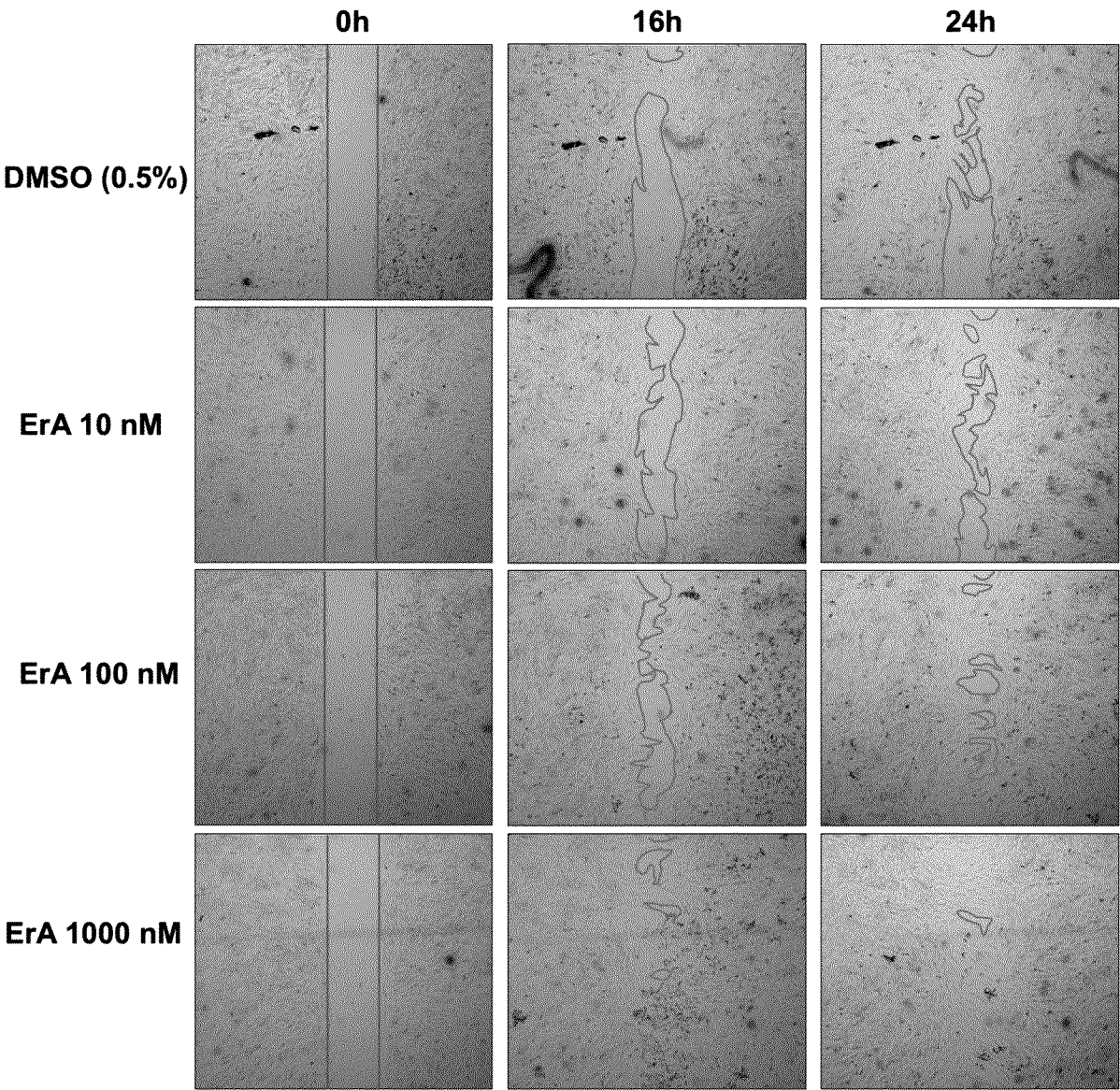


FIGURE 15

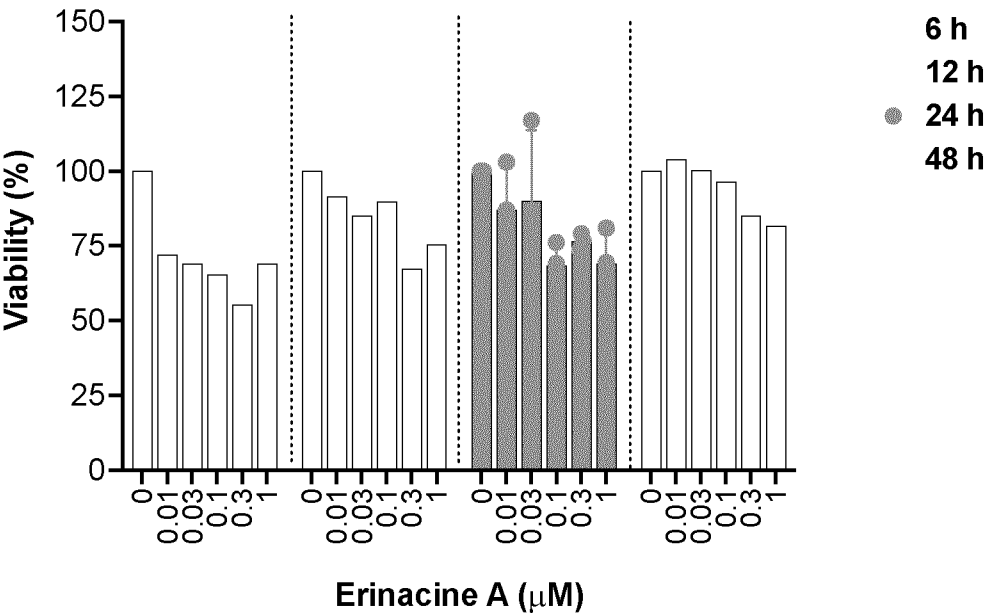


FIGURE 16

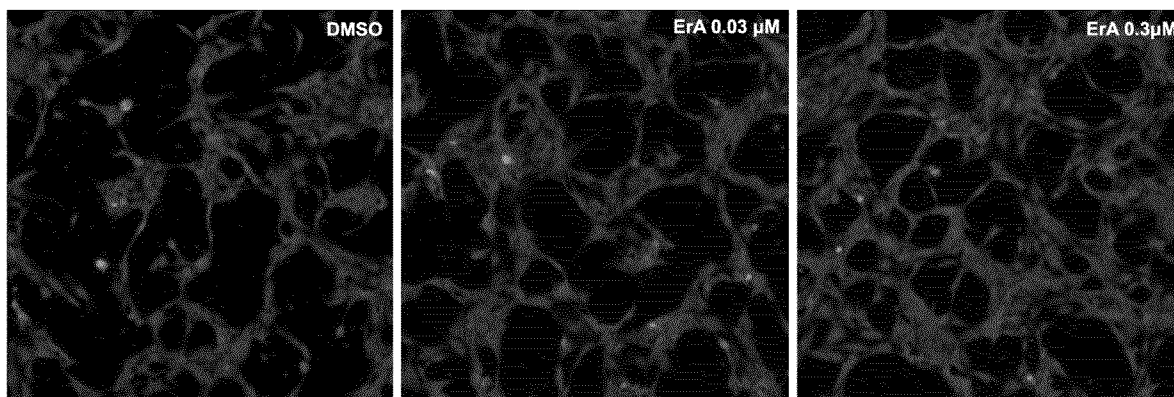


FIGURE 17

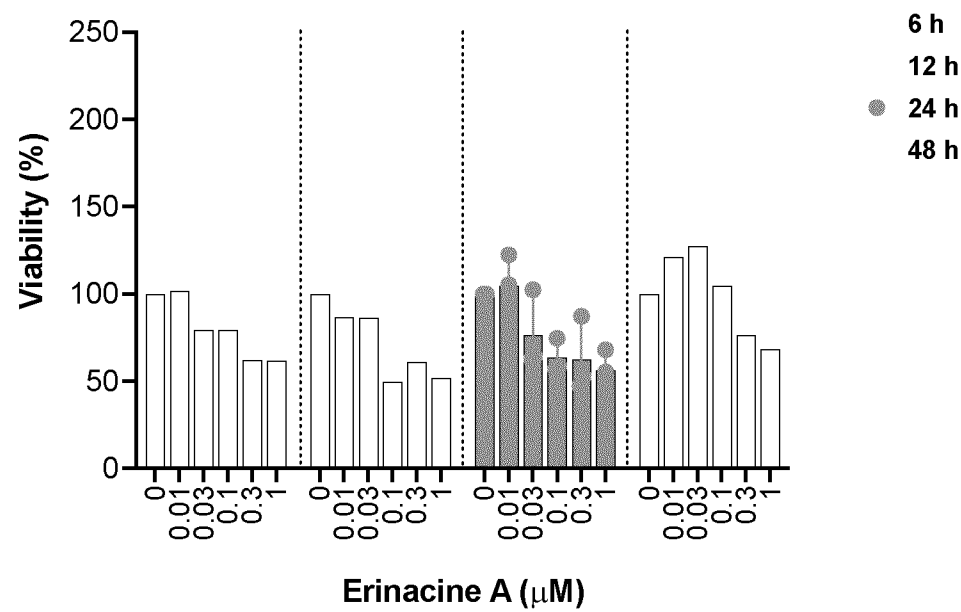


FIGURE 18

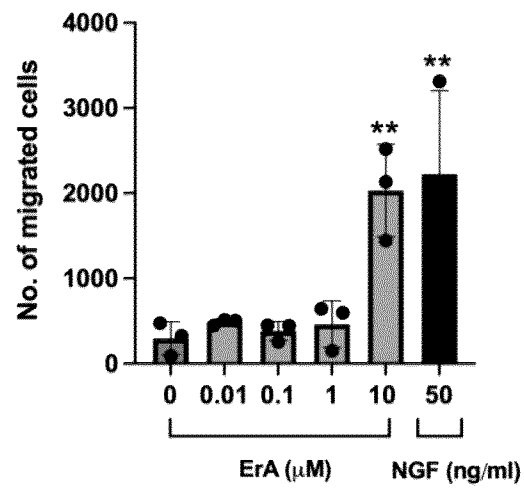
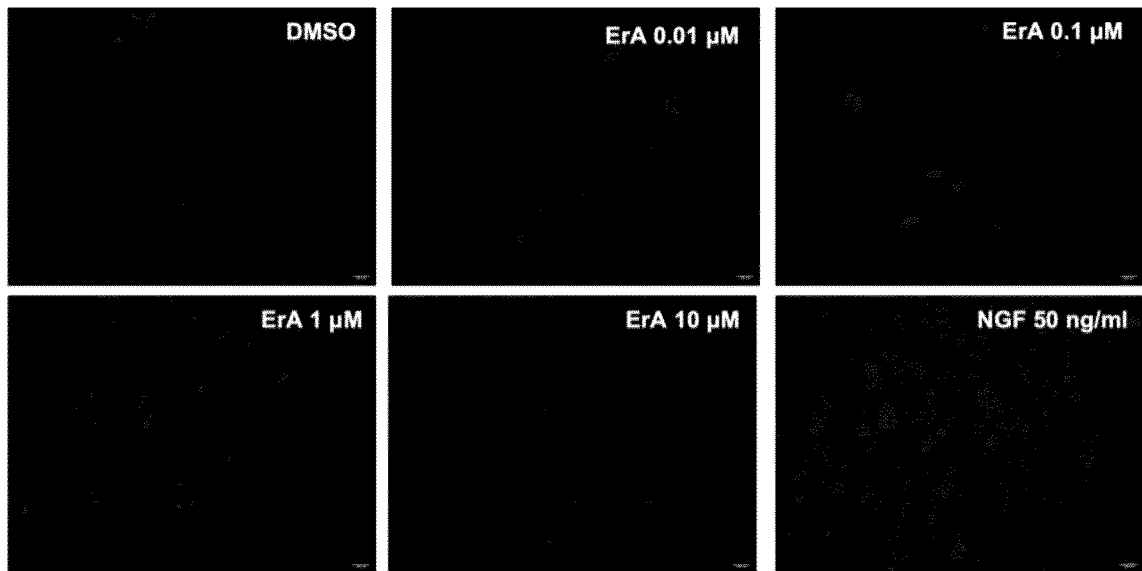


FIGURE 19

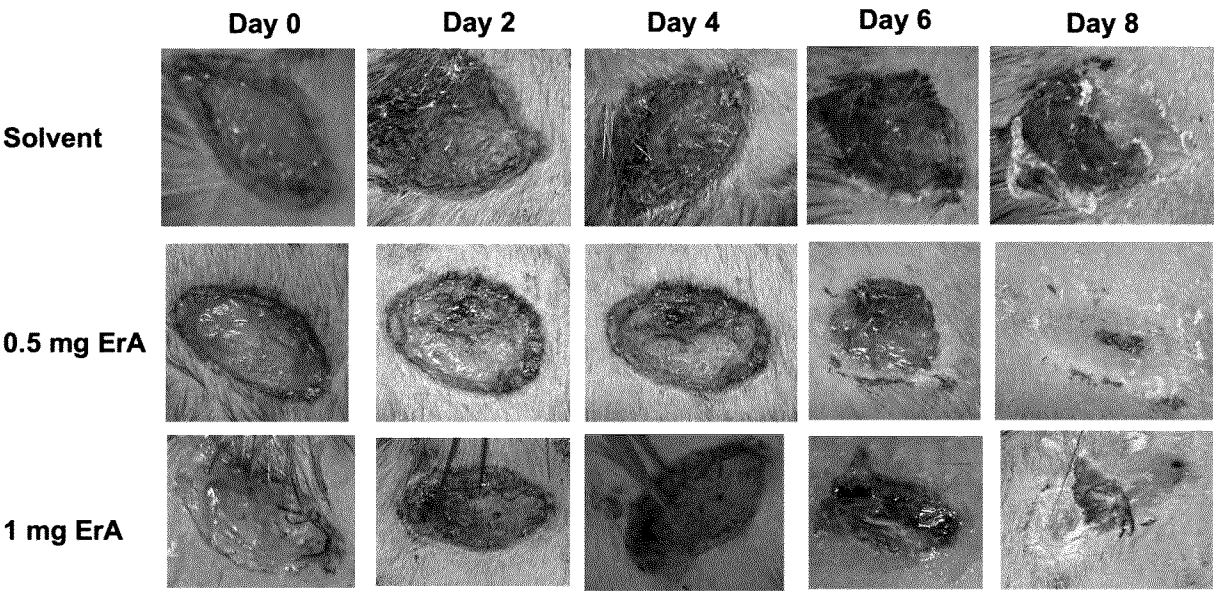


FIGURE 20

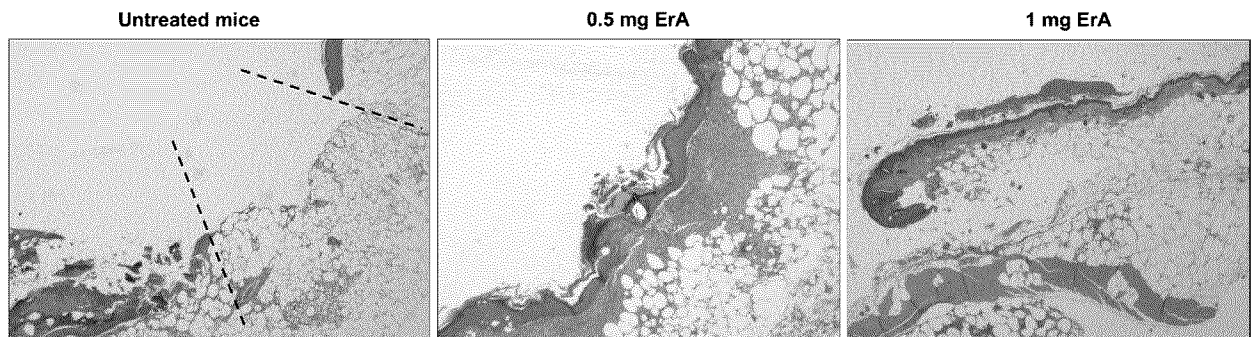


FIGURE 21

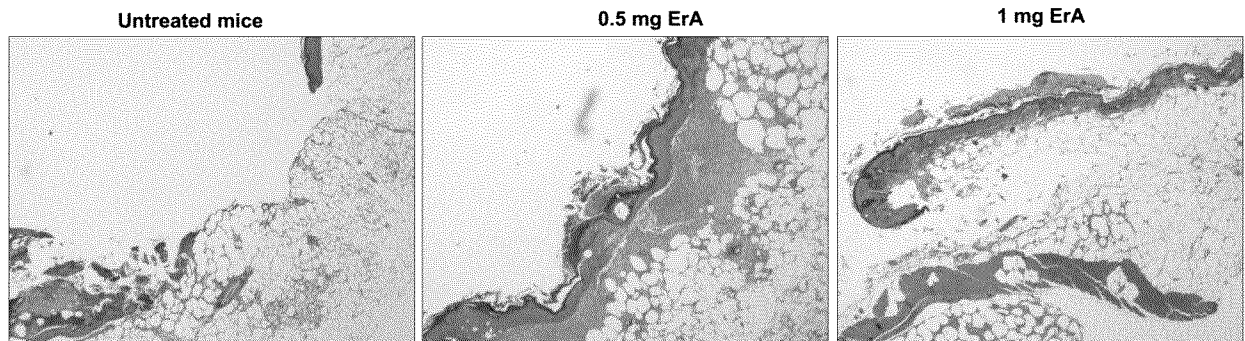


FIGURE 22

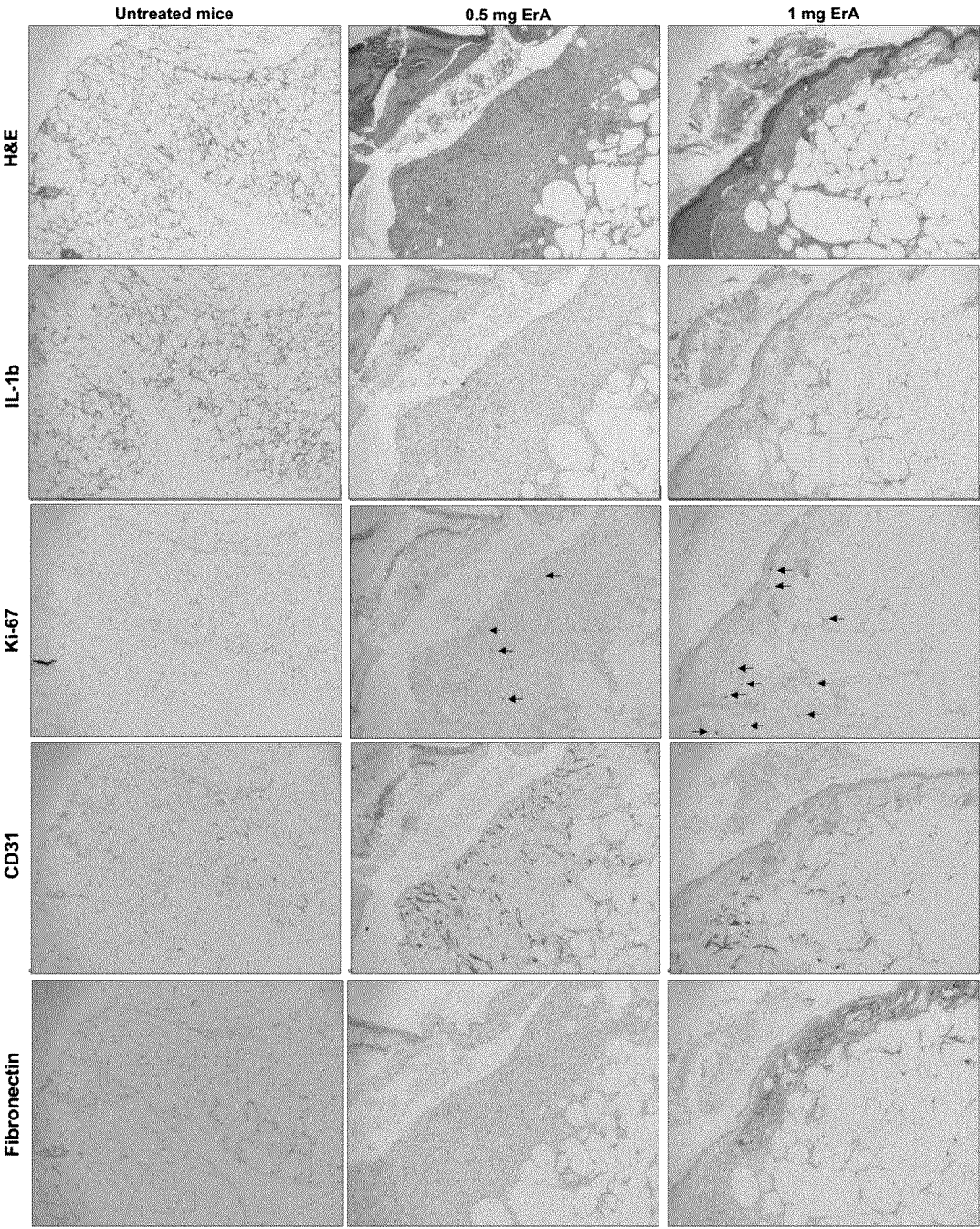
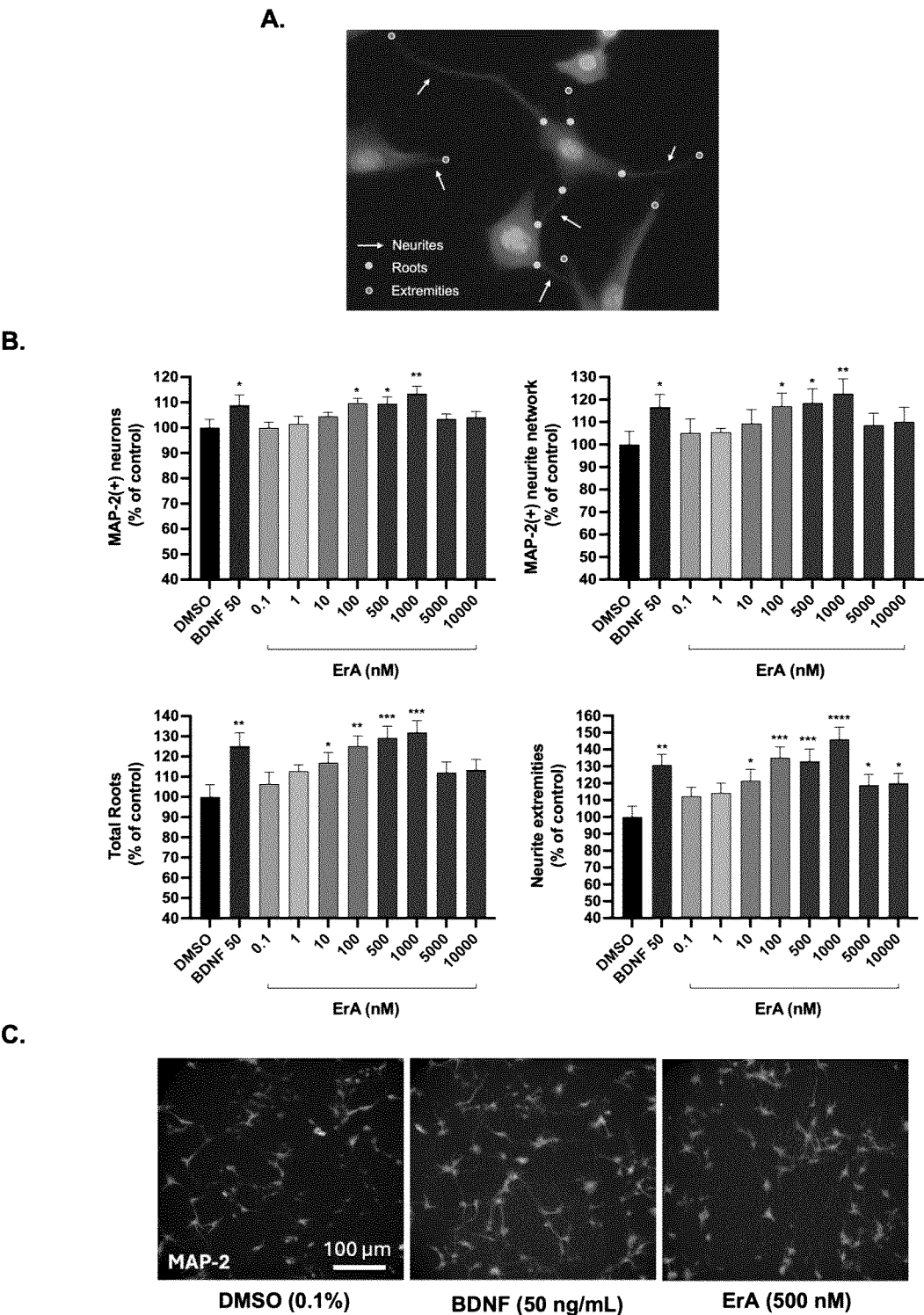


FIGURE 23



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/051717

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07C 235/40** (2006.01), **A61K 31/00** (2006.01), **A61K 31/16** (2006.01), **A61K 31/18** (2006.01),**A61K 31/19** (2006.01), **A61K 31/27** (2006.01) (more IPCs on the last page)

CPC: A61P 25/00 (2018.01), A61P 25/00 (2018.01), A61P 25/16 (2018.01),

A61P 25/28 (2018.01), A61P 25/28 (2018.01) (more CPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **C07C 235/40** (2006.01), **A61K 31/00** (2006.01), **A61K 31/16** (2006.01), **A61K 31/18** (2006.01), **A61K 31/19** (2006.01), **A61K 31/27** (2006.01) (more IPC's on the last page)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

STN: Structure search of Formula (I); SCOPUS (inventor and affiliation search); Orbit Intelligence (inventor, applicant and keywords: Erinacine A, wound, neurological, nerve growth factor, CNS, erinacine)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2021/0346346 A1 (CHADEAYNE, A. R.) 11 November 2021 (11-11-2021) *Erinacine H on page 42*	1-10
X	AYER, W.A. et al. "Metabolites of Bird's Nest Fungi. Part 11. Diterpenoid Metabolites of <i>Cyathus earlei</i> Lloyd" <i>Can. J. Chem.</i> 1979, 57 (24), 3332-3337 (01-12-1979) *compounds 12-14 and 17-18*	1-10
X	TORI, M. et al. "Total Synthesis of Allocyathin B ₂ , a Metabolite of Bird's Nest Fungi" <i>J. Org. Chem.</i> 1998, 63(2), 306-313 (01-01-1998) *compound 32*	1, 3, 4 and 6-10

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
05 March 2025 (05-03-2025)Date of mailing of the international search report
10 April 2025 (10-04-2025)Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Karla Randell (819) 635-5133

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/051717

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SNIDER, B.B. et al. "Synthesis of (±)-Allocyathin B ₂ and (+)-Erinacine A" <i>J. Org. Chem.</i> 1998, 63(14), 4732-4740 (12-06-1998) *compounds 4, 25 and 41-44*	1-10
X	WEI, J. et al. "Unprecedented Neoverrucosane and Cyathane Diterpenoids with Anti-Neuroinflammatory Activity from Cultures of the Culinary-Medicinal Mushroom <i>Hericium erinaceus</i> " <i>Molecules</i> 2023, 28(17) #6380 (31-08-2023) *whole document*	1-19
X	RUPCIC, Z. et al. "Two New Cyathane Diterpenoids from Mycelial Cultures of the Medicinal Mushroom <i>Hericium erinaceus</i> and the Rare Species, <i>Hericium flagellum</i> " <i>Int. J. Molecular Sciences</i> 2018, 19(3) #740 (06-03-2018) *whole document*	1-19
X	TSAI, P.-C. et al. "Preclinical Bioavailability, Tissue Distribution, and Protein Binding Studies of Erinacine A, a Bioactive Compound from <i>Hericium erinaceus</i> Mycelia Using Validated LC-MS/MS Method" <i>Molecules</i> 2021 26(15), #4510 (27-07-2021) *whole document*	1-19

International application No.
PCT/CA2024/051717

Form PCT/ISA/210 (patent family annex) (July 2022) Page 4 of 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/051717

IPC:

A61K 31/133 (2006.01), *A61K 31/235* (2006.01), *A61K 31/343* (2006.01), *A61K 31/415* (2006.01),
A61K 31/423 (2006.01), *A61K 31/505* (2006.01), *A61K 31/704* (2006.01), *A61K 31/4164* (2006.01),
A61K 31/4402 (2006.01), *A61K 31/4409* (2006.01), *A61K 31/7056* (2006.01), *A61P 25/00* (2006.01),
A61P 25/00 (2006.01), *A61P 25/16* (2006.01), *A61P 25/28* (2006.01), *A61P 25/28* (2006.01),
C07C 215/42 (2006.01), *C07C 233/72* (2006.01), *C07C 235/68* (2006.01), *C07C 237/24* (2006.01),
C07C 307/02 (2006.01), *C07D 203/04* (2006.01), *C07D 213/75* (2006.01), *C07D 213/81* (2006.01),
C07D 231/40 (2006.01), *C07D 239/34* (2006.01), *C07D 263/56* (2006.01), *C07D 307/79* (2006.01),
C07H 15/24 (2006.01)

CPC:

C07C 215/42 (2013.01), *C07C 233/72* (2013.01), *C07C 235/40* (2013.01), *C07C 235/68* (2013.01),
C07C 237/24 (2013.01), *C07C 307/02* (2013.01), *C07D 203/04* (2013.01), *C07D 213/75* (2013.01),
C07D 213/81 (2013.01), *C07D 231/40* (2013.01), *C07D 239/34* (2013.01), *C07D 263/56* (2013.01),
C07D 307/79 (2022.08), *C07H 15/24* (2015.07), *A61K 2121/00* (2013.01)