



(51) International Patent Classification:

A61K 47/60 (2017.01) A61P 17/00 (2006.01)

A61P 11/00 (2006.01) A61P 27/02 (2006.01)

A61P 1/00 (2006.01)

(21) International Application Number:

PCT/US2018/023109

(22) International Filing Date:

19 March 2018 (19.03.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/473,972 20 March 2017 (20.03.2017) US

62/501,567 04 May 2017 (04.05.2017) US

62/590,131 22 November 2017 (22.11.2017) US

(71) Applicant: SIENNA BIOPHARMACEUTICALS, INC.

[US/US]; 30699 Russell Ranch Road, Suite 140, Westlake Village, CA 91362 (US).

(72) Inventors: TRAVERSA, Silvio; 30699 Russell Ranch

Road, Suite 140, Westlake Village, CA 91362 (US).

MAINERO, Valentina; 30699 Russell Ranch Road, Suite

140, Westlake Village, CA 91362 (US). **HARRIS, Todd, James**; 30699 Russell Ranch Road, Suite 140, Westlake Village, CA 91362 (US). **RAVA ROSSA, Luisa, Bertarione**; 30699 Russell Ranch Road, Suite 140, Westlake Village, CA 91362 (US).

(74) Agent: **CHRISTENSEN, Michael, R.**; Knobbe, Martens, Olson & Bear, LLP, 2040 Main Street, 14th Floor, Irvine, CA 92614 (US).

(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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

(54) Title: POLYMER CONJUGATES TARGETING C-SRC WITH REDUCED EXPOSURE

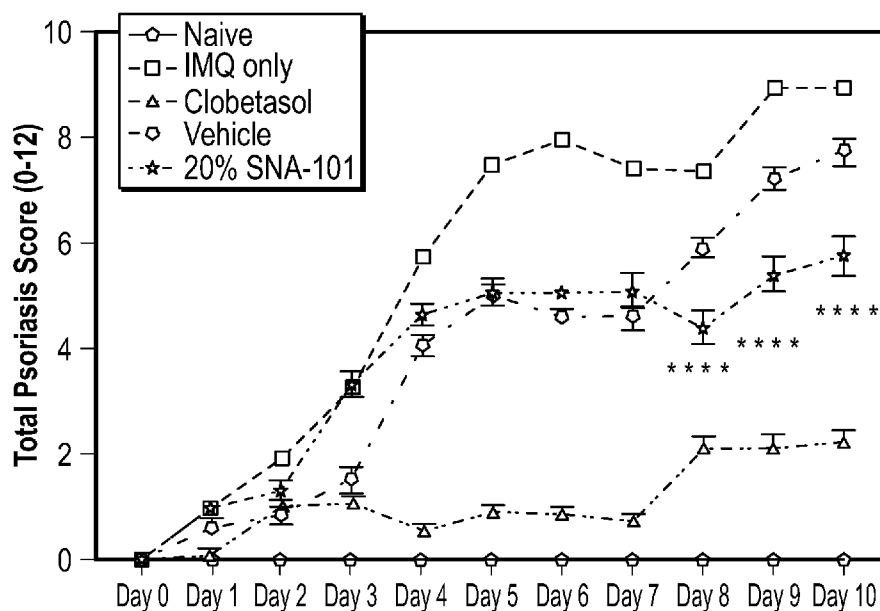


FIG. 74B

(57) Abstract: Disclosed herein are polymer conjugates, such as SNA-101, comprising an active agent linked to a polymer, wherein the active agent comprises an inhibitor, antagonist, or inverse agonist of c-Src. The disclosed polymer conjugates reduce exposure of the active agent at non-target sites.

TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

POLYMER CONJUGATES TARGETING C-SRC WITH REDUCED EXPOSURE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US provisional patent application Serial No. 62/473,972 filed March 20, 2017, and claims priority to US provisional patent application Serial No. 62/501,567 filed May 4, 2017, and claims priority to US provisional patent application Serial No. 62/590,131 filed November 22, 2017. Each of these applications is incorporated by reference in their entirety herein.

FIELD

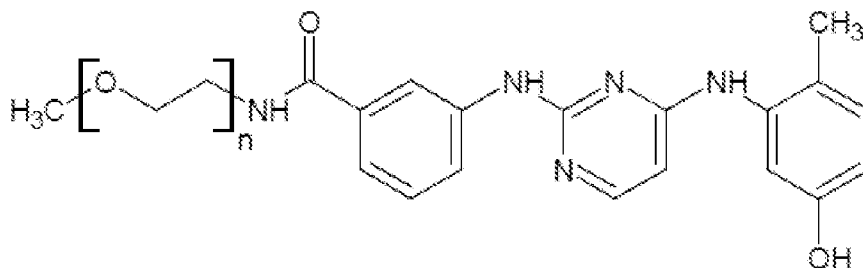
[0002] Disclosed herein are polymer conjugates, comprising active agents linked to polymers, and therapeutic uses thereof. More particularly, a polymer conjugate which exhibits reduced exposure to non-target sites and inhibits kinase mediators of various pathological conditions is described.

BACKGROUND

[0003] Inhibitors of c-Src have been described for possible therapeutic use in the prevention, alleviation and treatment of kinase-associated pathologies. However, such compounds are associated with broad kinase specificity, as well as undesirable and toxic effects. Accordingly, strategies to render these active kinase inhibitors more specific and less toxic are needed.

SUMMARY OF EMBODIMENTS

[0004] In several embodiments, a polymer conjugate (such as SNA-101) is provided having the following structure:



[0005] Effective delivery of pharmacologically active agents may be hindered by unwanted exposure of those agents to non-desired locations (such as the systemic circulation and/or lymphatic system). For example, topical agents useful in treating various skin disorders may result in toxic side effects because of systemic exposure. One issue with delivering compositions comprising one or more active agents topically (or non-topically) is the concern that such agents need to be delivered in an amount and at a location sufficient to have a therapeutic effect. At the same time however, exposure (e.g., absorption or longevity of the composition in the systemic circulation, lymphatic system, or other non-targeted sites) may not be desirable for multiple reasons, including, but not limited to, safety reasons. There remains an unmet need for compounds with reduced exposure at non-target sites that result in a clinically therapeutic effect.

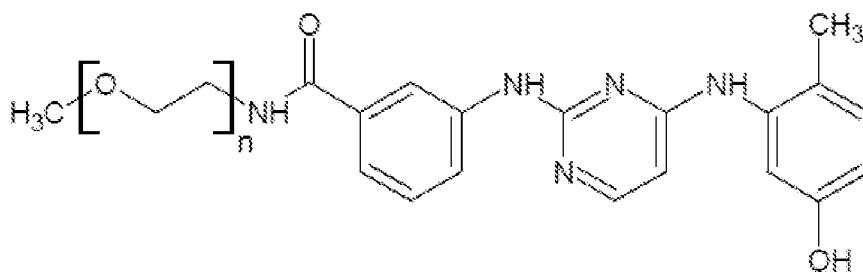
[0006] In several embodiments of the invention, the compositions described herein are both therapeutically efficacious and minimize non-target (e.g., systemic or bloodstream) exposure. In some embodiments, the active agents are PEGylated or otherwise coupled to large molecules, and surprisingly, are effective in crossing biological membranes such that the active agents are effectively delivered to the target location. Although inflammatory skin conditions are disclosed in several embodiments, other embodiments are used to treat non-dermal inflammation, as well as other several conditions (e.g., those conditions that would benefit from treatment with reduced exposure at non-target sites). For example, in some embodiments, the compositions and technology described herein are used in the gastrointestinal and pulmonary systems. Ophthalmic treatments are provided in some embodiments. In yet other embodiments, compositions for treating joints are provided. Treatment of the nose and ear are provided in other embodiments. Inflammatory and non-inflammatory conditions are contemplated herein.

[0007] Reduced exposure compounds and compositions are provided in several embodiments. "Reduced exposure" compounds are those compounds that, when delivered to a target location, are formulated to act at the target location with reduced exposure (e.g., entry and/or longevity) in non-target sites. Exposure is reduced as compared to active agents not formulated according to the embodiments described herein. As a non-limiting example, a PEGylated topical dermal active agent has reduced exposure to the bloodstream as compared to the active agent alone. Reduced exposure compounds include topical compounds that can be delivered to body surfaces and cavities such as the skin, eyes, ears, nose, mouth, vagina, rectum, etc., as well as oral (e.g., enteric coated) compounds for oral delivery that treat the gastrointestinal system (e.g., the GI lining), inhalants that treat the lungs, injections for joints, and other modes of delivery that target one location with the goal of reducing exposure to a non-desired site.

Non-desired target sites include, for example, the systemic system, the lymphatic system, non-target tissue, etc. “Reduced exposure compositions” comprise or consist essentially of one or more “reduced exposure compounds.”

[0008] Reduced exposure topical compositions are provided in many embodiments. In some embodiments, a reduced exposure composition is delivered orally, e.g., for treatment of the gastrointestinal system. The active agent remains in the lining of the gastrointestinal tract and is able to achieve pharmacological specificity. Because the active agent is conjugated with PEG or another molecule as described herein, the active agent is absorbed more slowly into the non-target site (e.g., the systemic circulation and/or lymphatic system). In some cases, less or none of the active agent is absorbed into the non-target site (e.g., systemic circulation and/or lymphatic system). Further, once the composition enters the systemic circulation and/or lymphatic system, clearance (e.g., by the kidney) occurs at a much faster rate. One or more of the advantages of (i) *reduced* absorption into the non-target site (e.g., systemic circulation and/or lymphatic system), (ii) *slower* absorption into the non-target site (e.g., systemic circulation and/or lymphatic system), and (iii) *faster* clearance rates from the non-target site (e.g., systemic circulation and/or lymphatic system) are also achieved when using the compositions (formulated according to the methods described herein) for treating the eye (e.g., via eye drops), the lungs (e.g., via inhalants), the skin (e.g., via dermal topicals), joints (e.g., via injectables), nasal passageways, and the ear (such as the ear canal and other structures). Vaginal and rectal tissues are treated in some embodiments via, for example suppositories.

[0009] In several embodiments, there is provided in a reduced exposure composition, a polymer conjugate comprising a warhead (e.g., at least one active agent) linked to a polymer, wherein the warhead comprises an inhibitor, antagonist, or inverse agonist of, for example, c-Src. In some embodiments, at least one inhibitor, antagonist, or inverse agonist of c-Src comprises or consists of a composition that includes any one of compounds 1-71 (and derivatives thereof) disclosed herein in Table 1 coupled to a polymer. In some embodiments, the warhead of the polymer conjugate is compound 1. In some embodiments, the polymer conjugate is SNA-101 (also referred to as CT101), having the following formula:



[0010] Non-dermal (non-skin) inflammation or other conditions may also be treated in some embodiments with compositions comprising these compounds. Non-inflammatory conditions may also be treated with some embodiments.

[0011] As described above, several embodiments disclosed herein provide reduced or minimized exposure (e.g., entry into and/or longevity in a non-target site such as the systemic circulation and/or lymphatic system). In some embodiments, exposure at a non-target site is less than 90%, 75%, 50%, 25%, 15%, 10%, 5% or 2% (or less) of the polymer conjugate as compared to a similar active entity that has not been produced according to the embodiments described herein. In some embodiments, desirable rate of clearance from the non-target site (e.g., systemic circulation and/or lymphatic system) for the compositions described herein is increased by at least 10%, 25%, 50%, or 75% or more as compared to non-conjugated controls. As an example, a PEGylated active agent described herein not only penetrates the desired membranes to reach a desired target, but has reduced non-target exposure by at least 20-80% or more as compared to the non-PEGylated active agent. In some embodiments, blood concentrations measured post administration of the compositions described herein are less than about 0.1 ng/ml, less than 1 ng/ml, or less than 10 ng/ml after, e.g., 15 minutes, 30 minutes, 1 hour, 6 hours or 12 hours.

[0012] In some embodiments, reduced exposure at non-target sites contributes to enhanced efficacy. Efficacy may be enhanced because lower concentrations/amounts/dosing schedules are required to achieve the same or similar therapeutic efficacy at the target site (because, for example, the active ingredient stays at the desired target site for a longer time). In one embodiment, concentrations/amounts/dosing schedules are reduced by 25%-75% or more.

[0013] More rapid clearance rates of the active agent once in the non-target site(s) (such as systemic circulation and/or lymphatic system) are also beneficial because this may allow for a *higher* concentration or *more* doses to be delivered. This is especially beneficial for active agents in which a subject would benefit from a higher dose but cannot tolerate the higher dose due to toxicity at the non-target site (e.g., systemic toxicity). Faster clearance rates would permit the desired higher dose to be

delivered according to the desired schedule. For example, a subject may be able to tolerate daily doses rather than weekly doses because of the reduced exposure.

[0014] In some embodiments, the active agents of the compositions described herein (e.g., the compounds in Table 1 conjugated e.g., with PEG or other polymers) are measured in non-target sites (e.g., the systemic circulation and/or lymphatic system) at less than amounts found when the active agent is delivered without conjugation (e.g., less than 0.5%, 1% or 2% after 6 or 12 hours, as compared with 3-15% (e.g., 3-6%) when the active agent is delivered without conjugation). In some embodiments, the active agents of the compositions described herein (e.g., the compounds in Table 1 conjugated e.g., with PEG or other polymers) are measured in non-target sites (e.g., the systemic circulation and/or lymphatic system) at less than 0.5%, 1% or 2% after 3-24 hours, as compared to an amount 2-20 times greater when the active agent is delivered without conjugation.

[0015] In some embodiments, clearance of the compositions (e.g., the conjugated polymer compounds) occurs within minutes of exposure to the non-target site (e.g., systemic circulation and/or lymphatic system), as opposed to hours. In other embodiments, 50% clearance of the conjugated polymer compounds occurs in less than 5 minutes, 15 minutes, 30 minutes, 1 hour, 6 hours, and 12 hours of exposure to the systemic circulation and/or lymphatic system. Clearance times of the conjugated polymer compounds are reduced by more than 25%, 50%, 75% and 90%, as compared to the non-conjugated active agents or other formulations. These reduced clearance times are beneficial to reduce toxicity and undesired side effects.

[0016] In some embodiments, an active agent may be increasingly toxic as it is metabolized in the non-target site (e.g., systemic circulation and/or lymphatic system) because the metabolites exhibit more toxicity than the original agent. Thus, faster clearance rates, in some cases even before the toxic metabolites are created, are especially beneficial.

[0017] The term "active entity" as used herein should not be understood as limiting the participation of the polymer itself and/or the chemical linking moiety between the polymer and the warhead in defining the pharmacology of the polymer conjugate. In some embodiments, the polymer influences the selectivity and/or inhibitory activity of the polymer conjugate. In some embodiments, the chemical linking moiety between the polymer and warhead influences the selectivity and/or inhibitory activity of the polymer conjugate. In some embodiments, the polymer conjugates exhibit no change in selectivity or inhibitory activity against the therapeutic target in comparison with the unconjugated active agent. In some embodiments, the polymer conjugates exhibit a significant increase in selectivity against the therapeutic target in comparison with the

unconjugated active agent. In some embodiments, the polymer conjugates exhibit a significant increase in inhibitory activity against the therapeutic target in comparison with the unconjugated active agent. In some embodiments, the polymer conjugates exhibit a significant increase in selectivity and inhibitory activity against the therapeutic target in comparison with the unconjugated active agent. In some embodiments, the increased selectivity and/or inhibitory activity of the polymer conjugate against the therapeutic target in comparison with the unconjugated active agent causes decrease in undesired biological effects. In some embodiments, the increased selectivity of the polymer conjugate is caused by an increase of the hydrodynamic volume resulting from the conjugated polymer chain. In some embodiments, the polymer chain creates a higher steric hindrance which allows discrimination among the diverse shapes and sizes of the binding sites of different proteins, thus improving selectivity with respect to the active agent alone.

[0018] In several embodiments, various inflammatory skin diseases are treated. The inflammatory skin disease comprises, in some embodiments, psoriasis, psoriasis guttata, inverse psoriasis, pustular psoriasis, psoriatic erythroderma, acute febrile neutrophilic dermatosis, eczema, xerotic eczema, dyshidrotic eczema, vesicular palmar eczema, acne vulgaris, atopic dermatitis, contact dermatitis, allergic contact dermatitis, dermatomyositis, exfoliative dermatitis, hand eczema, pompholyx, rosacea, rosacea due to sarcoidosis, rosacea due to scleroderma, rosacea due to Sweet syndrome, rosacea due to systemic lupus erythematosus, rosacea due to urticaria, rosacea due to herpetic pain, Sweet's disease, neutrophilic hydrodenitis, sterile pustule, drug rash, seborrheic dermatitis, pityriasis rosea, Kikuchi's disease of the skin, pruritic urticarial papules and plaques of pregnancy, Stevens-Johnson syndrome and toxic epidermal necrolysis, tattoo reaction, Wells syndrome (eosinophilic cellulitis), reactive arthritis (Reiter syndrome), bowel-associated dermatosis-arthritis syndrome, rheumatoid neutrophilic dermatosis, neutrophilic eccrine hidradenitis, neutrophilic skin disease of dorsum of hand, balanitis circumscripta plasmacellularis, balanoposthitis, Behcet's disease, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiforme, granuloma annulare, dermatitis of hand, lichen nitidus, lichen planus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, nummular dermatitis, pyoderma gangrenosum, sarcoidosis, subcorneal pustular dermatosis, urticaria, skin fibrosis, and transient acantholytic dermatosis.

[0019] Also provided herein, in several embodiments, are polymer conjugates wherein the polymer is polyethylene glycol (PEG) or methoxy-polyethylene glycol (m-PEG). In several embodiments, there is provided a pharmaceutical composition comprising or consisting essentially of a polymer conjugate disclosed herein that is

formulated for topical and non-topical administration. In several embodiments, methods of making and using the compositions described herein are provided.

[0020] In several embodiments, the invention comprises a reduced exposure composition comprising at least one active entity linked to at least one polymer, wherein the composition has reduced exposure at a non-target site as compared to the active entity delivered without the polymer. The non-target site comprises the systemic system, the lymphatic system and/or another non-target tissue site in some embodiments.

[0021] In some embodiments, the active entity comprises an inhibitor, an antagonist, or an inverse agonist. For example, the active entity may be an inhibitor, antagonist, or inverse agonist of c-Src. In some embodiments, inflammatory conditions are treated. In other embodiments, non-inflammatory conditions are treated. The active entity comprises or consists essentially of any one or more of compounds 1-71 in some embodiments. In some embodiments, the active entity comprises compound 1. In some embodiments, the composition comprises CT101.

[0022] The active entity binds to c-Src in some embodiments. The binding may be partially or fully inhibitory or not.

[0023] In some embodiments, the polymer used in the reduced exposure compounds comprises polyethylene glycol (PEG) and/or methoxy-polyethylene glycol (m-PEG). In embodiments where the active entity has one or more carboxyl, hydroxyl, amino and/or sulfhydryl groups, the active entity is PEGylated (or conjugated/coupled to another polymer) at one or more of said carboxyl, hydroxyl, amino and/or sulfhydryl groups.

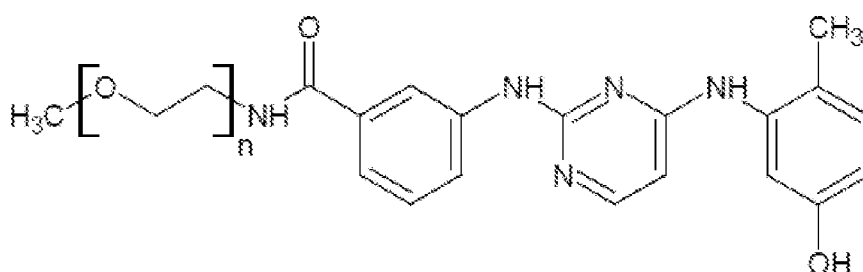
[0024] The reduced exposure compositions described herein are formulated for topical administration in several embodiments. Inhalants, injectables, eye drops, nasal sprays, oral administration etc. are provided in some embodiments. In several embodiments, methods of treating one or more of the following are provided: non-dermal inflammation, inflammatory skin disease, wounds, scars, autoimmune disorders, and cancerous or pre-cancerous lesions. Kits comprising one or more compounds and devices for administration (syringes, containers, inhalers, etc.), as well as instructions for use, are provided in certain embodiments.

[0025] Compositions may be administered via at least two routes of administration, either simultaneously or sequentially according to some embodiments. In one embodiment, the composition is administered via a first (e.g. topical dermal) route to a subject, wherein the subject further receives an additional agent via a second (e.g., non-dermal) route to achieve synergetic effects.

[0026] In several embodiments, the inventions comprises methods for reducing exposure of a composition at least one non-target site, wherein the method

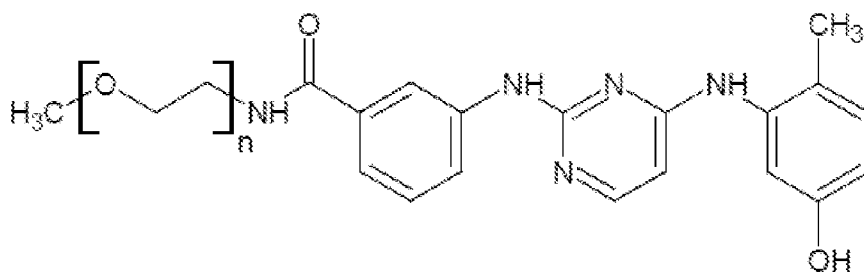
comprises applying a composition comprising at least one active entity linked to at least one polymer, wherein the combination of the active entity and polymer reduces exposure at the non-target site by more than 50% as compared to the active entity without the polymer. The composition may be applied topically, injected, inhaled, or administered orally. The non-target site includes non-target tissue at which pharmacological activity is not desired and/or not achieved. Non-target sites can include the bloodstream or systemic system. Non-target sites can also include the lymphatic system.

[0027] In several embodiments, a compound having the formula:



[0028] is provided. In some embodiments, n ranges from about 4 to about 1140 (e.g., 4-10, 10-20, 20-40, 40-60, 60-80, 80-100, 125-150-150-175, 175-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, and overlapping ranges therein). There is provided, in some embodiments, stereoisomers, enantiomers, and/or pharmaceutically acceptable salts of the compound.

[0029] In several embodiments, a reduced exposure composition for treating a target site is provided. In one embodiment, the composition comprises or consists essentially of a conjugate comprising or consists essentially of an active entity coupled (e.g., linked) to at least one polymer. Two, three or more active entities or two, three or more polymers may be used. In one embodiment, a pharmaceutically acceptable carrier formulated for delivering the conjugate to the target site is also provided. In several embodiments, the conjugate has reduced exposure at a non-target site as compared to the active entity delivered without the polymer. The non-target site includes for example the systemic system, the lymphatic system and/or other non-target tissue sites. In several embodiments, the non-target site comprises any site at which pharmacological activity is not desired and/or not achieved. In several embodiments, the conjugate has the formula:



[0030] In some embodiments, n ranges from about 4 to about 1140 (e.g., 4-10, 10-20, 20-40, 40-60, 60-80, 80-100, 125-150-150-175, 175-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, and overlapping ranges therein). There is provided, in some embodiments, stereoisomers, enantiomers, and/or pharmaceutically acceptable salts of the conjugate.

[0031] In several embodiments, a reduced exposure composition for treating a cell within a target site is provided. Methods for treating diseases, conditions, and disorders are also provided. In one embodiment, the composition comprises or consists essentially of a conjugate comprising or consists essentially of an active entity coupled (e.g., linked) to at least one polymer. Two, three or more active entities or two, three or more polymers may be used. The active entity may be for example, an inhibitor, antagonist, or inverse agonist of a cellular kinase. In several embodiments, the active entity is one or more of compounds 1-71. In one embodiment, the composition comprises compound 1. In one embodiment, the composition comprises SNA-101. The polymer can include, for example, polyethylene glycol (PEG) and/or methoxy-polyethylene glycol (m-PEG). In one embodiment, a pharmaceutically acceptable carrier formulated for delivering the conjugate to the target site is also provided. In several embodiments, the conjugate has reduced exposure at a non-target site as compared to the active entity delivered without the polymer. The non-target site includes for example the systemic system, the lymphatic system and/or other non-target tissue sites. In several embodiments, the non-target site comprises any site at which pharmacological activity is not desired and/or not achieved. In one embodiment, the conjugate can advantageously traverse plasma membranes of cells at the target site, thereby promoting interactions between the active entity and the cellular kinase. This traversal may include the crossing of cellular lipid bilayers to, e.g., distribute the active entity among both lipophilic and hydrophilic cellular compartments. Membranes include the lipid bilayer, plasma membrane and the nuclear membrane as examples. In several embodiments, the conjugate interacts with a kinase associated with the plasma membrane, cytoplasm and/or nucleus. The conjugate may exhibit a depot effect across

cellular compartments, thereby reducing the dose of the active entity required to inhibit the cellular kinase compared to the active entity without conjugation to the polymer. In several embodiments, the cellular kinase may be c-Src. In some embodiments, c-Src is bound and/or inhibited by the active entity. In some embodiments, the active entity has one or more carboxyl, hydroxyl, amino and/or sulfhydryl groups. In one embodiment, at least one polymer is conjugated to the active entity at the one or more carboxyl, hydroxyl, amino and/or sulfhydryl groups.

[0032] In some embodiments, the reduced exposure composition may be formulated for topical, oral, local ocular (e.g., eye drop), inhalation, injection or suppository delivery. Topical, oral, injection, inhalation, local ocular, and suppository administration is provided in several embodiments. In several embodiments, the administration is daily. In the methods of treatment, effective amounts of the active entity are delivered to a subject (e.g., human or veterinary). In several embodiments, the composition may be administered via at least two routes of administration, either simultaneously or sequentially. In some embodiments, the composition is administered via a topical route to a subject, and the subject further receives an additional agent via a non-topical route. In some such embodiments, this co-administration achieves synergetic effects. The composition may further comprise one or more additional ingredients, such as, for example, a protective agent, an emollient, an astringent, a humectant, a sun screening agent, a sun tanning agent, a UV absorbing agent, an antibiotic agent, an anti-angiogenesis agent, a preventive or therapeutic agent for inflammatory bowel disease, a physiological cooling agent, an antifungal agent, an antiviral agent, an antiprotozoal agent, an anti-acne agent, an anesthetic agent, a steroidal anti-inflammatory agent, a non-steroidal anti-inflammatory agent, an antipruritic agent, an additional antioxidant agent, a chemotherapeutic agent, an anti-histamine agent, a vitamin or vitamin complex, a hormone, an anti-dandruff agent, an anti-wrinkle agent, an anti-skin atrophy agent, a skin whitening agent, and/or a cleansing agent.

[0033] In several embodiments, the active entity and/or conjugate may have a longer residence time within a cell or other tissue at the target site compared to the active entity without conjugation to the polymer. For example, the residence time of the active entity and/or conjugate within a cell or other tissue at the target site is, as compared to the active entity without conjugation to the polymer, (i) at least 25% (e.g., 25-50%, 50-75%, 75-100%, 100-150%, or higher and overlapping ranges therein) longer and/or (ii) at least 2-20 fold (e.g., 2-10 fold, 2-4 fold, 4-6 fold, 6-8 fold, 8-10 fold, 10-12 fold, 12-14 fold, 14-16 fold, 16-18 fold, 18-20 fold, 20-30 fold, 40-50 fold, 10-50 fold, 50-100 fold, and overlapping ranges therein) longer. In one embodiment, the residence time is over 100 fold longer.

[0034] In some embodiments, a smaller dose of the conjugate may be needed to achieve a therapeutic effect comparable to the active entity without conjugation to the polymer. For example, in several embodiments, the dose of the conjugate needed to achieve a therapeutic effect comparable to the active entity without conjugation to the polymer is at least 10% (e.g., 10-15%, 15-20%, 20-25%, 25-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-100%, 100%-125%, 125-150%, or higher and overlapping ranges therein) lower. In one embodiment, the dose is over 200% lower. In some embodiments, fewer doses and/or smaller doses of the conjugate are required as compared to the active entity delivered without the polymer.

[0035] In several embodiments, the active entity and/or conjugate may have an increased concentration, activity and/or bioavailability within a cell or tissue at the target site compared to the active entity without conjugation to the polymer. In some such embodiments, the therapeutically effective amount of the active entity is at the target site. For example, the concentration, activity and/or bioavailability within a cell or other tissue at the target site is, as compared to the active entity without conjugation to the polymer, at least 2-20 fold (e.g., 2-4 fold, 4-6 fold, 6-8 fold, 8-10 fold, 10-12 fold, 14-16 fold, 18-20 fold, 20-30 fold, 30-40 fold, 40-50 fold, 50-100 fold, and overlapping ranges therein) greater than within a cell or tissue at a non-target site (e.g., the systemic system, the lymphatic system, the circulatory system, bone marrow). In one embodiment, the concentration, activity and/or bioavailability within a cell or tissue at the target site is over 100 fold greater.

[0036] In several embodiments, the active entity and/or conjugate may have reduced concentration, activity and/or bioavailability within a cell or tissue at a non-target site compared to the active entity without conjugation to the polymer. In several embodiments, the active entity and/or conjugate is present at a biologically inactive concentration within a cell or tissue at a non-target site. In several embodiments, reduced concentration, activity and/or bioavailability within a cell or tissue at a non-target site (e.g., the systemic system, the lymphatic system, bone marrow, the circulatory system) advantageously reduces toxicity and/or other side effects, such as, for example, immunosuppression. For example, in some embodiments, the active entity and/or conjugate has reduced systemic absorption and/or little or no systemic toxicity when the composition is formulated for oral delivery and is administered orally (e.g., a single administration, administration on a daily basis).

[0037] In several embodiments, the conjugate is amphiphilic and/or amphipathic. In some embodiments, the conjugate is more amphiphilic and/or amphipathic than the active entity without conjugation to the polymer. For example, in several embodiments, the conjugate, as compared to the active entity without

conjugation to the polymer, is at least 25% (e.g., 20-25%, 25-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-100%, 100%-125%, 125-150%, or higher and overlapping ranges therein) more amphiphilic. In one embodiment, the amphiphilicity is over 200% greater. Additionally, in some embodiments, the conjugate is more hydrophilic than the active entity without conjugation to the polymer. For example, in several embodiments, the conjugate, as compared to the active entity without conjugation to the polymer, is at least 25% (e.g., 20-25%, 25-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-100%, 100%-125%, 125-150%, or higher and overlapping ranges therein) more hydrophilic. In one embodiment, the hydrophilicity is over 200% greater. In some embodiments, the greater hydrophilicity of the conjugate advantageously facilitates one or more of: non-compartmentalization within a cell or tissue at the target site; access to and activity in both the lipid bilayer and the cytosol of the cell; access to and/or activity in both the lipid bilayer and the cytoplasm of the cell; and/or access to and/or activity across the lipid bilayer. In some embodiments, the conjugate exhibits greater access to the kinase compared to the active entity without conjugation to the polymer.

[0038] In several embodiments, the method of treatment and/or use of the compositions described herein are provided for the prophylaxis or treatment of one or more of the following in a subject in need thereof: a joint, an eye, an autoimmune disorder, the gastrointestinal system, a lung, a cancerous or pre-cancerous lesion, a scar, a wound, non-dermal inflammation, an inflammatory condition, an inflammatory skin condition, and/or an inflammatory skin disease.

[0039] In several embodiments, the method of treatment and/or use of the compositions described herein are provided for the prophylaxis or treatment of one or more of the following conditions: psoriasis, psoriasis guttata, inverse psoriasis, pustular psoriasis, psoriatic erythroderma, acute febrile neutrophilic dermatosis, eczema, xerotic eczema, dyshidrotic eczema, vesicular palmar eczema, acne vulgaris, atopic dermatitis, contact dermatitis, allergic contact dermatitis, dermatomyositis, exfoliative dermatitis, hand eczema, pompholyx, rosacea, rosacea due to sarcoidosis, rosacea due to scleroderma, rosacea due to Sweet syndrome, rosacea due to systemic lupus erythematosus, rosacea due to urticaria, rosacea due to herpetic pain, Sweet's disease, neutrophilic hydrodenitis, sterile pustule, drug rash, seborrheic dermatitis, pityriasis rosea, Kikuchi's disease of the skin, pruritic urticarial papules and plaques of pregnancy, Stevens-Johnson syndrome and toxic epidermal necrolysis, tattoo reaction, Wells syndrome (eosinophilic cellulitis), reactive arthritis (Reiter syndrome), bowel-associated dermatosis-arthritis syndrome, rheumatoid neutrophilic dermatosis, neutrophilic eccrine hidradenitis, neutrophilic skin disease of dorsum of hand, balanitis circumscripta

plasmacellularis, balanoposthitis, Behcet's disease, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiforme, granuloma annulare, dermatitis of hand, lichen nitidus, lichen planus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, nummular dermatitis, pyoderma gangrenosum, sarcoidosis, subcorneal pustular dermatosis, urticaria, skin fibrosis, and transient acantholytic dermatosis, alopecia, alopecia areata, androgenic alopecia, and/or dry eye.

BRIEF DESCRIPTION OF THE FIGURES

[0040] The Figures below are illustrative for some embodiments and should not be construed as overly limiting.

[0041] Figure 1 depicts the chemical structure of benzamide ChEMBL 249097.

[0042] Figure 2 depicts the synthesis scheme of CT101.

[0043] Figure 3 depicts the HPLC chromatogram of carboxylic intermediate 2 (280nm).

[0044] Figure 4 depicts the report of the flash purification of carboxylic intermediate 2.

[0045] Figure 5 depicts the chromatogram of CT101 reaction mixture at 23h (280nm).

[0046] Figure 6 depicts the chromatogram of CT101 reaction mixture at 23h (ELSD).

[0047] Figure 7 depicts the report of the flash purification of CT101.

[0048] Figure 8 depicts the preparative HPLC chromatogram of CT101 (280nm).

[0049] Figure 9 depicts the analytical HPLC of CT101 (280nm).

[0050] Figure 10 depicts CT101.

[0051] Figure 11 depicts CT101 synthesis.

[0052] Figure 12 depicts the MW 229 Da by-product.

[0053] Figure 13 depicts the hypothesized MW 258 Da by-product structures.

[0054] Figure 14 depicts the hypothesized MW 272 Da by-product structures.

[0055] Figure 15 depicts the HPLC analysis of hydrolysis reaction mixture/ 66 h aging time (HPLC method MI).

[0056] Figure 16 depicts the HPLC analysis of crude Carboxylic acid intermediate (HPLC method MI CT101 001).

[0057] Figure 17 depicts the HPLC analysis of amidation reaction mixture/21 h aging time (HPLC method MI CT101 001).

[0058] Figure 18 depicts the HPLC analysis of crude CT101 (HPLC method MI CT101 001).

[0059] Figure 19 depicts the UV Profile (@270 and 210 nm) of CT101 purification by reversed phase flash chromatography.

[0060] Figure 20 depicts the HPLC analysis of final CT101 lot n° 2013RB20/S48 (HPLC method MI CT101 002).

[0061] Figure 21 depicts the NMR analysis of final CT101/lot n° 2013RB20/S48.

[0062] Figure 22 depicts the ESI MS analysis of CT101 lot n° 2013RB20/S48/single charged ion.

[0063] Figure 23 depicts the certificate of analysis of final CT101/ lot n° 2013BR20/S48.

[0064] Figure 24 depicts the HPLC analysis of final CT101 lot n° 2013RB20/S49 (HPLC method MI CT101 002).

[0065] Figure 25 depicts the NMR analysis of final CT101/lot n° 2013RB20/S49.

[0066] Figure 26 depicts the ESI MS analysis of CT101 lot n° 2013RB20/S49/single charged ion.

[0067] Figure 27 depicts the certificate of analysis of final CT101/lot n° 2013RB20/S49.

[0068] Figure 28 depicts representative chromatograms detected using UV analysis (A) and MS analysis (B).

[0069] Figure 29 depicts chromatograms of a CT101 plasma standard extract (100 µg/mL) detected using SIR (TIC, upper) and UV (lower).

[0070] Figure 30 depicts method validation.

[0071] Figure 31 depicts individual chromatograms used for the analysis of CT101 (sample: plasma spiked with CT101 for 100 µg/mL). Top chromatogram: 1167.5; middle chromatogram: 778.6, bottom chromatogram: 584.2.

[0072] Figure 32 depicts mouse plasma concentrations of CT101. Data are presented as Mean ± CI 95%.

[0073] Figure 33 depicts representative chromatograms showing CT101 in extracted murine plasma following intra-venous administration. Top chromatogram: 2 hours. Middle chromatogram: 10 minutes, bottom chromatogram: blank murine plasma.

[0074] Figure 34 depicts representative chromatograms showing CT101 in extracted murine plasma following epicutaneous administration. Top chromatogram: 8 hours, middle chromatogram: 0 hours, bottom chromatogram: blank murine plasma.

[0075] Figure 35 depicts raw luminescence values for a CT101 pre-incubation time of 6 hours and a stimulation time of 24 hours. Each symbol represents an individual well. Each condition was tested in sextuplicate.

[0076] Figure 36 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 6 hours and a stimulation time of 24 hours.

[0077] Figure 37 depicts raw luminescence values for a CT101 pre-incubation time of 18 hours and a stimulation time of 24 hours. Each condition was tested in sextuplicate except for Jurkat cells where conditions were tested in triplicate.

[0078] Figure 38 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 18 hours and a stimulation time of 24 hours.

[0079] Figure 39 depicts raw luminescence values for a CT101 pre-incubation time of 24 hours and a stimulation time of 24 hours. Each condition was tested in sextuplicate except for Jurkat cells where conditions were tested in triplicate.

[0080] Figure 40 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 24 hours and a stimulation time of 24 hours.

[0081] Figure 41 depicts raw luminescence values for a CT101 pre-incubation time of 6 hours and a stimulation time of 6 hours. Each condition was tested in sextuplicate.

[0082] Figure 42 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 6 hours and a stimulation time of 6 hours.

[0083] Figure 43 depicts raw luminescence values for a CT101 pre-incubation time of 18 hours and a stimulation time of 6 hours. Each condition was tested in sextuplicate except for Jurkat cells where conditions were tested in triplicate.

[0084] Figure 44 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 18 hours and a stimulation time of 6 hours.

[0085] Figure 45 depicts raw luminescence values for a CT101 pre-incubation time of 24 hours and a stimulation time of 6 hours. Each condition was tested in sextuplicate.

[0086] Figure 46 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 24 hours and a stimulation time of 6 hours.

[0087] Figure 47 depicts percentage cytotoxicity for a CT101 pre-incubation time of 6 hours and a stimulation time of 24 hours.

[0088] Figure 48 depicts percentage cytotoxicity for a CT101 pre-incubation time of 18 hours and a stimulation time of 24 hours.

[0089] Figure 49 depicts percentage cytotoxicity for a CT101 pre-incubation time of 24 hours and a stimulation time of 24 hours.

[0090] Figure 50 depicts percentage cytotoxicity for a CT101 pre-incubation time of 24 hours and a stimulation time of 6 hours.

[0091] Figure 51 depicts percentage cytotoxicity for a CT101 pre-incubation time of 6 hours and a stimulation time of 6 hours.

[0092] Figure 52 depicts bodyweights. Data are presented as Mean $\pm$ SEM percentages of the initial bodyweights. # $p < 0.05$ and ### $p < 0.001$ when compared to Day 0. ° $p < 0.05$, °° $p < 0.01$ and **** $p < 0.0001$ when compared to the vehicle-treated group.

[0093] Figure 53 depicts ear swelling. Data are presented as Mean $\pm$ SEM. ### $p < 0.0001$ in the vehicle-treated group when compared to Day 0. °° $p < 0.01$, °°° $p < 0.001$ and °°°° $p < 0.0001$ when compared to the vehicle-treated group. Betamethasone 0.1 % induced a significant reduction of ear swelling when compared to the vehicle-treated group on Day 8, Day 11 and Day 14 ($p < 0.0001$).

[0094] Figure 54 depicts macroscopic scores. Data are presented as Mean $\pm$ SEM. ##### $p < 0.0001$ when compared to Day 0 in the vehicle-treated group. °°° $p < 0.01$ and °°°°, °°°°° $p < 0.0001$ when compared to the vehicle-treated group.

[0095] Figure 55 depicts cytokine levels in ears (pg/ml). A. IL-1 β B. IFN- γ C. IL-4 D. IL-10. Data are presented as Mean $\pm$ SEM. **** $p < 0.0001$, ** $p < 0.001$, * $p < 0.01$ when compared to the Vehicle treated group. ##### $p < 0.0001$, ### $p < 0.001$ when compared to the right ear within the same group.

[0096] Figure 56 depicts bodyweights. Data are presented as Mean $\pm$ SEM of the initial (Day -13) bodyweights. * $p < 0.05$, ** $p < 0.01$.

[0097] Figure 57 depicts ear swelling. Data are presented as Mean $\pm$ SEM of the difference between ovalbumin challenged and contralateral (saline-injected) ears. Statistical significances: # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ when compared to the baseline (0 hours) values. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when comparing to the Control group.

[0098] Figure 58 depicts ear swelling at peak disease (fifteen minutes after the ovalbumin challenge). Data are presented as Mean $\pm$ SEM of the difference between ovalbumin-challenged and contralateral (saline-injected) ears. Statistical significances: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to the vehicle-treated group.

[0099] Figure 59 depicts erythema scores (Challenged ears). Data are presented as Mean $\pm$ SEM.

[0100] Figure 60 depicts erythema scores (Challenged ears). Data are presented as Mean $\pm$ SEM.

[0101] Figure 61 depicts histopathology scores (Left ears). Data are presented as Mean $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$ when compared to the Control group.

[0102] Figure 62 depicts representative histopathology pictures. Left panel: Left ears. Right panel: Right ears. Top line: Control Group. Middle line: Betamethasone 0.1%-treated group. Bottom line: Vehicle-treated group. Magnification: x100.

[0103] Figure 63 depicts representative histopathology pictures. Left panel: Left ears. Right panel: Right ears. Top line: CT101_5%-treated group. Middle line: CT101_10%-treated group. Bottom line: CT101_20%-treated group. Magnification: x100.

[0104] Figure 64 depicts the BioMAP profile of SNA-101 in the Diversity PLUS Panel at the indicated concentrations. The X-axis lists the quantitative protein-based biomarker readouts measured in each system. The Y-axis represents a log-transformed ratio of the biomarker readouts for the drug-treated sample ($n = 1$) over vehicle controls ($n \geq 6$). The grey region around the Y-axis represents the 95% significance envelope generated from historical vehicle controls. Biomarker activities are annotated when 2 or more consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope, and have at least one concentration with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxicity is indicated on the profile plot by a thin black arrow above the X-axis, and antiproliferative effects are indicated by a thick grey arrow. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation. Other BioMAP profiles disclosed herein are also depicted in a similar manner.

[0105] Figure 65 depicts a Reference Benchmark Overlay of SNA-101 and Benchmark Apremilast. Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction.

[0106] Figure 66 depicts an overlay of SNA-101 (29 μM) and Topiramate (3.3 μM), which was the top similarity match from a search of the BioMAP Reference Database of $> 4,000$ agents. Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction. Similarity search results are filtered and ranked. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 .

[0107] Figure 67 depicts Mechanism HeatMAP Analysis for SNA-101. HeatMAP analysis of the 148 biomarker readouts (rows) within the Diversity PLUS panel

by SNA-101 in comparison to 19 consensus mechanism class profiles (columns). Horizontal grey lines separate the 12 Diversity PLUS systems, while the vertical grey line separates SNA-101 from the 19 consensus mechanism profiles. Biomarker activities outside of the significance envelope are red if protein levels are increased, blue if protein levels are decreased and white if levels are within the envelope or unchanged. Darker shades of color represent greater change in biomarker activity relative to vehicle control.

[0108] Figure 68 depicts the BioMAP profile of SNA-101 in the Diversity PLUS Panel at the indicated concentrations. The X-axis lists the quantitative protein-based biomarker readouts measured in each system. The Y-axis represents a log-transformed ratio of the biomarker readouts for the drug-treated sample ($n = 1$) over vehicle controls ($n \geq 6$). The grey region around the Y-axis represents the 95% significance envelope generated from historical vehicle controls. Biomarker activities are annotated when 2 or more consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope, and have at least one concentration with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxicity is indicated on the profile plot by a thin black arrow above the X-axis, and antiproliferative effects are indicated by a thick grey arrow. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation. Other BioMAP profiles disclosed herein are also depicted in a similar manner.

[0109] Figure 69 depicts a Reference Benchmark Overlay of SNA-101 and Benchmark Staurosporine. Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction.

[0110] Figure 70 depicts an overlay of SNA-101 (300 μM) and N037 (490 ng/ml), which was the top similarity match from a search of the BioMAP Reference Database of $> 4,000$ agents for SNA-101 (300 μM). Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction. Similarity search results are filtered and ranked. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 .

[0111] Figure 71 depicts an overlay of SNA-101 (100 μM) and Infliximab (30000 ng/ml), which was the top similarity match from a search of the BioMAP Reference Database of $> 4,000$ agents for SNA-101 (100 μM). Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction. Similarity

search results are filtered and ranked. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 .

[0112] Figure 72 depicts Mechanism HeatMAP Analysis for SNA-101. HeatMAP analysis of the 148 biomarker readouts (rows) within the Diversity PLUS panel by SNA-101 in comparison to 19 consensus mechanism class profiles (columns). Horizontal grey lines separate the 12 Diversity PLUS systems, while the vertical grey line separates SNA-101 from the 19 consensus mechanism profiles. Biomarker activities outside of the significance envelope are red if protein levels are increased, blue if protein levels are decreased and white if levels are within the envelope or unchanged. Darker shades of color represent greater change in biomarker activity relative to vehicle control.

[0113] Figure 73 depicts a schematic showing how the IMQ-induced psoriasis study was performed.

[0114] Figure 74 depicts the total psoriasis clinical scores over time for all groups (A), the SNA-101 group (B), the SNA-125 group (C), and the SNA-352 group (D). The mean score for each group is displayed for each day $\pm$ SEM.

[0115] Figure 75 depicts the erythema scores over time for all groups (A), the SNA-101 group (B), the SNA-125 group (C), and the SNA-352 group (D). The mean score for each group is displayed for each day $\pm$ SEM.

[0116] Figure 76 depicts the plaque scores over time for all groups (A), the SNA-101 group (B), the SNA-125 group (C), and the SNA-352 group (D). The mean score for each group is displayed for each day $\pm$ SEM.

[0117] Figure 77 depicts the punctate redness/scabbing scores over time for all groups (A), the SNA-101 group (B), the SNA-125 group (C), and the SNA-352 group (D). The mean score for each group is displayed for each day $\pm$ SEM.

[0118] Figure 78A depicts the weight of spleens upon experimental termination on day 10. Mean spleen weight for each group is displayed $\pm$ SEM. Figure 78B depicts left ear thickness as measured with a caliper on days 0, 4, 6, 8, and 10. Mean thickness for each group is displayed for each day $\pm$ SEM. Figure 78C depicts the daily weight of mice. Body weight changes are displayed for each day as a percent of their weight measured on day 0. Mean values for each group are displayed $\pm$ SEM.

[0119] Figure 79 depicts the levels of IL-17F (A), TNF- α (B), IL-22 (C), and IL-17A (D) as measured in left ears biopunched on day 4. After tissue homogenization, the cytokine levels in tissue lysates were measured via multiplex and then normalized with total protein amounts. Mean values for each group are displayed $\pm$ SEM.

[0120] Figure 80 depicts CT101 (SNA-101).

[0121] Figure 81 depicts the CT101 synthesis scheme.

[0122] Figure 82 depicts HPLC analysis of starting benzamide (HPLC method MI CT101 001).

[0123] Figure 83 depicts HPLC analysis of hydrolysis reaction mixture/69 h aging time (HPLC method MI CT101 001).

[0124] Figure 84 depicts HPLC analysis of crude Carboxylic acid intermediate (HPLC method MI CT101 001).

[0125] Figure 85 depicts HPLC analysis of amidation reaction mixture/20 h aging time (HPLC method MI CT101 001).

[0126] Figure 86 depicts HPLC analysis of crude CT101 (HPLC method MI CT101 001).

[0127] Figure 87 depicts the UV profile (@270 and 210 nm) of CT101 purification by reversed phase flash chromatography.

[0128] Figure 88 depicts HPLC analysis of final CT101 lot n° 2017CG07/S4 (HPLC method MI CT101 002).

[0129] Figure 89 depicts ¹H NMR analysis of final CT101/lot n° 2017CG07/S4.

[0130] Figure 90 depicts ESI MS analysis of final CT101 lot n° 2017CG07/S4 /single charged ion.

[0131] Figure 91 depicts ESI MS analysis of final CT101 lot n° 2017CG07/S4 /double charged ion.

[0132] Figure 92 depicts the certificate of analysis of final CT101/ lot n° 2017CG07/S4.

[0133] Figure 93 depicts HPLC analysis of final CT101 lot n° 2017CG07/S2A (HPLC method MI CT101 002).

[0134] Figure 94 depicts ¹H NMR analysis of final CT101/lot n° 2017CG07/S2A.

[0135] Figure 95 depicts ESI MS analysis of final CT101 lot n° 2017CG07/S2A /single charged ion.

[0136] Figure 96 depicts ESI MS analysis of final CT101 lot n° 2017CG07/S2A /double charged ion.

[0137] Figure 97 depicts the certificate of analysis of final CT101/ lot n° 2017CG07/ S2A.

DETAILED DESCRIPTION

Platform Technology

[0138] Several embodiments relate to the use of agents that were developed using Applicant's proprietary Low Systemic Exposure™ ("LSE™") platform technology to generate LSE molecules (also generally referred to herein as polymer conjugates or compositions). In several embodiments, the LSE platform creates polymer conjugates optimized for topical applications. In several embodiments, the polymer conjugates developed by LSE or more generally the reduced exposure technology exhibit enhanced penetration. In still further embodiments, the enhanced penetration leads to delivery of a high local concentration of the drug. In further embodiments, the polymer conjugates show a limited non-target absorption upon topical administration due to their increased molecular size and amphiphilicity and/or amphipathicity. In still further embodiments, side-effects are minimized by limiting or eliminating non-target (e.g., systemic) absorption.

[0139] In several embodiments of the reduced exposure compositions/compounds, the polymer conjugate comprises a "warhead" linked to a polymer. In some embodiments, the warhead is a pharmacologically active entity selected according to the particular target or pathway of interest. As discussed herein, there are also provided, in several embodiments, polymer conjugates for use in the treatment of conditions (including but not limited to inflammatory skin diseases). In several embodiments, the polymer is directly coupled to the warhead without a separate chemical linking moiety between the polymer and the warhead; such direct coupling may involve without limitation ester, ether, acetal, ketal, vinyl ether, carbamate, urea, amine, amide, enamine, imine, oxime, amidine, iminoester, carbonate, orthoester, phosphonate, phosphinate, sulfonate, sulfinic acid, sulfide, sulfate, disulfide, sulfinamide, sulfonamide, thioester, aryl, silane, siloxane, heterocycles, thiocarbonate, thiocarbamate, and phosphoramidate bonds. In several embodiments, the linker is a separate chemical linking moiety between the polymer and the warhead. In several embodiments, the polymer is polyethylene glycol (PEG), wherein the terminal OH group can optionally be modified e.g. with C1-C5 alkyl or C1-C5 acyl groups, e.g., with C1-, C2- or C3-alkyl groups or C1-, C2- or C3 groups. In several embodiments, the modified PEG is a terminally alkoxy-substituted PEG. In several embodiments, the modified PEG is a methoxy-PEG (mPEG). In some embodiments, the polymer has a molecular weight ranging from about 100 to about 100,000 Da. In some embodiments, the polymer is polydisperse with respect to molecular weight (e.g., has a distribution of molecular weights) and the indicated molecular weight of the polymer represents an average molecular weight. In other embodiments, the polymer has a molecular weight ranging from about 200 to about 50,000 Da. In several embodiments, the polymer has a

molecular weight ranging from about 500 to about 10,000 Da (e.g., 500-1000, 1000-2000, 2000-3000, 3000-5000, 5000-7000, 7000-10,000 Da, and overlapping ranges therein).

[0140] In several embodiments, the polymer is a short-chain PEG, and in some embodiments a terminally alkoxy-substituted PEG, such as a mPEG with a molecular weight ranging from about 200 to about 4,000 Da, from about 400 to about 3,000 Da, from about 500 to about 2,000 Da, from about 700 to about 3,000 Da, from about 900 to about 4,000 Da, or from about 1,000 to about 5,000 Da. In several embodiments, the short-chain PEG or mPEG has an average molecular weight of about 1,000-3,000 Da. (e.g., 2,000 Da).

[0141] In some embodiments, the polymer is a long-chain PEG. The long-chain PEG may be a terminally alkoxy-substituted PEG, such as methoxy-substituted PEG, with a molecular weight ranging greater than about 4,000 Da. In several embodiments, the molecular weight ranges from about 4,500-10,000Da (e.g., 4,500 to about 5,500 Da). In several embodiments, the long-chain PEG or mPEG has an average molecular weight of about 2,000 Da or of about 5,000 Da. In several embodiments, the polymer is of natural or semi-synthetic or synthetic origin. In several embodiments, the polymer has a linear or branched structure. In several embodiments, the polymer is selected from poly(alkylene oxides) or from (polyethylene) oxides. In several embodiments, the polymer selected may include, without limitation, one or more of the following: polyacrylic acid, polyacrylates, polyacrylamide or N-alkyl derivatives thereof, polymethacrylic acid, polymethacrylates, polyethylacrylic acid, polyethylacrylates, polyvinylpyrrolidone, poly(vinylalcohol), polyglycolic acid, polylactic acid, poly(lactic-co-glycolic) acid, dextran, chitosan, and hydroxyethyl starch.

[0142] In an embodiment, the polymer conjugates provided herein are administered to the skin by topical application. In one embodiment, the polymer conjugates provided herein treat inflammatory skin diseases.

[0143] In one embodiment, active agents useful for stimulating hair follicles (for hair growth) are provided as oral applications or topical applications for the scalp. Hair removal agents and anti-acne agents are provided in other embodiments. Hair growth, hair removal and anti-acne therapies can all involve active agents that, if exposed to the non-target site (e.g., systemic circulation and/or lymphatic system) for long periods, result in toxicity or undesired side effects. Thus, the reduced exposure compositions described herein provides benefits for these applications as well.

[0144] In alternative embodiments, the polymer conjugates configured for reduced exposure are administered to other areas of the body besides the skin. For example, in one embodiment, administration comprises treatment of the lung and

respiratory conditions via inhalation of the polymer conjugates. Eye drops are provided in some embodiments to treat eye inflammation or ophthalmic disorders and diseases. Treatment to the joints to treat inflammation or other joint conditions is also provided. In yet another embodiment, administration comprises treatment of the gastro-intestinal tract via, for example, an enteric coated capsule comprising the polymer conjugates taken orally. Reduced exposure provides benefits in these applications. Applications for the nose and ear, such as inhalants, ointments and drops are provided in several embodiments. Treatment to the nasal passage to treat allergies or allergic rhinitis is also provided. Vaginal and rectal compounds are provided in some embodiments, including as suppositories, creams, ointments, etc.

[0145] In some embodiments, conjugating the warhead to a polymer (e.g., PEG) in the disclosed molecular weight ranges may slow diffusion of the molecule in the tissue, thereby potentially increasing residence time of the molecule in the target tissue, e.g. epidermis and dermis for skin, associated epithelial and sub-epithelial layers in other topical surfaces like gut, eye, mucosa, lungs etc. This “depot” effect may also lead to lower concentrations needing to be applied or for products to be applied with lower frequency, or both.

[0146] In other embodiments, conjugating the warhead to a polymer (e.g., PEG) in the disclosed molecular weight ranges may be useful in reducing the diffusion or extravasation of the molecule out of the circulatory system after it enters it via injection and or diffusion from the target tissue. Indeed, changes in the tissue distribution of polymer conjugates compared to unconjugated drug have been observed in IV injection studies. In general, the unconjugated drug tends to have a long half-life and a volume of distribution within tissues AND blood, suggesting that the unconjugated drug extravasates out of the blood vessels into the tissue prior to being cleared. Whereas, in some instances, the PEGylated drug has a volume of distribution that is largely restricted to the blood, indicating that very little extravasation occurs with the polymer conjugates prior to being renally cleared. This reduced extravasation may explain at least in part the observed shorter half-life for the polymer conjugates.

[0147] The compositions described herein may be combined with other modalities to achieve synergic effects. These other modalities include, but are not limited to, energy delivery (such as laser, radiofrequency, ultrasound, microwave, etc.), thermal therapy, light therapy, radiation, intravenous chemotherapy, and others. In some embodiments, the compositions are applied with pressure, heat, massage etc. to facilitate localization to the desired target site. In some embodiments, the compositions are administered in combination with one or more additional therapeutics that may not be reduced exposure compounds.

[0148] Santi et al. state that “permanent PEGylation is generally not applicable to small-molecule drugs because the bulky carrier usually prevents their binding to targets and cell penetration.” (Proceedings of the National Academy of Sciences 109.16 (2012): 6211-6216). Further, Nakagami et al. state that “hydrophilic polymers on the surface of particles...prevents the close interactions between particles and target cell membranes, inhibiting the cellular uptake and, subsequently, preventing endosomal escape. All of these factors combine to decrease the biological efficacy of PEGylated particles” (Gene therapy (2013): 2-4). In several embodiments, the polymer conjugate exhibits unexpected permeability across the plasma membrane. In several embodiments, the polymer conjugate exhibits unexpected permeability across the nuclear membrane. In several embodiments, the polymer conjugate exhibits unexpected permeability across both the nuclear and plasma membranes.

[0149] The reduced exposure compounds, comprising a hydrophobic drug conjugated to a short chain PEG, exhibit surprising accessibility across cellular compartments, compared to the unconjugated drug. This accessibility is thought to result for the amphipathic nature of the conjugate, allowing it to traverse and distribute evenly among both lipophilic and hydrophilic cellular compartments. Accordingly, the conjugate can cross and reside within the lipid bilayer of the cell membrane, accumulate within the cytosol, and even traverse the nuclear envelope – thereby providing access both membrane, cytosolic and nuclear molecular targets. This property of the reduced exposure compounds result in excellent depo'ing, longer residence times within target cells, and relative non-compartmentalization. Consequently, these compounds are biologically active at lower concentrations and require less frequent dosing – thereby reducing potential drug toxicity.

Polymer Conjugates Targeting c-Src

[0150] In several embodiments, the warhead employed in the LSE polymer conjugate is a small molecule targeting c-Src. There is also provided, in several embodiments, methods of treating an inflammatory skin disease in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is a small molecule targeting c-Src. As used herein, the term “inflammatory skin disease” refers to a skin condition accompanied by inflammation that is mediated, in part, by immune cells, including in some embodiments, T-cells. Non-limiting examples of inflammatory skin diseases include psoriasis, psoriasis guttata, inverse psoriasis, pustular psoriasis, psoriatic erythroderma, acute febrile neutrophilic dermatosis, eczema, xerotic eczema, dyshidrotic eczema, vesicular palmar eczema,

acne vulgaris, atopic dermatitis, contact dermatitis, allergic contact dermatitis, dermatomyositis, exfoliative dermatitis, hand eczema, pompholyx, rosacea, rosacea due to sarcoidosis, rosacea due to scleroderma, rosacea due to Sweet syndrome, rosacea due to systemic lupus erythematosus, rosacea due to urticaria, rosacea due to herpetic pain, Sweet's disease, neutrophilic hydrodenitis, sterile pustule, drug rash, seborrheic dermatitis, pityriasis rosea, Kikuchi's disease of the skin, pruritic urticarial papules and plaques of pregnancy, Stevens-Johnson syndrome and toxic epidermal necrolysis, tattoo reaction, Wells syndrome (eosinophilic cellulitis), reactive arthritis (Reiter syndrome), bowel-associated dermatosis-arthritis syndrome, rheumatoid neutrophilic dermatosis, neutrophilic eccrine hidradenitis, neutrophilic skin disease of dorsum of hand, balanitis circumscripta plasmacellularis, balanoposthitis, Behcet's disease, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiforme, granuloma annulare, dermatitis of hand, lichen nitidus, lichen planus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, nummular dermatitis, pyoderma gangrenosum, sarcoidosis, subcorneal pustular dermatosis, urticaria, skin fibrosis, and transient acantholytic dermatosis.

[0151] The c-Src kinase is the most widely studied member of the largest family of nonreceptor protein tyrosine kinases, known as the Src family kinases (SFKs). Other SFK members include Lyn, Fyn, Lck, Hck, Fgr, Blk, Yrk, and Yes. The Src kinases can be grouped into two sub-categories, those that are ubiquitously expressed (Src, Fyn, and Yes), and those which are found primarily in hematopoietic cells (Lyn, Lck, Hck, Blk, Fgr). (Benati, D. Src Family Kinases as Potential Therapeutic Targets for Malignancies and Immunological Disorders. *Current Medicinal Chemistry*. 2008; 15: 1154-1165). SFKs are key messengers in many cellular pathways, including those involved in regulating proliferation, differentiation, survival, motility, and angiogenesis. The activity of SFKs is highly regulated intramolecularly by interactions between the SH2 and SH3 domains and intermolecularly by association with cytoplasmic molecules. This latter activation may be mediated by focal adhesion kinase (FAK) or its molecular partner Crk-associated substrate (CAS), which plays a prominent role in integrin signaling, and by ligand activation of cell surface receptors, e.g. epidermal growth factor receptor (EGFR). These interactions disrupt intramolecular interactions within Src, leading to an open conformation that enables the protein to interact with potential substrates and downstream signaling molecules. Src can also be activated by dephosphorylation of tyrosine residue Y530. Maximal Src activation requires the autophosphorylation of tyrosine residue Y419 (in the human protein) present within the catalytic domain. Elevated Src activity may be caused by increased transcription or by deregulation due to overexpression of upstream growth factor receptors such as EGFR, HER2, platelet-

derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor, ephrins, integrin, or FAK.

[0152] Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease with a dramatically increasing incidence over the last decades. Clinically AD is characterized by highly pruritic often excoriated plaques and papules that show a chronic relapsing course. The diagnosis of AD is mostly based on major and minor clinical findings. See Hanifin J. M., Arch Dermatol: 135, 1551 (1999). Histopathology reveals spongiosis, hyper and focal parakeratosis in acute lesions, whereas marked epidermal hyperplasia with hyper and parakeratosis, acanthosis/hypergranulosis and perivascular infiltration of the dermis with lymphocytes and abundant mast cells are the hallmarks of chronic lesions.

[0153] Psoriasis is characterized by frequent episodes of redness, itching, and thick, dry, silvery scales on the skin. Psoriasis comprises lesions that can involve primary and secondary alterations in epidermal proliferation, inflammatory responses of the skin, and an expression of regulatory molecules such as lymphokines and inflammatory factors. Psoriatic skin is morphologically characterized by an increased turnover of epidermal cells, thickened epidermis, abnormal keratinization, inflammatory cell infiltrates into the epidermis and polymorphonuclear leukocyte and lymphocyte infiltration into the epidermis layer. Psoriasis is often associated with other inflammatory disorders, for example arthritis, including rheumatoid arthritis, inflammatory bowel disease (IBD), and Crohn's disease.

[0154] Compositions comprising compounds Nos 1-71 shown in Table 1 are used, in several embodiments, as inhibitors, antagonists, and inverse agonists of c-Src. Several embodiments relate to polymer conjugates of compounds 1-71, optimized for topical applications while also minimizing side-effects caused by exposure at non-target sites (e.g., systemic absorption). Non-topical applications are provided in other embodiments.

[0155] In several embodiments, the warhead of the polymer conjugate is a small molecule disclosed in Table 1 targeting c-Src. There is also provided, in several embodiments, methods of treating an inflammatory skin disease in a subject, the method comprising administering to the subject an effective amount of an LSE polymer conjugate wherein the warhead is a small molecule disclosed in Table 1 targeting c-Src. In some embodiments, the warhead of the LSE polymer conjugate is compound 1. In some embodiments, the LSE polymer conjugate is CT101.

[0156] A growing body of research suggests that dry eye is the result of an underlying cytokine and receptor-mediated inflammatory process. There is provided, in several embodiments, methods of treating dry eye in a subject, the method comprising

administering to the subject an effective amount of a polymer conjugate, wherein the warhead is a small molecule targeting c-Src. In some embodiments the composition is formulated as an eye drop. In some embodiments, one or two drops of the composition are used per application. In other embodiments, three or four drops of the composition are used per application. In additional embodiments, six drops of the composition are used per application. In some embodiments, the composition is applied for a period of 60 seconds before flushing. In other embodiments, the composition is applied for a period of 120 seconds before flushing. In additional embodiments, the composition is applied for a period of 360 seconds before flushing. In some embodiments, the composition may be administered one or more times a day. In some embodiments, the composition is administered daily. In some embodiments, the composition may be administered once a week.

[0157] In some embodiments, alopecia is treated. Non-limiting examples include androgenic alopecia and alopecia areata. Androgenic alopecia (also known as hereditary baldness, male pattern baldness, and seborrheic alopecia) is a non-scarring hair loss of telogen hairs caused by an excessive androgen effect in genetically susceptible men and women. Alopecia areata is known to be associated with autoimmune activities; hence, topically administered immunomodulatory compounds demonstrate efficacy for treating that type of hair loss.

[0158] There is provided, in several embodiments, methods of treating an alopecia in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is a small molecule targeting c-Src. In some embodiments, hair regeneration compositions are in the form of a liquid. In other embodiments, hair regeneration compositions are in the form of a lotion. In additional embodiments, hair regeneration compositions are in the form of a cream. In some embodiments, hair regeneration compositions are in the form of a gel. In other embodiments, the hair regeneration composition is administered twice daily. In other embodiments, the hair regeneration composition is administered one daily. In additional embodiments, the hair regeneration composition is administered once weekly. In some embodiments, the hair regeneration composition is administered directly to the scalp. In some embodiments, the hair regeneration composition is administered directly non-scalp areas.

[0159] Allergic inflammatory diseases are characterized by an immune response against a sensitizing agent, such as an allergen, resulting in the release of inflammatory mediators that recruit cells involved in inflammation in a subject, potentially leading to tissue damage and sometimes death. Allergic inflammatory diseases of the eye, skin, upper and lower airways, and gastrointestinal tract, lung, including, but not

limited to, atopic dermatitis, atopic keratoconjunctivitis, allergic conjunctivitis, asthma, and allergic rhinitis. There is provided, in several embodiments, methods of treating an allergic inflammatory disease in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is a small molecule targeting c-Src.

[0160] There is also provided, in several embodiments, methods of treating the following conditions in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is a small molecule targeting c-Src: nail dystrophy; seborrheic keratosis; androgenic alopecia; contact dermatitis; actinic keratosis; acne; asthma; eczema (atopic dermatitis); onychomycosis; sinusitis; allergic rhinitis; rosacea; COPD; pruritus; early AMD; urticaria; diabetic retinopathy; psoriasis; alopecia areata; dry eye; vitiligo; glaucoma; late AMD; ulcerative colitis; Crohn's disease; ocular rosacea; hair growth and cycling; skin neoplasias; squamous cell carcinoma; basal cell carcinoma; malignant melanoma; malignant cutaneous lymphomas; vascular tumors; angiosarcoma; Kaposi's sarcoma; infantile hemangiomas; hemangioendothelioma; inflammatory dermatoses; dermatitis (atopic, contact); psoriasis; keloids; rosacea; bullous diseases; bullous pemphigoid; erythema multiforme; UV irradiation therapy; age-related macular degeneration; diabetic retinopathy; macular and corneal edema.

[0161] There is also provided, in several embodiments, methods of treating a respiratory disease in a subject via delivery of the polymer conjugates (wherein the warhead is a small molecule targeting c-Src) to the lungs and/or airways. Delivery routes may include for example intratracheal instillation or inhalation. The formulation may include liquids, nebulized or aerosolized liquids or suspensions, dry powder, nanocomposites, nanoparticles or microparticles, etc. Respiratory disorders, include treatable obstructive, restrictive or inflammatory airways diseases of whatever type, etiology, or pathogenesis. Non-limiting examples of respiratory conditions include: acute bronchitis; acute laryngotracheal bronchitis; arachidic bronchitis; catarrhal bronchitis; croupus bronchitis; dry bronchitis; infectious asthmatic bronchitis; productive bronchitis; staphylococcus or streptococcal bronchitis; vesicular bronchitis; cylindric bronchiectasis; sacculated bronchiectasis; fusiform bronchiectasis; capillary bronchiectasis; cystic bronchiectasis; dry bronchiectasis; follicular bronchiectasis; chronic obstructive pulmonary disease (COPD), chronic obstructive lung disease (COLD), chronic obstructive airways disease (COAD) or small airways obstruction of whatever type, etiology, or pathogenesis, in particular chronic bronchitis, pulmonary emphysema, bronchiectasis, cystic fibrosis, bronchiolitis obliterans, organizing pneumonia (BOOP), chronic organizing pneumonia (COP), bronchiolitis fibrosa obliterans, follicular

bronchiolitis or dyspnea associated therewith; cough of whatever type, etiology, or pathogenesis in particular idiopathic cough or cough associated with gastro-esophageal reflux disease (GERD), drugs, bronchial hyper-responsivity, asthma, COPD, COLD, COAD, bronchitis, bronchiectasis, pulmonary eosinophilic syndromes, pneumoconiosis, interstitial lung disease, pulmonary fibrosis, aspiration disorders, rhinitis, laryngitis or pharyngitis; pulmonary eosinophilic syndromes of whatever type, etiology, or pathogenesis, in particular acute eosinophilic pneumonia (idiopathic or due to drugs or parasites), simple pulmonary eosinophilia, Loeffler's syndrome, tropical pulmonary eosinophilia, chronic eosinophilic pneumonia, allergic bronchopulmonary mycosis, allergic bronchopulmonary aspergillosis (ABPA), Churg-Strauss syndrome or idiopathic hypereosinophilic syndrome; asthma of whatever type, etiology, or pathogenesis, in particular asthma that is a member selected from the group consisting of atopic asthma, non-atopic asthma, allergic asthma, atopic bronchial IgE-mediated asthma, bronchial asthma, essential asthma, true asthma, intrinsic asthma caused by pathophysiologic disturbances, extrinsic asthma caused by environmental factors, essential asthma of unknown or inapparent cause, non-atopic asthma, bronchitic asthma, emphysematous asthma, exercise-induced asthma, allergen induced asthma, cold air induced asthma, occupational asthma, infective asthma caused by bacterial, fungal, protozoal, or viral infection, non-allergic asthma, incipient asthma and wheezy infant syndrome; alveolar hemorrhage of whatever type, etiology, or pathogenesis, in particular a member of the group consisting of idiopathic pulmonary hemosiderosis, alveolar hemorrhage due to drugs or other exogenous agents, alveolar hemorrhage associated with HIV or bone marrow transplant or autoimmune alveolar hemorrhage (e.g. associated with systemic lupus erythematosus, Goodpasture's syndrome, Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, pauci-immune glomerulonephritis); pneumoconiosis of whatever type, etiology, or pathogenesis, in particular pneumoconiosis that is a member selected from the group consisting of aluminosis or bauxite workers' disease, anthracosis or miners' asthma, asbestosis or steam-fitters' asthma, chalicosis or flint disease, ptilosis caused by inhaling the dust from ostrich feathers, siderosis caused by the inhalation of iron particles, silicosis or grinders' disease, byssinosis or cotton-dust asthma and talc pneumoconiosis; interstitial lung diseases (ILD) or pulmonary fibrosis of whatever type, etiology, or pathogenesis, in particular idiopathic pulmonary fibrosis, cryptogenic fibrosing alveolitis, fibrosing alveolitis, ILD or pulmonary fibrosis associated with connective tissue disease (systemic lupus erythematosus, mixed connective tissue disease, polymyositis, dermatomyositis, Sjörgen's syndrome, systemic sclerosis, scleroderma, rheumatoid arthritis), usual interstitial pneumonia (UIP), desquamative interstitial pneumonia (DIP), granulomatous

lung disease, sarcoidosis, Wegener's granulomatosis, histiocytosis X, Langerhan's cell granulomatosis, hypersensitivity pneumonitis, extrinsic allergic alveolitis, silicosis, chronic eosinophilic pneumonia, lymphangiolymphomatosis, drug-induced ILD or pulmonary fibrosis, radiation-induced ILD or pulmonary fibrosis, alveolar proteinosis, graft-versus-host-disease (GVHD), lung transplant rejection, ILD or pulmonary fibrosis due to environmental/occupational exposure, BOOP, COP, bronchiolitis fibrosa obliterans, follicular bronchiolitis, idiopathic acute interstitial pneumonitis (Hamman Rich syndrome) or alveolar hemorrhage syndromes; seasonal allergic rhinitis or perennial allergic rhinitis or sinusitis of whatever type, etiology, or pathogenesis, in particular sinusitis that is a member selected from the group consisting of purulent or nonpurulent sinusitis, acute or chronic sinusitis and ethmoid, frontal, maxillary, or sphenoid sinusitis; Acute Respiratory Distress Syndrome (ARDS), adult respiratory distress syndrome or acute lung injury of whatever type, etiology, or pathogenesis; progressive massive fibrosis (PMF); pulmonary hypertension of whatever type, etiology or pathogenesis including primary pulmonary hypertension, essential hypertension, pulmonary hypertension secondary to congestive heart failure, pulmonary hypertension secondary to COPD, pulmonary venous hypertension, pulmonary arterial hypertension and hypoxia-induced pulmonary hypertension. Respiratory disorders also include, in some embodiments, malignancies and tumors of the respiratory system, non-limiting examples of which include lung adenocarcinoma, squamous cell carcinoma, large cell carcinoma, bronchioloalveolar carcinoma (BAC), pulmonary adenocarcinoma (AIS), non-small-cell carcinoma, small cell carcinoma, and mesothelioma.

[0162] In some embodiments, for those compounds Nos 1-71 having one amino group, the compound is modified (e.g., PEGylated) at that location (e.g., a PEG or modified PEG is linked to the compound by reaction with the amino group). If two or more amino groups are present, either location is PEGylated in some embodiments. In other embodiments, the amino group located the furthest away from the moieties interacting with the target is used. In some embodiments, the amino group that causes the least hindrance on activity is used (whether or not it is located the furthest way from the moieties interacting with the target). The effect of conjugation on the activity of the compound can be determined based on various methods, such as bioassays, mass spectroscopy, surface plasmon resonance, in vivo assays, clinical assays, and predictive in silico modeling programs.

[0163] In some embodiments, for those compounds Nos 1-71 having one sulfhydryl group, the compound is modified (e.g., PEGylated) at that location. If two or more sulfhydryl groups are present, either location is PEGylated in some embodiments. In other embodiments, the sulfhydryl group located the furthest away from the moieties

interacting with the target is used. In some embodiments, the sulfhydryl group that causes the least hindrance on activity is used (whether or not it is located the furthest way from the moieties interacting with the target).

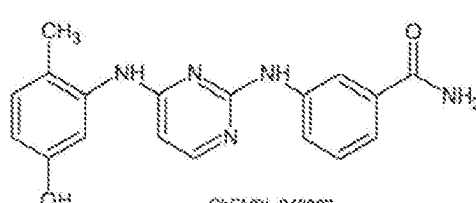
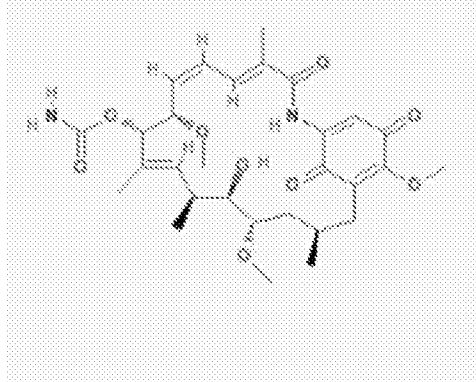
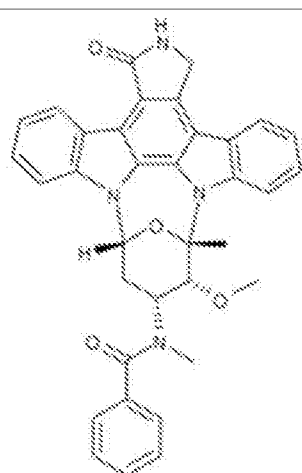
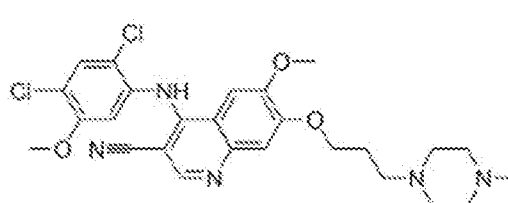
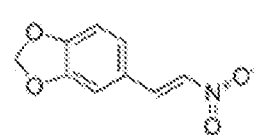
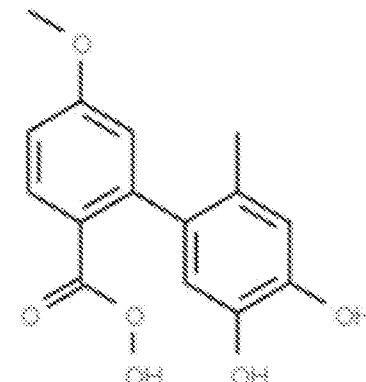
[0164] In some embodiments, for those compounds Nos 1-71 having one hydroxyl group, the compound is modified (e.g., PEGylated) at that location. If two or more hydroxyl groups are present, either location is PEGylated in some embodiments. In other embodiments, the hydroxyl group located the furthest away from the moieties interacting with the target is used. In some embodiments, the hydroxyl group that causes the least hindrance on activity is used (whether or not it is located the furthest way from the moieties interacting with the target).

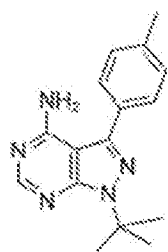
[0165] In some embodiments, for those compounds Nos 1-71 having one carboxyl group, the compound is modified (e.g., PEGylated) at that location. If two or more carboxyl groups are present, either location is PEGylated in some embodiments. In other embodiments, the carboxyl group located the furthest away from the moieties interacting with the target is used. In some embodiments, the carboxyl group that causes the least hindrance on activity is used (whether or not it is located the furthest way from the moieties interacting with the target).

[0166] In some embodiments, for those compounds Nos 1-71 having two or more carboxyl, hydroxyl, amino and/or sulfhydryl groups, the compound is modified (e.g., PEGylated) at the site furthest away from the active site. In some embodiments, the site that causes the least hindrance on activity is used (whether or not it is located the furthest way from the moieties interacting with the target).

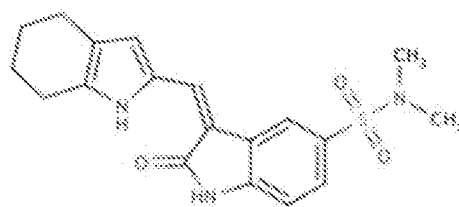
[0167] Methods for conjugating the PEG or modified PEG to the small molecule warheads in Table 1, through reaction between functional groups (or functionalized groups), including reaction with the above-mentioned functional groups (amino, sulfhydryl, hydroxyl, carboxyl) are used in several embodiments. Methods of conjugation can be found for example in "Bioconjugate Techniques" (3rd Edition) 2013 by Greg T. Hermanson (<http://www.sciencedirect.com/science/book/9780123822390>); incorporated herein in its entirety by reference. Although PEGylation is used as an example, other polymers are used in some embodiments.

TABLE 1

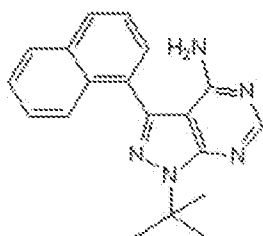
 ChEMBL 249097 (1)	 (2)
 (3)	 (4)
 (5)	 (6)



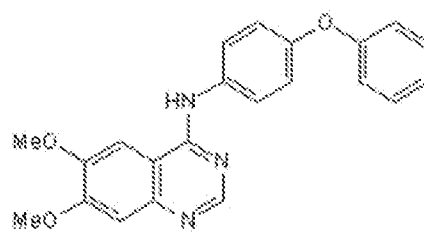
(10)



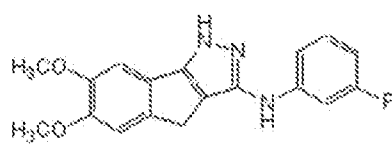
(11)



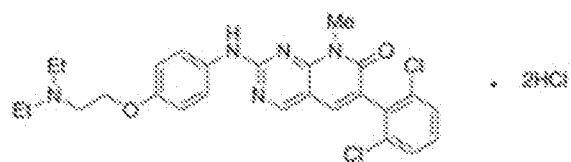
(12)



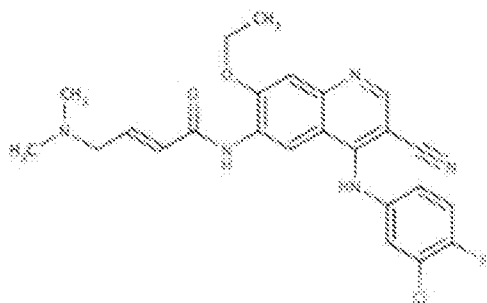
(13)



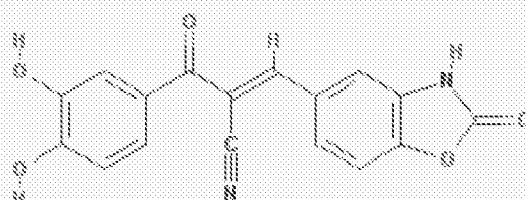
(14)



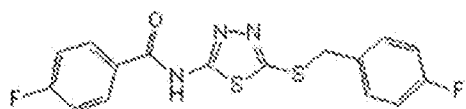
(15)



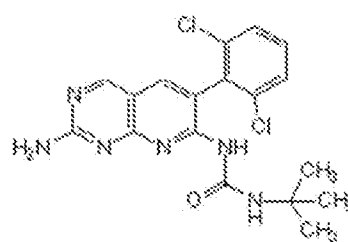
(16)



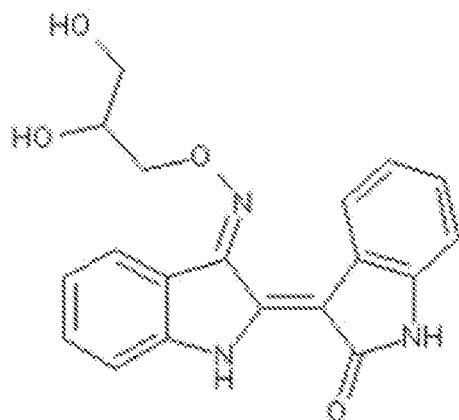
(17)



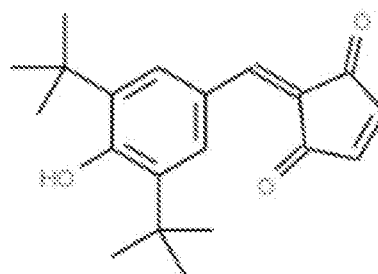
(18)



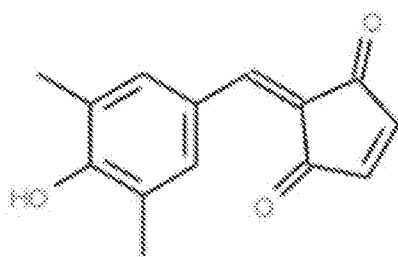
(19)



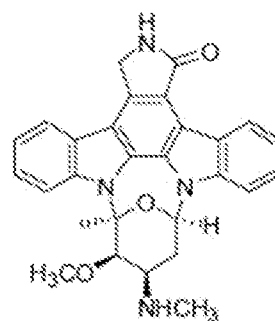
(20)



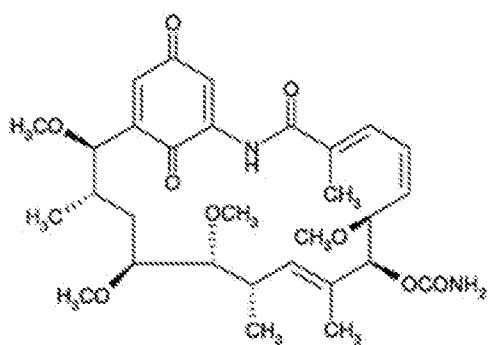
(21)



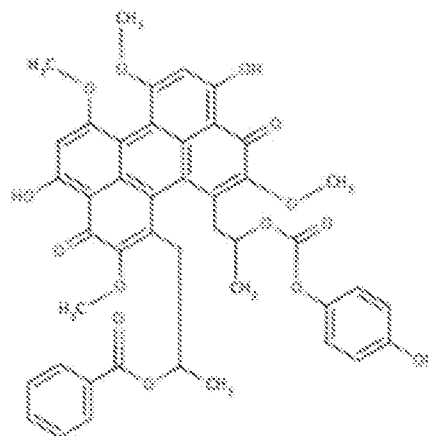
(22)



(23)



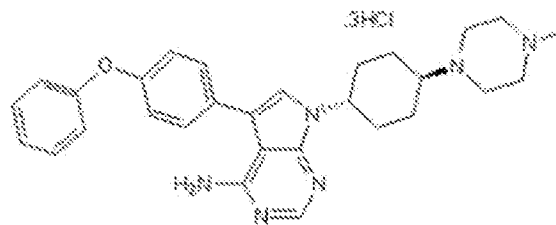
(24)



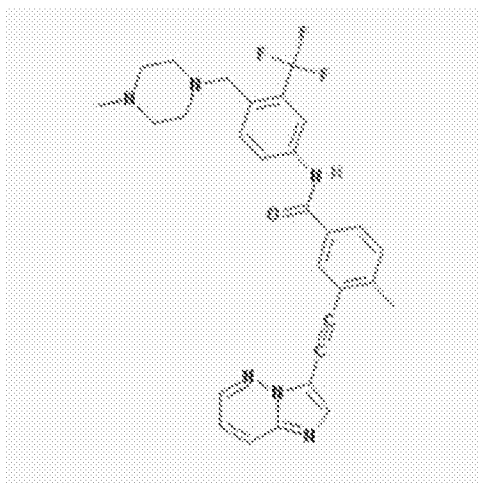
(25)



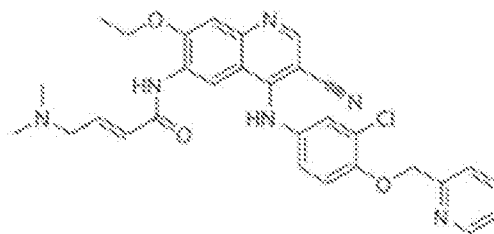
(26)



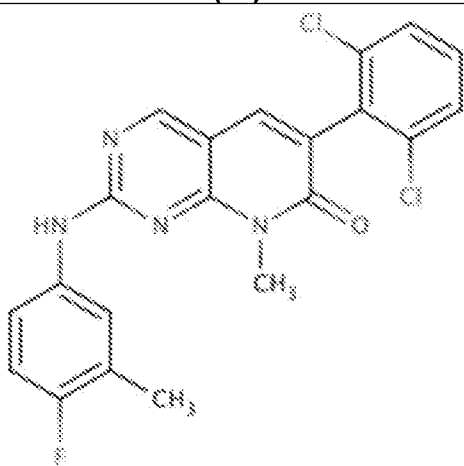
(27)



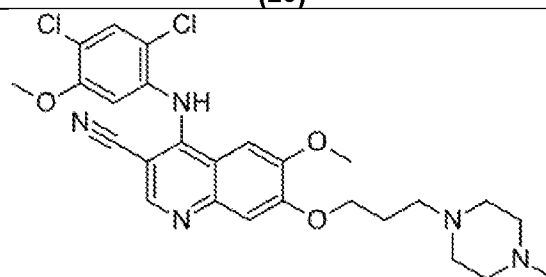
(28)



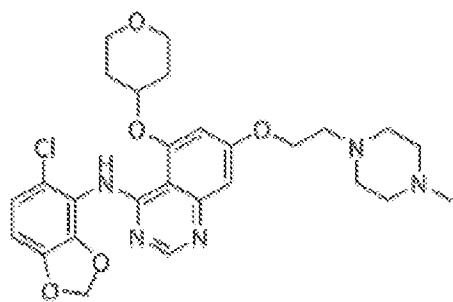
(29)



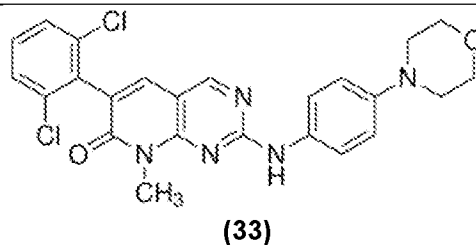
(30)



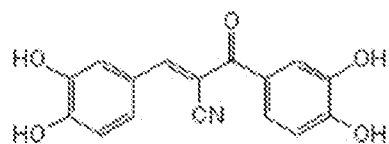
(31)



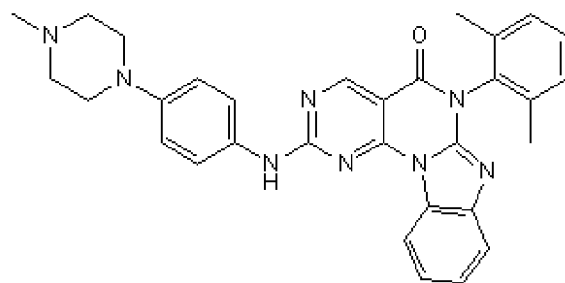
(32)



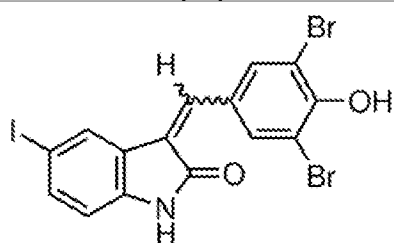
(33)



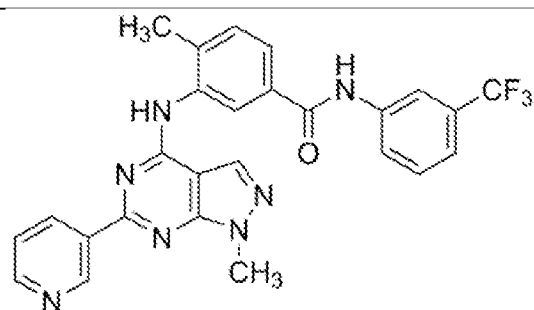
(34)



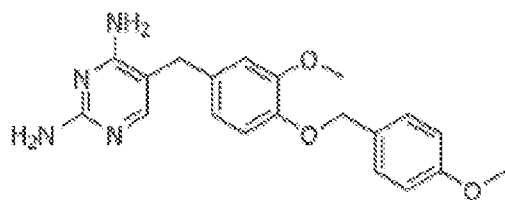
(35)



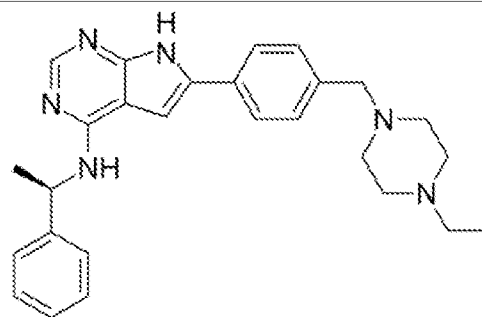
(36)



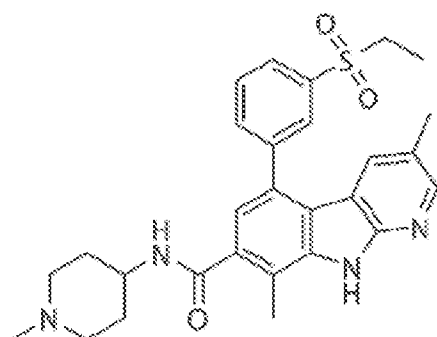
(37)



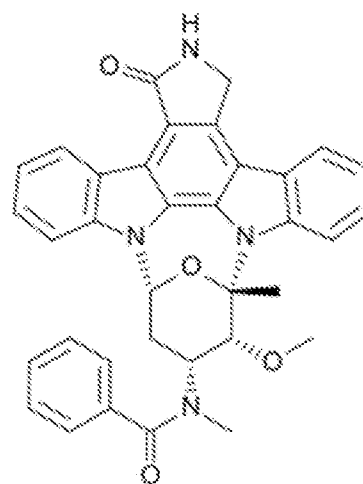
(38)



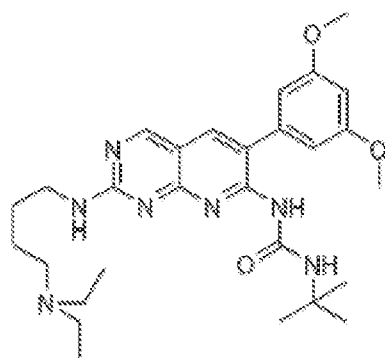
(39)



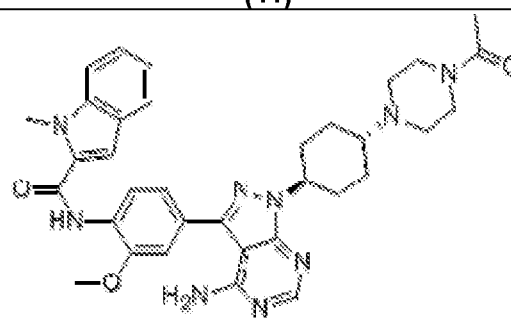
(40)



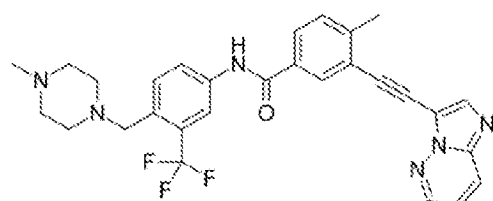
(41)



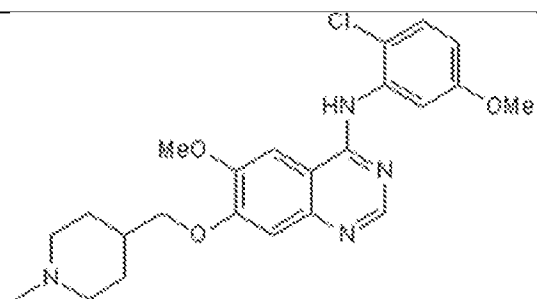
(42)



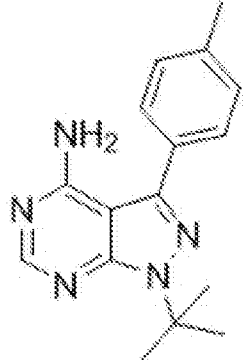
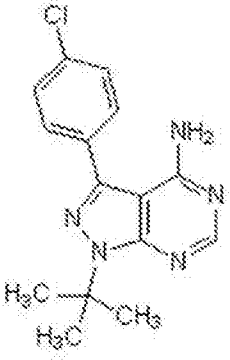
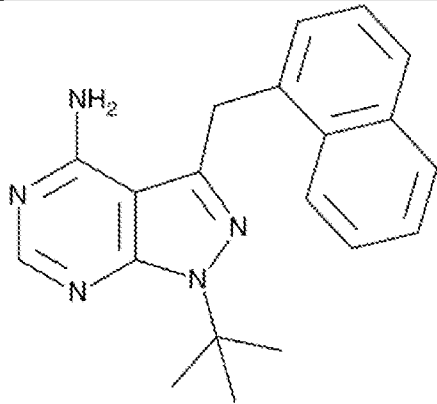
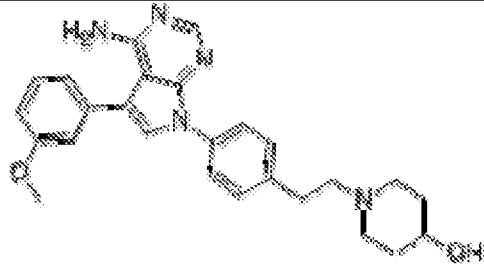
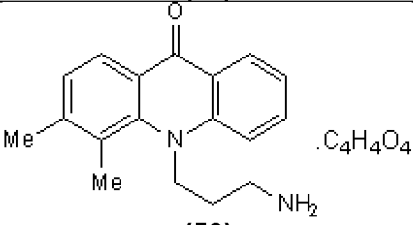
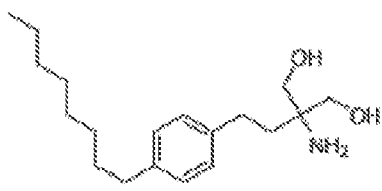
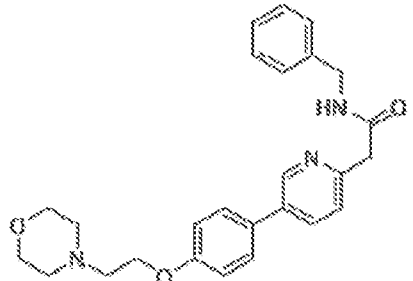
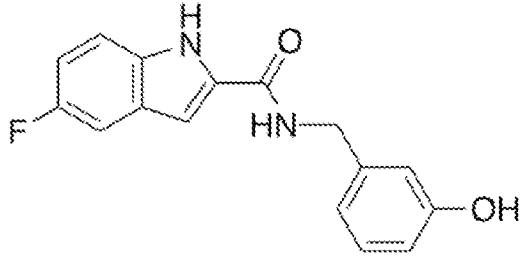
(43)

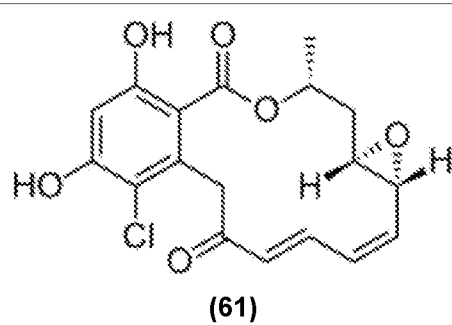
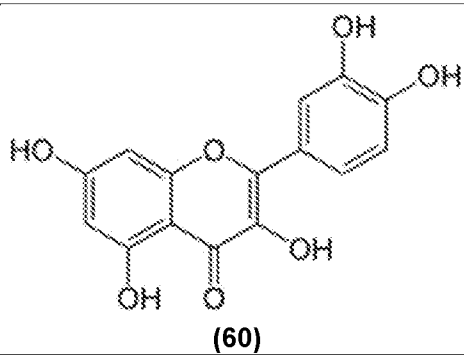
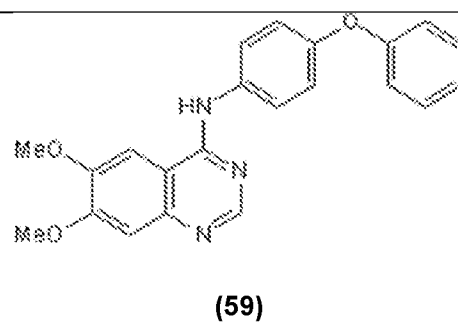
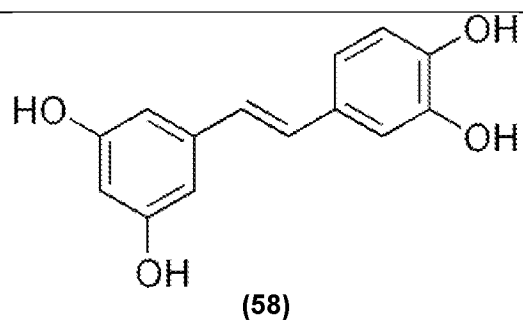
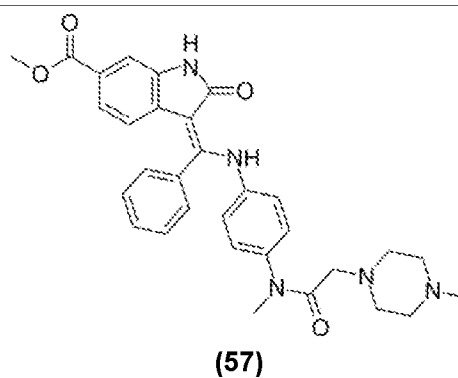
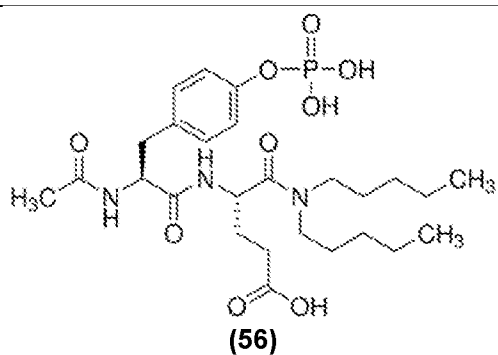
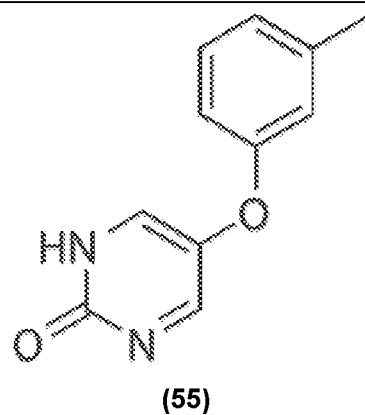
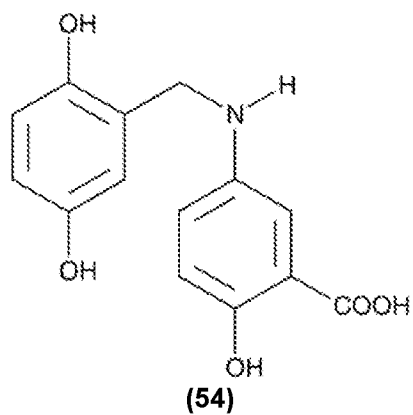


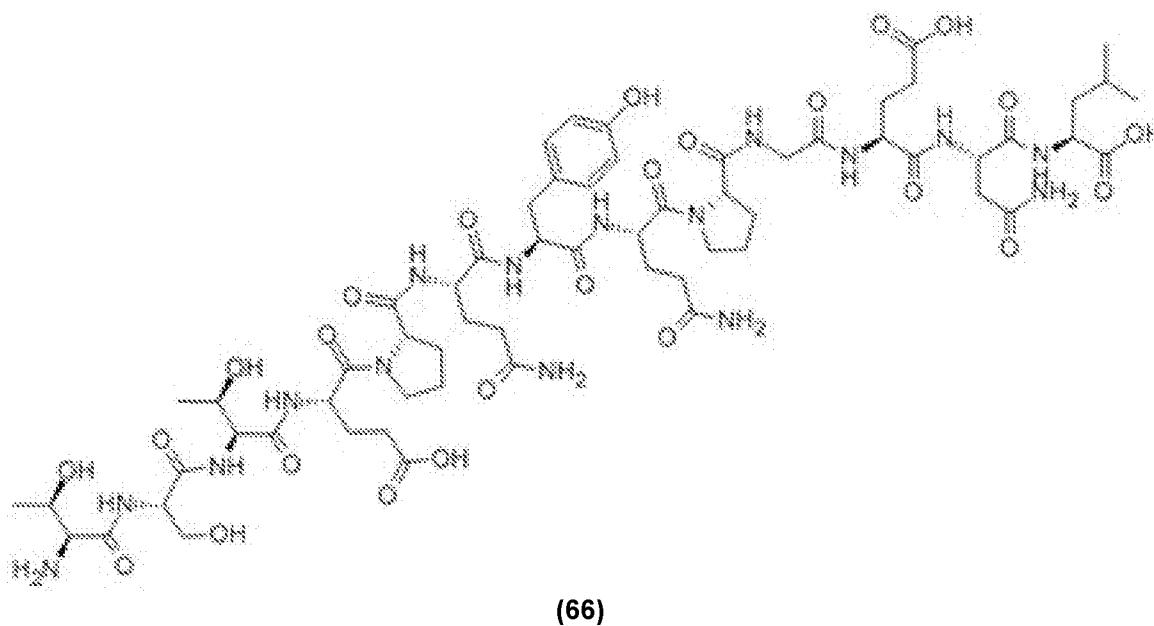
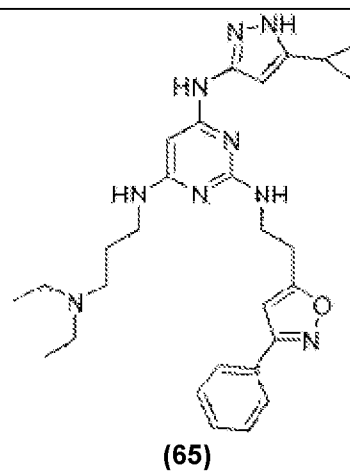
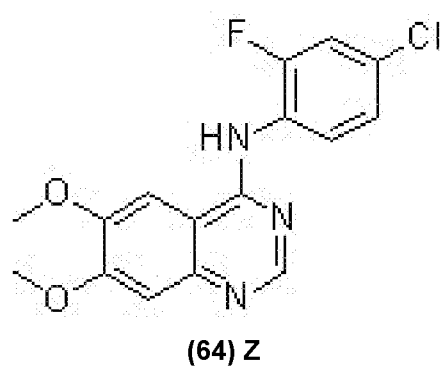
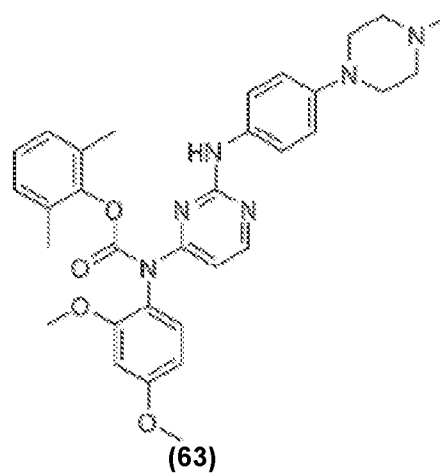
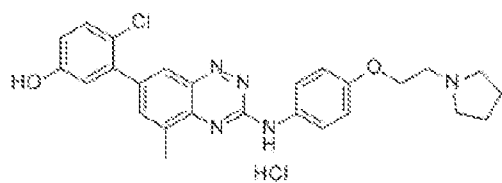
(44)

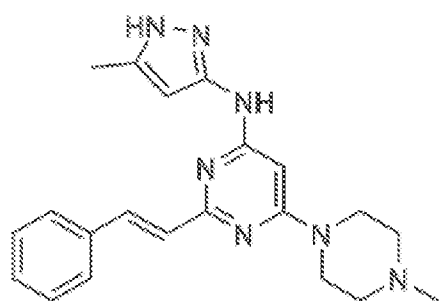


(45)

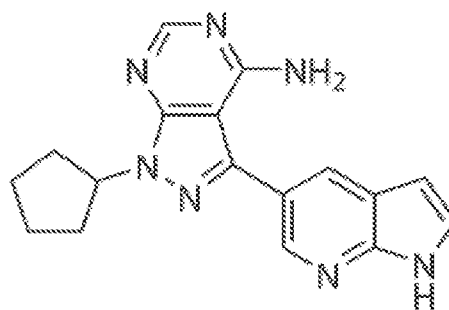
 (46)	 (47)
 (48)	 (49)
 (50)	 (51)
 (52)	 (53)



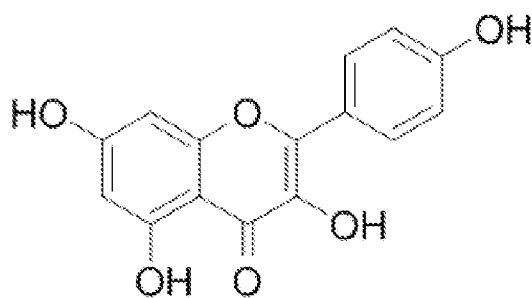




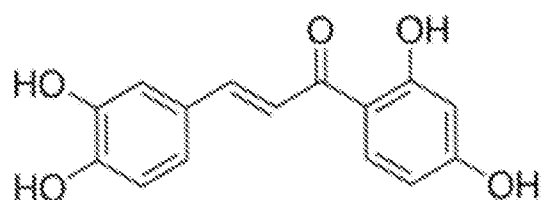
(67)



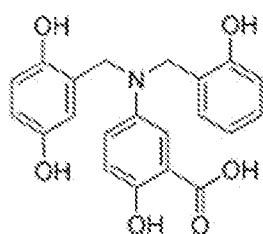
(68)



(69)



(70)



(71)

[0168] Non-limiting examples of conjugation sites according to some embodiments and chemistries for compounds in Table 1 are disclosed. For example, for structures 54, 56, 66, and 71 of Table 1, the existing carboxylic moiety (-COOH) could be conjugated to PEG-amine through formation of an amide bond using any one of several possible coupling agents (including, e.g., TBTU, HBTU, HOBT, DCC, and N-hydroxysuccinimide). Further, for example, for structures 7, 10, 12, 19, 23, 27, 38, 43, 46, 47, 48, 49, 50, 51, 66, and 68 of Table 1, the existing amino group (-NH₂) could be conjugated to PEG-COOH through formation of an amide bond using any one of several possible coupling agents (including, e.g., TBTU, HBTU, HOBT, DCC, and N-hydroxysuccinimide). Further, for example, for structures 1, 6, 9, 17, 20, 21, 22, 25, 26, 34, 36, 49, 51, 53, 54, 58, 60, 61, 62, 66, 69, 70, and 71 of Table 1, the existing hydroxyl moiety (-OH) could be conjugated to PEG-halide through formation of an ether bond in the presence of a strong base (including, e.g. NaH, KH, and n-BuLi). These conjugation sites and chemistries are neither exhaustive nor limiting, and are included herein as examples only, and not intended to limit the scope of the embodiments described herein.

[0169] Identifying a conjugation site and developing a conjugation strategy and/or chemistry does not require that all the atoms and the structures of the starting compound are maintained. Once the active part of the compound has been identified or hypothesized, some atoms, groups and structures of the compound can be removed or modified while maintaining sufficient or similar target site binding and activity in several embodiments.

[0170] In alternative embodiments, the warhead employed in the LSE polymer conjugate is a small molecule targeting c-Src selected from one or more of the following: substituted 2-anilinopyrimidines; imidazoles, oxazoles and thiazoles with protein kinase inhibiting activities; heterocyclic substituted pyrazoles; aryl-amino substituted pyrrolopyrimidine multi-kinase inhibiting compounds; 2-phenylamino-4- (5-pyrazolylamino)-pyrimidine derivatives; bicyclic heteroaryls; 2-amino-6-anilino-purines; triazolopyridazines; substituted amides; pyrimidine derivatives; pyridinyl-pyrimidinylamino-benzamide derivatives; pyrrolo[2,3-d]pyrimidines; amino-substituted dihydropyrimido[4,5-d]pyrimidinone derivatives; 2-heteroaryl-amino-pyrimidine derivatives; isoxazole derivatives; pyrazolo [3,4-d] pyrimidines; 1, 2, 5, 6-tetraaza- as-indacenes; pyrimido compounds having antiproliferative activity; fused polycyclic 2-aminopyrimidine derivatives; 2-aminopyridine kinase inhibitors; benzothiazole derivatives; pyrazolyl-ureas; pyrido[2,3-d]pyrimidin-7-carboxylic acid derivatives; isoxazolyl-pyrimidines; derivatives of azaindoles; caprolactam derivatives; pyrrolopyridine kinase inhibiting compounds; pyrazole derivatives; kinase inhibitors based upon n-alkyl pyrazoles; thioazepinone derivatives; 2,3-benzodiazepin-2-one

derivatives; azdo530; na/k-atpase-derived peptide src inhibitors; 5- (4- (haloalkoxy) phenyl) pyrimidine-2-amine compounds; pyrimidylaminobenzamide compounds; quinazoline derivatives; diaryl urea derivatives; 1 -(5-tert-butyl-2-aryl-pyrazol-3-yl)-3-[2-fluoro-4-[(3-oxo-4h-pyrido[2, 3-b]pyrazin-8-yl)oxy]phenyl]urea derivatives; bufalin phosphate prodrugs; hydroxyindole carboxylic acid based inhibitors; fused pyridine and pyrimidine bicyclic compounds; dichloro-phenyl-pyrido [2,3-d] pyrimidine derivatives; and quinazolines.

[0171] In alternative embodiments, the LSE polymer conjugate comprises an ROR γ t antagonist/inverse agonist warhead selected from one or more of the following: stearic acid; All-trans retinoic acid; ALTA 1550; Ursolic acid; Digoxin; T0901317; SR1001; SR1078; SR3335; SR1555; SR2211; ML209; N-(1-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)benzyl)-1,2,3,4-tetrahydroquinolin-6-yl)acetamide; 2,4-difluoro-N-(1-((4-fluorophenyl)sulfonyl)-1,2,3,4-tetrahydroquinolin-7-yl)benzenesulfonamide; 2-Chloro-6-fluoro-N-(1-((4-fluorophenyl)sulfonyl)-1,2,3,4-tetrahydroquinolin-7-yl)benzamide; (S)-2-fluoro-N-(3-methyl-1-(m-tolylsulfonyl)-2,3-dihydro-1H-pyrido[2,3-b][1,4]oxazin-7-yl)-6-(trifluoromethyl)benzamide; 4-(1-(2-Chloro-6-cyclopropylbenzoyl)-7-fluoro-1H-indazol-3-yl)-3-fluorobenzoic acid; 4-(1-(2-Chloro-6-(trifluoromethyl)benzoyl)-7-fluoro-1H-indazol-3-yl)-2-hydroxycyclohex-3-enecarboxylic acid; GSK-6a; GSK-8h; GSK-9g; GSK-13; GSK-21; 2-(4-(Ethylsulfonyl)phenyl)-N-(6-(3-fluorophenoxy)-[1,1'-biphenyl]-3-yl)acetamide; N-(6-(3,5-difluorophenoxy)-3'-fluoro-[1,1'-biphenyl]-3-yl)-2-(4-(N-methylsulfamoyl)phenyl)acetamide; N-(4-Ethylphenyl)-3-(hydroxymethyl)-N-isobutyl-4-((tetrahydro-2H-pyran-4-yl)methoxy)benzenesulfonamide; N-(4-chlorophenyl)-4-((3,5-dimethylisoxazol-4-yl)methoxy)-N-isobutylbenzenesulfonamide; N-(2,4-dimethylphenyl)-4-(2-hydroxy-2-(pyridin-4-yl)ethoxy)-N-isobutylbenzenesulfonamide; N-isobutyl-N-((5-(4-(methylsulfonyl)phenyl)thiophen-2-yl)methyl)-1-phenylmethanesulfonamide; N-(4-(4-acetyl)piperazin-1-yl)benzyl)-N-isobutyl-1-phenylmethanesulfonamide; N-(3,4-dimethoxyphenyl)-1-ethyl-2-oxo-1,2-dihydrobenzo[cd]indole-6-sulfonamide; JTE-151; substituted 2,3-dihydro-1H-inden-1-one Retinoic acid-related orphan nuclear receptor Antagonists; Methylene linked quinolinyl modulators of ROR-gamma-t; Fused pyridine and pyrimidine derivatives; heteroaromatic compounds; SEVEN-MEMBERED SULFONAMIDES; Substituted dihydro-benzimidazole compounds; Pyrrolo sulfonamide compounds; Tetrahydroquinoline and related bicyclic compounds; Amide compounds; Heterocyclic compounds; and amido compounds..

[0172] In alternative embodiments, the LSE polymer conjugate comprises a Src family tyrosine kinase inhibitor warhead selected from dasatinib and saracatinib.

[0173] In alternative embodiments, the LSE polymer conjugate comprises an IL-23 inhibitor warhead selected from SCH-90222, STA-5326, and STA-5326.

[0174] In alternative embodiments, the LSE polymer conjugate comprises a STAT3 inhibitor warhead selected from cucurbitacin I, niclosamide, cryptotanshinone, SD 1008, Stat3 Inhibitor III, WP1066, Nifuroxazide, Stat3 Inhibitor, Stattic, Stat3 Inhibitor, S3I-201; Stat3 Inhibitor VIII, 5,15-DPP, 2-Hydroxy-4-(((4-methylphenyl)sulfonyloxy)acetyl)amino)-benzoic acid (NSC74859) and Kahweol.

[0175] In alternative embodiments, the LSE polymer conjugate comprises a JAK inhibitor warhead selected from ruxolitinib, fedratinib, tofacitinib, baricitinib, pacritinib, decernotinib, XL019, AZD1480, INCB039110, LY2784544, BMS911543, NS018, GLPG0634, GLPG0788, or N-(cyanomethyl)-4-2-(4-morpholinoanilino)pyrimidin-4-yl)benzamide; or a pharmaceutically acceptable salt thereof.

[0176] Spleen tyrosine kinase (SYK) is a non-receptor linked protein tyrosine kinase which plays a role as a mediator of immunoreceptor signaling in a host of inflammatory cells including mast cells, B-cells, macrophages and neutrophils. In alternative embodiments, the LSE polymer conjugate comprises a SYK inhibitor warhead selected from Cerdulatinib (4-(cyclopropylamino)-2-((4-(4-(ethylsulfonyl)piperazin-1-yl)phenyl)amino)pyrimidine-5-carboxamide), entospletinib (6-(1H-indazol-6-yl)-N-(4-morpholinophenyl)imidazo[1,2-a]pyrazin-8-amine), fostamatinib ([6-({5-Fluoro-2-[(3,4,5-trimethoxyphenyl)amino]-4-pyrimidinyl}amino)-2,2-dimethyl-3-oxo-2,3-dihydro-4H-pyrido[3,2-b][1,4]oxazin-4-yl)methyl dihydrogen phosphate), fostamatinib disodium salt (sodium (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-3-oxo-2H-pyrido[3,2-b][1,4]oxazin-4(3H)-yl)methyl phosphate), BAY 61-3606 (2-(7-(3,4-Dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino)-nicotinamide HQ), RO9021 (6-[(1R,2S)-2-Amino-cyclohexylamino]-4-(5,6-dimethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide), imatinib (Gleevec; 4-[(4-methylpiperazin-1-yl)methyl]-N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide), GSK143 (2-(((3R,4R)-3-aminotetrahydro-2H-pyran-4-yl)amino)-4-(p-tolylamino)pyrimidine-5-carboxamide), PP2 (1-(tert-butyl)-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine), PRT-060318 (2-(((1R,2S)-2-aminocyclohexyl)amino)-4-(m-tolylamino)pyrimidine-5-carboxamide), PRT-062607 (4-((3-(2H-1,2,3-triazol-2-yl)phenyl)amino)-2-(((1R,2S)-2-aminocyclohexyl)amino)pyrimidine-5-carboxamide hydrochloride), R112 (3,3'-((5-fluoropyrimidine-2,4-diyl)bis(azanediyl))diphenol), R348 (3-Ethyl-4-methylpyridine), R406 (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one), piceatannol (3-Hydroxyresveratrol), YM193306 (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643), 7-azaindole, piceatannol, ER-27319, Compound D, PRT060318, luteolin, apigenin, quercetin, fisetin, myricetin, or morin.

[0177] In several embodiments, the LSE polymer conjugate comprises a RAC1 inhibitor warhead selected from W56, NSC23760 and NSC 23766, or the inhibitors described in Yuan Gao, et al. PNAS, May 18, 2004, vol. 101, 7618-7623.

[0178] In several embodiments, the LSE polymer conjugate comprises an ENaC inhibitor warhead selected from triamterene, phenamil, amiloride and amiloride derivatives, particularly benzyl amiloride (Benzamil). Additional amiloride derivatives are described in WO2012035158; WO2009074575; WO201 1028740; WO2009150137; WO201 1079087; and WO2008135557, each of which are herein specifically incorporated by reference.

[0179] In several embodiments, the LSE polymer conjugate comprises a NFkB inhibitor warhead selected from Bithionol, Bortezomib, Cantharidin, Chromomycin A3, Daunorubicinum, Digitoxin, Ectinascidin 743, Emetine, Fluorosalan, Manidipine hydrochloride, Narasin, Ouabain, Sorafenib tosylate, Sunitinib malate, Tioconazole, Tribromsalan, Triclabendazolum, Zafirlukast, and Withaferin A.

[0180] In several embodiments, the LSE polymer conjugate comprises an IRAK inhibitor warhead selected from the inhibitors described in Wang, Zhulun, et al. "IRAK-4 inhibitors for inflammation." Current topics in medicinal chemistry 9.8 (2009): 724-737, which is herein specifically incorporated by reference.

[0181] In several embodiments, the LSE polymer conjugate comprises a PKC inhibitor warhead selected from sotrastaurin (also known as AEB071 and described in U.S. Pat. No. 6,645,970), 3-(1H-Indol-3-yl)-4-[2-(piperazin-1-yl)quinazolin-4-yl]-1H-pyrrole-2,5-dione (described in U.S. Pat. No. 6,645,970), 3-[2-chloro-7-[(dimethylamino)methyl]-1-naphthalenyl]-4-[7-[2-(2-methoxyethoxy)ethoxy]-1H-indol-3-yl]-1H-pyrrole-2,5-dione (described in PCT Publication No. WO07/006,533 and US Publication No. 2008/0318975), 3-[3-(4,7-diaza-spiro[2,5]oct-7-yl)-isoquinolin-1-yl]-4-(7-methyl-1H-indol-3-yl)-pyrrole-2,5-dione (described in Example 69 of U.S. Pat. No. 7,235,555); ruboxistaurin((9S)-9-[(dimethylamino)methyl]-6,7,10,11-tetrahydro-9H,18H-5,21:12,17-dimethenodibenzo-[e,k]pyrrolo[3,4-h][1,4,13]oxadiazacyclohexadecine-18,20(19H)-dione (also known as LY-333531 and described in U.S. Pat. No. 5,698,578)) and the mesylate salt of ruboxistaurin (described in European patent No. 0776895 B1). Each of the references cited above are incorporated herein by reference.

[0182] In several embodiments, the LSE polymer conjugate comprises a PKC α/β inhibitor warhead selected from 3-[2-chloro-7-[(dimethylamino)methyl]-1-naphthalenyl]-4-[7-[2-(2-methoxyethoxy)ethoxy]-1H-indol-3-yl]-1H-pyrrole-2,5-dione (CAS No. 919992-85-1 described in PCT Publication No. WO07/006,533 and US Publication No. 2008/0318975); 3-(1H-indol-3-yl)-4-[2-(piperazin-1-yl)quinazolin-4-yl]pyrrole-2,5-dione having the following structure and described in Example 70 of PCT

Publication No. WO 2002/038561 or U.S. Pat. No. 6,645,970; (9S)-9-[(dimethylamino)methyl]-6,7,10,11-tetrahydro-9H,18H-5, 21:12,17-dimethenodibenzo-[e,k]pyrrolo[3,4-h][1,4,13]oxadiazacyclohexadecine-18,20(19H)-dione (also referred to as ruboxistaurin or LY-333531, CAS No. 169939-94-0 described in U.S. Pat. No. 5,698,578); ruboxistaurin mesylate salt (described in U.S. Pat. No. 5,710,145 and EP Patent No. 776895 B1); and 12-(2-cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5H-indolo(2,3-a)pyrrolo(3,4-c)-carbazole (CAS No. 136194-77-9, available from Calbiochem® and described in U.S. Pat. No. 5,489,608). Each of the references cited above are incorporated herein by reference.

[0183] In several embodiments, the LSE polymer conjugate comprises a specific SIP receptor agonist warhead selected from SIP itself, SEW2871, JTE-013, VPC23019, R- 3477 (Actelion), KRP-203 (Kyorin Pharmaceutical Co.), sonopizumab (Lpath), BAF-312 (Novartis), ONO-4641 (Ono Pharmaceutical Co.), ES-285 (PharmaMar SA), 2-amino-2-[2- (4-octylphenyl)ethyl]propane-1,3-diol (FTY720; fingolimod), phospho-FTY720, and pharmaceutically acceptable salts thereof.

[0184] In several embodiments, the LSE polymer conjugate comprises a PI3K inhibitor warhead selected from Compound 1 ((S)-3-(1-((9H-purin-6-yl)amino)ethyl)-8-chloro-2-phenylisoquinolin-1(2H)-one), AMG-319, GSK 2126458, GSK 1059615, GDC-0032, GDC-0980, GDC-0941, XL147, XL499, XL765, BKM 120, GS1101, CAL 263, SF1126, PX-866, BEZ235, CAL-120, BYL719, RP6503, RP6530, TGR1202, INK1117, PX-886, BAY 80-6946, IC87114, Palomid 529, ZSTK474, PWT33597, TG100-115, GNE-477, CUDC-907, AEZS-136, BGT-226, PF-05212384, LY3023414, PI-103, LY294002, INCB-040093, CAL-130 and wortmannin.

[0185] In several embodiments, the LSE polymer conjugate comprises an AKT inhibitor warhead selected from AZD5363, miltefosine, perifosine, VQD-002, MK-2206, GSK690693, GDC-0068, triciribine, CCT128930, PHT-427, or honokiol, or a combination thereof. In one embodiment, the AKT inhibitor is MK-2206 or perifosine.

[0186] In several embodiments, the LSE polymer conjugate a mTOR inhibitor warhead selected from AP23841, AZD8055, BEZ235, BGT226, deferolimus (AP23573/MK-8669), EM101/LY303511, everolimus (RAD001), EX2044, EX3855, EX7518, GDC0980, INK-128, KU-0063794, NV-128, OSI-027, PF-4691502, rapalogs, rapamycin, ridaforolimus, SAR543, SF1126, temsirolimus (CCI-779), WYE-125132, XL765, zotarolimus (ABT578), torin 1, GSK2126458, AZD2014, GDC-0349, or XL388, or a combination thereof.

[0187] In several embodiments, the LSE polymer conjugate comprises a PDE4 inhibitor warhead selected from rolipram, mesembrine, drotaverine, roflumilast, ibudilast, piclamilast, luteolin, cilomilast, diazepam, arofyline, CP-80633, denbutylline,

drotaverine, etazolate, filaminast, glaucine, HT-0712, ICI-63197, irsogladine, Mesembrine, Ro20-1724, RPL-554, and YM-976.

[0188] Suitable protecting groups, in some embodiments, are for protecting functional groups during the conjugation of warhead and polymer. Various protecting groups as well as suitable means and conditions for protecting and deprotecting the substituents are used in several embodiments. The means and conditions of protecting and deprotecting employed depend on the nature of the involved functional groups. Protecting groups for hydroxy-, amino-, and/or carboxy residues are selected in several embodiments from acetone, ethylidene methoxymethyl, 2-methoxyethoxymethyl, benzyloxymethyl, tetrahydropyranyl, methyl, ethyl, isopropyl, t-butyl, benzyl, triphenylmethyl, t-butyldimethylsilyl, triphenylsilyl, methoxycarbonyl, t-butyloxycarbonyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, acetyl, benzoyl, toluenesulfonyl, dimethoxybenzyl, nitrophenyloxycarbonyl, nitrobenzyloxycarbonyl, allyl, fluorenylmethyl, tetrahydrofuranyl, phenacyl, acetol, phenyl, trimethylsilyl, pyrrolidyl, indolyl, hydrazino and other protecting groups such as those that can be found in Greene T. W., et al., *Protective Groups in Organic Synthesis*, 4th ed., John Wiley and Son, New York, N.Y. (2007); incorporated herein in its entirety by reference. The reagents and conditions of protecting and deprotecting reactions are in particular selected for their suitability at selectively attaching and removing the protecting group without adversely affecting the rest of the compound.

[0189] The polymer conjugates disclosed herein may also be prepared as pharmaceutically acceptable salts including salts of inorganic acids such as hydrochloric, hydroiodic, hydrobromic, phosphoric, metaphosphoric, nitric acid and sulfuric acids as well as salts of organic acids, such as tartaric, acetic, citric, malic, benzoic, glycolic, gluconic, succinic, aryl sulfonic, (e.g., p-toluene sulfonic acids, benzenesulfonic), phosphoric, malonic, and the like. Suitable acids for formation of pharmaceutically acceptable salts are used in some embodiments. Further, pharmaceutically acceptable salts of compounds may be formed with a pharmaceutically acceptable cation. Pharmaceutically acceptable cations include, but are not limited to, alkali cations (Li⁺, Na⁺, K⁺), earth alkali cations (Mg²⁺, Ca²⁺, Ba²⁺), ammonium and organic cations, such as quaternary ammonium cations.

Synthesis Of Polymer Conjugates

[0190] The description above is not intended to be limiting and should be viewed as an example to guide the manufacture of the other compounds identified herein. The polymer conjugates may also be made as described in US Patent Nos. 8,673,347 and 8,926,955, both herein incorporated by reference. Several embodiments

provide a method for the production of polymer conjugates of the active agents that result in a highly pure reaction product, obtained in high and consistent yields.

[0191] In one embodiment, the conjugation reaction of the process to synthesize a conjugate polymer compound is catalysed by a base in an organic solvent. The base may be a strong base. In one embodiment, the base is selected from the group of alkali metal hydrides, tertiary amines and/or alkoxide. In another embodiment, the base catalysing the polymer conjugation reaction is sodium hydride. Other bases, such as sodium methoxide, or triethylamine can also be used. In several embodiments, the molar ratio of the base catalyst to the compound is between about 1:1 and about 4:1, about 1:1 to about 1.5:1 and about 1:1. The reaction may be carried out in an organic solvent, such as in anhydrous conditions (e.g., in a dry organic solvent). The water content in the solution mixture of the conjugation process may be equal or less than 200 ppm. The organic solvent may be selected from the group of dichloromethane, chloroform, N,N-dimethylformamide. In certain embodiments, the organic solvent is dichloromethane or anhydrous dichloromethane.

[0192] The conjugation reaction may be carried out under inert gas atmosphere, such as nitrogen or argon atmosphere. The reaction of the process may be carried out at a temperature of about -10° to about 60° C, about 0° to about 25° C or at room temperature after an initial step at 0° C.

[0193] Following the production of the target compound, the polymer conjugate may then be separated and purified from the reaction mixture. In one embodiment, the compound is obtained by purification of the crude mixture by flash chromatography. An automated gradient flash purification system may be used and may be equipped with a suitable column and solvent. The purification method may be selected from reverse phase and direct phase columns and the conditioning/elution solvent may be selected from dichloromethane, water, methanol, acetonitrile, ammonium formate buffer solution at different mixture ratios. In one embodiment, the compound is purified by a reverse phase flash chromatography equipped with a C18 cartridge and the purification is carried out by gradient elution with acetonitrile/water. In one embodiment, the compound is purified by a normal phase flash chromatography.

[0194] The product may then be dried e.g. over sodium sulphate and filtered off and the solvent is removed by evaporation under reduced pressure at 25° C. Purification of the target product is carried out in several embodiments. After the purification step the resultant polymer compound has a purity of at least about 95%, about 96%, about 97%, about 98%, about 98.5%, about 99% or about 99.5%. The disclosed process results in an overall mass yield of the compound from about 40% to

about 98% by weight, or from about 50% to about 95% by weight based on the weight of a reactant compound.

[0195] In several embodiments, the polymer moiety which is covalently attached to the active entity is biocompatible, can be of natural or semi-synthetic or synthetic origin and can have a linear or branched structure. The polymer may be selected from poly(alkylene oxides), or from (polyethylene) oxides. However, other polymers include without limitation polyacrylic acid, polyacrylates, polyacrylamide or N-alkyl derivatives thereof, polymethacrylic acid, polymethacrylates, polyethylacrylic acid, polyethylacrylates, polyvinylpyrrolidone, poly(vinylalcohol), polyglycolic acid, polylactic acid, poly(lactic-co-glycolic) acid, dextran, chitosan, hydroxyethyl starch.

[0196] In some embodiments, the above-mentioned polymer moiety can carry an amino functional end-group or can be functionalized to carry an amino functional end-group. Hence, the polymer moiety can be an amino-activated polymer of general formula X—NH₂.

[0197] The reaction of formation of the compositions identified herein may be carried out at a temperature of about 10° to about 60° C., about 15° to about 25° C. or at room temperature. The polymer moiety X may be a polyethylene glycol (PEG) moiety, wherein the terminal OH group can optionally be modified e.g. with C1-C5 alkyl or C1-C5 acyl groups, such as with C1-, C2- or C3-alkyl groups or C1-, C2- or C3 groups. The modified polyethylene glycol may be a terminally alkoxy-substituted polyethylene glycol, including a methoxy-polyethylene-glycol (mPEG).

[0198] In other aspects, the conjugated polymer compounds may be used as active agents in a topical medicament useful for the prevention, alleviation and/or treatment of dermal pathologies. It has been shown that the conjugated polymer compounds described herein are very advantageously used as topical medicament since they do not show adverse or toxic effects (e.g. irritation) when dermally administered or any phototoxic effect (e.g. photomutagenicity, phototoxicity or photosensitisation) (as shown in the studies described in the following examples).

[0199] The dermal pathologies for such treatment may be pathologies characterized by hyperproliferation of the keratinocytes, such as psoriasis, atopic dermatitis, chronic eczema, acne, pityriasis rubra pilaris, keloids, hypertrophic scars and skin tumors, such as keratoacanthoma, squamous cell carcinoma, basal cell carcinoma.

[0200] The compounds disclosed herein or pharmaceutically acceptable salts thereof can be administered as they are, or in the form of various pharmaceutical compositions according to the pharmacological activity and the purpose of administration. Yet another aspect is a pharmaceutical composition comprising an effective amount of at least one compound in Table 1 optionally together with

pharmaceutically acceptable carriers, adjuvants, diluents or/and additives. Pharmaceutical carriers, adjuvants, diluents or/and additives are applied in the formulation of the pharmaceutical composition comprising a compound of embodiments identified herein.

[0201] The disclosed compounds can be employed as the sole active agent in a pharmaceutical composition. Alternatively, the compounds of Table 1 may be used in combination with one or several further active agents, e.g. other active pharmaceutical agents in the treatment of the conditions described herein.

[0202] In particular, the polymer conjugate compounds may be used in combination with at least one steroidal anti-inflammatory drug and/or one further agent capable of inhibiting an early mediator of the inflammatory cytokine cascade, e.g. an antagonist or inhibitor of a cytokine selected from the group consisting of TNF, IL-1 α , IL-1 β , IL-Ra, IL-8, MIP-1 α , MIF-1 β , MIP-2, MIF and IL-6. Particularly useful anti-inflammatory drugs are selected from alclometasone dipropionate, amcinonide, beclomethasone dipropionate, betamethasone, betamethasone benzoate, betamethasone dipropionate, betamethasone sodium phosphate, betamethasone sodium phosphate and acetate, betamethasone valerate, clobetasol butyrate, clobetasol propionate, clocortolone pivalate, cortisol (hydrocortisone), cortisol (hydrocortisone) acetate, cortisol (hydrocortisone) butyrate, cortisol (hydrocortisone) cypionate, cortisol (hydrocortisone) sodium phosphate, cortisol (hydrocortisone) sodium succinate, cortisol (hydrocortisone) valerate, cortisone acetate, desonide, desoximetasone, dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, diflorasone diacetate, diflucortolone valerate, fludrocortisone acetate, fludroxycortide, flumetasone pivalate, flunisolide, fluocinolone acetonide, fluocinonide, fluocortolone, fluorometholone, flurandrenolide, fluticasone propionate, halcinonide, halobetasol propionate, medrysone, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, mometasone furoate, paramethasone acetate, prednisolone, prednisolone acetate, prednisolone sodium phosphate, prednisolone tebutate, prednisone, triamcinolone, triamcinolone acetate, triamcinolone acetonide, triamcinolone diacetate, triamcinolone hexacetonide. Useful antagonists or inhibitors of a cytokine are selected from infliximab, etanercept or adalimumab.

[0203] The polymer conjugate compounds may be used in combination with at least one natural extract or essential oil which is anti-itching agent, for example and not restricted to, extracts of *Abelmoschus esculentus*, *Actaea alba*, *Aglaia odorata*, *Alkanna tinctoria*, *Althaea officinalis*, *Altingia excelsa*, *Andropogon virginicus*, *Aralia nudicaulis*, *Aralia racemosa*, *Argemone mexicana*, *Barleria prionitis*, *Camelia sinensis*, *Caesalpinia digyna*, *Campsis grand/flora*, *Carissa congesta*, *Carthamus oxyacantha*,

Cassia tora, Chrysanthemum indicum, Cimicifuga racemosa, Cinnamomum camphora, Clematis vitalba, Cuscuta reflexa, Diospyros peregrina, Enicostema axillare, Hammamelis virginiana, Jatropha multifida, Lavandula officinalis, Lavandula latifolia, Liquidambar orientalis, Lithospermum officinale, Madhuca longifolia, Martynia annua, Medicago sativa, Michelia champaca, Mikania glomerata, Mimosa pudica, Oryza sativa, Phaseolus vulgaris, Phyllanthus urinaria, Phyllanthus virgatus, Pistacia vera, Polygonum hydropiper, Quercus ilex, Rauvolfia caffra, Ricinus communis, Rubus idaeus, Sagittaria sagittifolia, Sandoricum koetjape, Sapindus mukorossi, Schleicheria oleosa, Sesbania grandiflora, Spondias dulcis, Tilia sp., Toona ciliata, Tragia involucrata, Trichosanthes quinqueangulata, Vaccaria pyramidata, Ventilago madraspatana, Veratrum album or Xanthium strumarium among others

[0204] The polymer conjugate compounds may be used in combination with at least one synthetic compound or product of biotechnological origin which is an anti-itching agent, for example and not restricted to mepyramine (pyrilamine), antazoline, diphenhydramine, carbinoxamine, doxylamine, clemastine, dimenhydrinate, pheniramine, chlorphenamine (chlorpheniramine), dexchlorpheniramine, brompheniramine, triprolidine, cyclizine, chlorcyclizine, hydroxyzine, meclizine, cetirizine, levocetirizine, promethazine, thenaldine, alimemazine (trimeprazine), cyproheptadine, azatidine, ketotifen, acrivastine, astemizole, cetirizine, loratadine, desloratadine, mizolastine, terfenadine, fexofenadine, fexofenadine, azelastine, levocabastine, olopatadine, corticosteroids such as cortisone, hydrocortisone dexamethasone, prednisone; Neutragen™ [INCI: Water, Butylene Glycol, Dextran, Palmitoyl Tripeptide-8] marketed by Atrium Innovations/Unipex Group, Meliprene [INCI: Dextran, Acetyl Heptapeptide-1] marketed by Institut Europeen de Biologie Cellulaire/Unipex Group, Skinasensyl™ [INCI: Acetyl Tetrapeptide-15] marketed by Laboratoires Serobiologiques/Cognis, SymSitive® 1609 [INCI: 4-t-Butylcyclohexanol] marketed by Symrise, Symbiocell™ [INCI: Extract from Cestrum Latifolium] marketed by BASF, Gatuline®/Derma-Sensitive [INCI: Octyldodecyl Myristate, Capparis Spinosa Fruit Extract] marketed by Gattefosse or MAXnolia [INCI: Magnolia Officinalis Bark Extract, Vitis Vinifera/Vitis Vinifera (Grape) Seed Extract, Tocopherol] marketed by Mibelle among others.

[0205] The polymer conjugate compounds may be used in combination with at least one physiological cooling agent, for example and not restricted to menthone glycerol acetal, menthyl lactate, menthyl ethyl oxamate, substituted menthyl-3-carboxylic acid amides (e.g. menthyl-3-carboxylic acid N-ethylamide, Na-(L-menthanecarbonyl)glycine ethyl ester, 2-isopropyl-N-2,3-trimethylbutanamide, substituted cyclohexanecarboxylic acid amides, 3-menthoxypropane-1,2-diol, 2-

hydroxyethyl menthyl carbonate, 2-hydroxypropyl menthyl carbonate, N- acetylglycine menthyl ester, isopulegol, menthyl hydroxycarboxylic acid esters (e.g. menthyl 3-hydroxybutyrate), monomenthyl succinate, monomenthyl glutarate, 2-mercaptocyclodecanone, menthyl 2-pyrrolidin-5-onecarboxylate, 2,3-dihydroxy-p-menthane, 3,3,5-trimethylcyclohexanone glycerol ketal, 3-menthyl 3,6-di- and -trioxaalkanoates, 3-menthyl methoxyacetate and icilin.

[0206] Further agents which can be used in combination with the polymer compounds are e.g. antagonists and/or inhibitors of RAGE, antagonists and/or inhibitors of HMGB1, antagonists and/or inhibitors of the interaction of a Toll-like receptor (TLR) with HMGB1, the functional N-terminal lectin-like domain (D1) of thrombomodulin and/or a synthetic double-stranded nucleic acid or nucleic acid analogue molecule with a bent shape structure as described in the international patent application WO 2006/002971 which is herein incorporated by reference.

[0207] The compositions described herein may be administered by a physician or other professional. Patients may also be able to self-administer. In several embodiments, administration of the composition may be performed dermally, via, for example, ointments, creams, oils, liposomes or trans-dermal patches, or wherein the polymer conjugates are incorporated into liposomes.

[0208] In some embodiments, at least one excipient is provided. Excipients can include a nonaqueous or aqueous carrier, and one or more agents selected from moisturizing agents, pH adjusting agents, strontium ions (Sr^{2+}), deodorants, fragrances, chelating agents, preservatives, emulsifiers, thickeners, solubilizing agents, penetration enhancers, anti-irritants, colorants, surfactants, beneficial agents, pharmaceutical agents, and other components for use in connection with the compositions described herein (such as topical compositions for treatment of the skin). In several embodiments, the composition is an anhydrous formulation to prevent skin irritation such as water-based irritant contact dermatitis or stinging sensation upon application to damaged skin. In another embodiment, the composition is formulated such that preservatives need not be employed (e.g., a preservative-free formulation) so as to avoid skin irritation associated with certain preservatives.

[0209] To facilitate application, the composition may be provided as an ointment, an oil, a lotion, a paste, a powder, a gel, a foam, or a cream. The composition may also include additional ingredients such as a protective agent, an emollient, an astringent, a humectant, a sun screening agent, a sun tanning agent, a UV absorbing agent, an antibiotic agent, an antifungal agent, an antiviral agent, an antiprotozoal agent, an anti-acne agent, an anesthetic agent, a steroidal anti-inflammatory agent, a non-steroidal anti-inflammatory agent, an antipruritic agent, an additional antioxidant agent, a

chemotherapeutic agent, an anti-histamine agent, a vitamin or vitamin complex, a hormone, an anti-dandruff agent, an anti-wrinkle agent, an anti-skin atrophy agent, a skin whitening agent, a cleansing agent, additional peptides, additional modified peptides, and combinations thereof. In a further embodiment, the composition may avoid irritants (such as animal or cellular-based materials) to avoid skin irritation.

[0210] In some embodiments, the compositions may be administered by injection or infusion, in particular by intravenous, intramuscular, transmucosal, subcutaneous or intraperitoneal injection or infusion and/or by oral, topical, dermal, nasal, inhalation, aerosol and/or rectal application, etc.

[0211] In a further embodiment, the compositions are administered reversibly immobilized on the surface of a medical device, in particular by binding, coating and/or embedding the compositions on a medical device, such as but not limited to, stents, catheters, surgical instruments, cannulae, cardiac valves, or vascular prostheses. After contacting the medical device with body fluid or body tissue, the reversibly immobilized compounds are liberated. Consequently, the coated medical devices act as drug delivery devices eluting the medicament, whereby the drug delivery kinetics can be controlled, providing an immediate release or a controlled, delayed or sustained drug delivery, for example.

[0212] In some embodiments, the composition further comprises an enteric coating that resists degradation under the prevailing pH of the stomach and permits delivery to specific regions of the gastrointestinal tract.

[0213] The pharmaceutical compositions may also be used for diagnostic or for therapeutic applications. For diagnostic applications, the compound may be present in a labelled form, e.g. in a form containing an isotope, e.g. a radioactive isotope or an isotope which may be detected by nuclear magnetic resonance. In some embodiments, a therapeutic application is, in the case of a topical application, the prevention, alleviation and treatment of psoriasis and dermatitis.

[0214] The concentrations of the compounds in the pharmaceutical composition can vary. The concentration will depend upon factors such as the total dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, the route of administration, the age, body weight and symptoms of a patient. The compounds typically are provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for topical administration. Typical dose ranges are from about 1 µg to about 1 g/kg of body weight per day; a dose range may be from about 0.01 mg/kg to 100 mg/kg of body weight per day, or about 0.1 to 20 mg/kg once to four times per day. In some embodiments, the dosage of the drug to be administered is likely to depend on variables such as the type

and extent of the progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the selected compound and the formulation of the compound excipient, and its route of administration.

[0215] Although the foregoing has been described in some detail by way of illustrations and examples for purposes of clarity and understanding, it will be understood by those of skill in the art that modifications can be made without departing from the spirit of the present disclosure. Therefore, it should be clearly understood that the forms disclosed herein are illustrative only and are not intended to limit the scope of the present disclosure, but rather to also cover all modification and alternatives coming with the true scope and spirit of the embodiments of the invention(s).

[0216] Terms and phrases used in this application, and variations thereof, especially in the appended claims, unless otherwise expressly stated, should be construed as open ended as opposed to limiting. As examples of the foregoing, the term 'including' should be read to mean 'including, without limitation,' 'including but not limited to,' or the like.

[0217] The indefinite article "a" or "an" does not exclude a plurality. The term "about" as used herein to, for example, define the values and ranges of molecular weights means that the indicated values and/or range limits can vary within $\pm 20\%$, e.g., within $\pm 10\%$. The use of "about" before a number includes the number itself. For example, "about 5" provides express support for "5".

[0218] The phrases "active agent" and "active entity" are synonyms and can be used interchangeably.

[0219] The terms "CT101" and "SNA-101" are synonyms and can be used interchangeably.

EXAMPLES

[0220] Non-limiting examples are provided below.

Example 1: A first synthesis scheme for CT101, a PEGylated variants of the kinase inhibitor ChEMBL id 249094 (CAS944795-06-6), by LSE Technology

[0221] ChEMBL 249097 (CAS No. 944795-06-6, illustrated in Figure 1) was identified as a member of the extensively studied protein kinase inhibitors class of 2,4-dianilino pyrimidines. After bibliographic data mining, ChEMBL 249097 resulted to inhibit several kinases whose misregulation is involved in atopic dermatitis, including CSK, Lyn, SYK and TXK kinases at low nanomolar range.

[0222] Because of its peculiar polypharmacology profile, together with the feasibility of LSE modification and its readily commercial availability, ChEMBL 249097 was considered an interesting starting point to research LSE-variants with improved therapeutic potential.

[0223] Figure 2 depicts the first synthesis scheme for CT101.

Materials

Acetonitrile for HPLC (Merck/VWR, Cat No 1.00030.2500)
ChEMBL id 249094 (CAS944795-06-6) (Proactive Molecular Res, Cat No P06-110062)
Sodium Hydroxide 32% solution (Sigma Aldrich, Cat No 30531)
Tetrahydrofuran (Sigma Aldrich, Cat No 401757)
Dichloromethane (VWR, Cat No. 23354.326)
Dichloromethane, anhydrous (Sigma Aldrich, Cat No 270997)
N,N-dicyclohexyl carbodiimide (Fluka, Cat No 36650)
N-hydroxysuccinimide (Aldrich Cat. No130672)
Iodine (Sigma-Aldrich, Cat No 03551 -100G)
Methanol (VWR, Cat No 20864320)
Ethyl acetate (VWR, Cat No 23882.321)
mPEG-NH₂ 2015 Da (Iris Biotech GmbH, Cat No PEG1152, Lot No 1411212)
Na₂SO₄ anhydrous (VWR, Cat No 28114.296)
NaCl (VWR, Cat No 443827W)
Silica column (Biotage Snap Ultra HP Sphere 10g, Cat. No FSUL-0442-0010)
TLC (Fluka, Cat No 99577-1 EA)
Trifluoroacetic acid (TFA) (Sigma Aldrich, Cat No302031)
Hydrochloric acid 32% (Merck, Cat No 1.00319.2511)

Methods

- A) Analytical HPLC with UV detection at 214-280 nm and ELS detectors (Waters 2695, 2487, 2424) (Reference Method: 5 to 100 2%min ELSD 80C)
HPLC analytical C18 column (eg. Phenomenex Jupiter C18 300Å, 5µm, 4.6×250mm, Cat No 00G-4053-EO)
Acetonitrile for HPLC
H₂O for HPLC

Time (min)	Flow (mL/min)	H ₂ O, 0.1%TFA (%)	CH ₃ CN, 0.1%TFA (%)	Gradient
0	1	95	5	
5	1	95	5	
50	1	0	100	Linear gradient
55	1	0	100	
55	1	95	5	Linear gradient
70	1	95	5	

- B) Preparative flash chromatograph with UV detection at 280nm (Biotage Isolera One) Silica column (Biotage SNAP Ultra 10g HP Sphere, Cat.no. FSUL-0442-0010) Dichloromethane Ethyl acetate

CV (endpoint)	Dichloromethane (%)	Ethyl acetate (%)	gradient
3	95	5	
15	0	100	Linear gradient
22	0	100	
3	0	100	
0.1	95	5	Linear gradient
3	95	5	

- C) Preparative flash chromatograph with UV detection at 280nm (Biotage Isolera One)
Silica column (Biotage SNAP Ultra 10g HP Sphere, Cat.no. FSUL-0442-0010)
Dichloromethane
Methanol

CV (endpoint)	Dichloromethane (%)	Methanol (%)	gradient
3	100	0	
10	80	20	Linear gradient
4	80	20	

- D) Preparative HPLC with UV detection at 214-280nm (e.g. AKTA Purifier 10) HPLC semipreparative C18 column (e.g. Phenomenex Jupiter C18 300Å, 5µm, 15×250mm, Cat No 00G-4053-AK)
HPLC water with 0.1 % trifluoroacetic acid
HPLC acetonitrile with 0.1 % trifluoroacetic acid

CV (endpoint)	H ₂ O 0.1% TFA (%)	CH ₃ CN, 0.1% TFA (%)	gradient
0	95	5	
1.2	95	5	
22	0	100	Linear gradient
3	0	100	
0.1	95	5	Linear gradient
3	95	5	

[0224] Step 1 – In a 25ml one neck round bottom flask with glass stopper, 50.81 mg of ChEMBL id 249097 (CAS944795-06-6, MW: 335.36 g/mol; 0.1515mmol) were dissolved in 3 ml of THF; then 5ml of water and 561 μ L of 32% NaOH solution (MW 40, d 1.35g/ml, 40 eq) were added dropwise under vigorous stirring. The mixture was shielded from direct light with an aluminum foil, heated to 70°C on oil bath and let under stirring for 48-60h. Reaction outcome was checked by HPLC (Method A) by injection of 5 μ L of a sample resulting from 10 μ L of reaction mixture diluted with 52.6 μ L of H₂O/THF 5/3 (vol/vol). Typical conversion range is 75-80% (Figure 3). Reaction mixture was neutralized with diluted hydrochloric acid, saturated with sodium chloride and extracted with 5×100mL of methylene chloride and 2×100mL of ethyl acetate. Collected organic layers were dried over anhydrous sodium sulfate and evaporated, yielding to 46mg of crude. The material was then purified by flash chromatography on a silica column (Method B, Figure 4). Fraction of interest were identified by TLC (dichloromethane/ethyl acetate 65/35 vol/vol, Benzamide 1: R_f~0.1, carboxylic intermediate 2: R_f~0.2), mixed and evaporated, yielding to 26.4mg of intermediate (MW336.4, 0.078mmol, yield 52%, purity by HPLC method A 97.6% @280nm, injection of 5 μ L of a 1 mg/ml solution in H₂O/THF 5/3 vol/vol).

[0225] Step 2 – In a one neck round bottom flask dried under nitrogen, 13.4mg of carboxylic intermediate 2 (MW 336.36, 0.04mmol) were suspended in 10ml of anhydrous dichloromethane under magnetic stirring. Subsequently, 8.23mg of dicyclohexyl carbodiimide (MW 206.33, 1 eq) and 4.59mg of N-hydroxy succinimide (MW 115.1, 1 eq) were added and the mixture left under stirring. After 15 min, 80.39mg of mPEG-amine (MW 2015, 1eq) were added and the reaction vessel shielded from direct light. After 23h, reaction outcome was evaluated by HPLC (Method A, Figures 5 and 6) by injection of 15 μ L of a sample prepared by evaporating 32 μ L of reaction mixture under nitrogen flow and re-dissolving the residue in 38 μ L of THF and 62 μ L of H₂O. The reaction mixture was directly loaded into a flash silica column previously equilibrated with dichloromethane, and then eluted with a gradient of methanol (Method C) as reported in Figure 7.

[0226] Fractions of interest were identified by TLC (Dichloromethane/methanol 95/5, CT101 Rf-0.15, Carboxylic intermediate 2 Rf- 0.19, mPEG-amine Rf-0.11) and by HPLC (Method A) by injection of 5µL of a sample prepared by evaporating 32µL of the selected fraction under nitrogen flow and re-dissolving the residue in 12µL of THF, 20µL of water and 68µL of water/THF mixture 5/3 vol/vol.

[0227] Fractions of interest were mixed and evaporated, yielding to 53.5mg of waxy solid that was dissolved in 0.8ml of HPLC water and finally purified by HPLC chromatography (Method D).

[0228] The main peak registered at 280 nm was fractionated as reported in Figure 8.

[0229] Fractions collected were analyzed by HPLC (Method A) and those of interest evaporated of the acetonitrile and lyophilized, yielding 48 mg of yellow waxy solid with a ~55% molar yield (MW 2199Da).

[0230] The product was characterized by analytical RP-HPLC (Method A) by injection of 15µL of a 1 mg/ml solution in water / methanol 1/1.

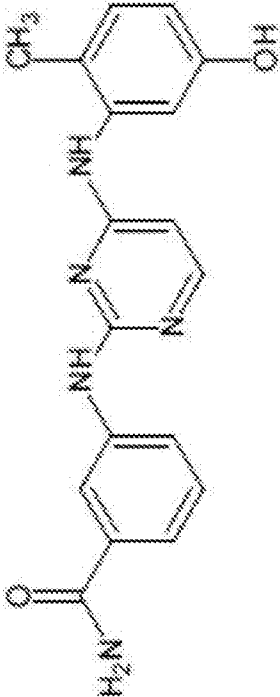
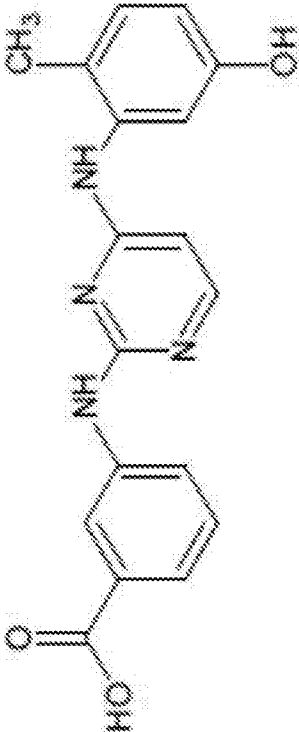
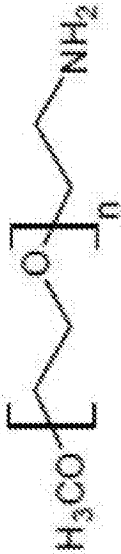
[0231] Figure 9 reports the resulting chromatographic profile at 280 nm.

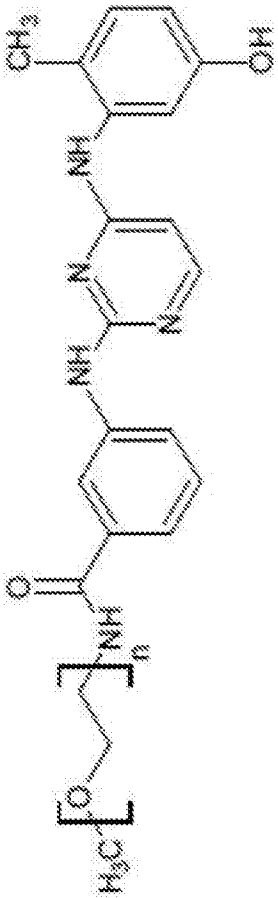
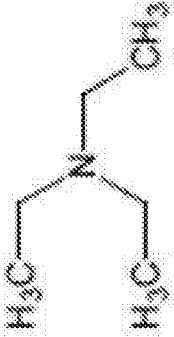
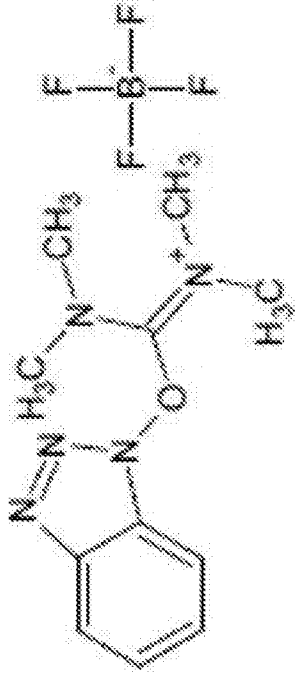
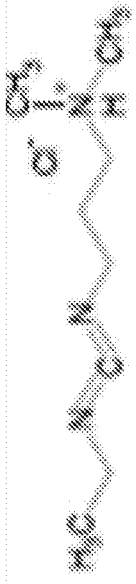
[0232] Purity detected at 280nm was 99.5% (RT 24.26 min).

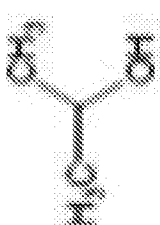
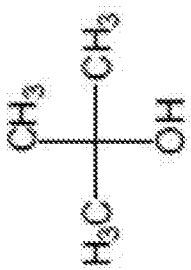
[0233] The molecular identity of CT101 was confirmed by LC/MS and NMR analyses. Figure 10 depicts the structure of CT101.

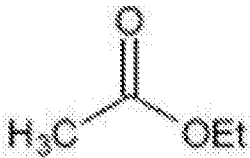
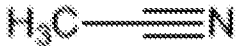
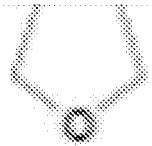
Example 2: A second synthesis scheme for CT101 by LSE Technology

Abbreviations

IUPAC name (nickname)	Formula	MW
3-({4-[(5-hydroxy-2-methylphenyl)amino]pyrimidin-2-yl}amino)benzamide (Benzamide)		335.36
3-({4-[(5-hydroxy-2-methylphenyl)amino]pyrimidin-2-yl}amino)benzoic acid (Carboxylic acid intermediate)		336.34
α -methoxy- ω -{2-aminoethyl}poly(ethyleneoxy) (mPEG ₂₀₀₀ -NH ₂)		(for n=44) 2013.42 (Average MW) 2015

IUPAC name (nickname)	Formula	MW
3-({4-((5-hydroxy-2-methylphenyl)amino)pyrimidin-2-yl}amino)-N-mPEG ₂₀₀₀ benzamide (CT101)		(for n=45) 2331.75 (Average MW) 2333
Triethylamine (TEA)		101.19
O-(1H-benzotriazole-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU)		321.08
N-[3-(dimethylamino)propyl]-N'-ethylcarbodiimide HCl (WSC)		191.70

IUPAC name (nickname)	Formula	MW
Dichloromethane (DCM)	CH_2Cl_2	84.93
Methanol (MeOH)	$\text{H}_3\text{C-OH}$	32.04
Ethanol (EtOH)		46.07
Propan-2-ol (iPrOH)		60.91
2-Methylpropan-2-ol (tBuOH)		74.12

IUPAC name (nickname)	Formula	MW
Ethyl acetate (AcOEt)		88.11
Acetonitrile (ACN)		41.05
Diethyl ether		74.12
Tetrahydrofuran (THF)		72.11

[0234] The second synthesis scheme for CT101 production starting from commercially available benzamide is shown in Figure 11. The selected synthetic scheme consists of hydrolysis to the carboxylic acid intermediate (Step 1) and amidation with mPEG₂₀₀₀-NH₂ (Step 2). Figure 11 depicts the second scheme for CT101 synthesis.

[0235] Two main batches of CT101, representative of the developed process, were produced: the first batch generated 1.50 g of CT101 with 41% overall yield from the starting benzamide and 99.5% HPLC purity, the second batch generated two CT101 samples (1.63 g and 0.36 g respectively) with 47% overall yield from the starting benzamide, and 99,7% HPLC purity. CT101 yield has been calculated assigning it 2333 Da as the average molecular weight, as the average molecular weight of MeO-PEG₂₀₀₀-NH₂ used for its preparation was 2015 Da.

[0236] A preliminary small scale synthesis of CT101 had been carried out, which produced the material used for the structural characterization and the *in vitro* activity tests.

[0237] The used synthetic procedure consisted of hydrolysis of the benzamide with a NaOH aqueous solution as base and THF as solvent. The carboxylic acid intermediate, isolated by flash chromatography, was amidated with mPEG₂₀₀₀-NH₂ in the presence of N-hydroxysuccinimide and N,N'-dicyclohexylcarbodiimide, producing the CT101 which was purified by normal phase flash chromatography first and then by preparative HPLC. The overall yield from the starting benzamide was nearly 26%.

[0238] Starting from this procedure the development studies aimed at defining a reproducible synthetic protocol for the production of CT101 samples with a representative quality, suitable for scaling up and, preferably, with higher yields.

[0239] The results of next outlined experiments for each step will be given as % area of product, as measured by HPLC analyses (@ 270nm).

Step 1: Benzamide hydrolysis

[0240] Preliminary hydrolysis trials were performed reproducing the conditions carried out in the preliminary small scale synthesis.

[0241] Benzamide dissolved in THF was treated with 40 equivalents of NaOH as a 4.2% aqueous solution, at 70°C.

[0242] Only a partial benzamide conversion was observed even at long reaction time (Exp. N° 1/Table 2). Further attempts to increase benzamide conversion (by working under pressure in sealed tube, and increasing temperature and NaOH aqueous solution concentration) were unsuccessful (Exp. N° 4, 5, 8/Table 2).

[0243] Results of HPLC analyses (@ 270nm) carried out on the reaction mixtures are shown in Table 2.

Table 2: HPLC results (@270nm) of benzamide hydrolysis using THF as solvent

Exp. N°	NaOH, %	Reactor	T, °C	Reaction time	% area carboxylic acid	% area benzamide	% area others
1	4,2	Open flask	70	5 days	54,0	38,1	7,9
		Sealed tube	90	1 day	15,1	11,7	72,2
4	4,2	Sealed tube	95-100	20 h	0,0	19,0	81,0
5	4,2	Sealed tube	90	5 days	19,2	23,4	57,4
8	10	Sealed tube	90	2 days	86,2	10,7	3,1

[0244] The low observed selectivity was mainly due to the formation of a by-product with 229 Da as nominal mass (according to HPLC-MS), which could derive from the reaction of the benzamide with the ammonia generated from the hydrolysis reaction. Figure 12 depicts a MW 229 Da by-product.

[0245] The adopted reaction system appeared as a biphasic mixture, which could represent a further drawback to the success of the reaction, so one issue was the selection of a solvent able to dissolve the starting benzamide and to generate a monophasic reaction mixture.

[0246] N-nitrosation with $\text{NaNO}_2/\text{H}_2\text{SO}_4$ to activate the benzamide towards the hydrolysis reaction was tested. The hydrolysis reaction occurred with almost complete conversion, but the observed selectivity was low due to the formation of nitrated by-products, may be on the phenol ring. The use of Na_2O_2 was checked too with no notable results.

[0247] Going back to more classical conditions, the benzamide hydrolysis was performed using NaOH aqueous solutions as base, at various concentrations, in different alcoholic solvents (EtOH, iPrOH, tBuOH).

[0248] Initially, EtOH appeared a very promising solvent, as good conversion and selectivity were achieved. However, the reaction apparently progressed only to a certain extent but never went to completion. Soon it was clear that the analytical result was affected by a resolution problem of the used HPLC method: the benzamide co-eluted with a by-product, whose nominal mass (according to HPLC-MS) was 258 Da (in Figure 13 the hypothesized structures).

[0249] A new HPLC method was developed, which was able to differentiate the co-eluting compounds, but, in the meantime, the results of the first trials with EtOH were undermined by this problem. The results of the analyses performed with the HPLC method that didn't differentiate the benzamide and the MW 258 Da by-product, are shown in Table 3.

Table 3: HPLC results (@270nm) of benzamide hydrolysis using EtOH as solvent

Exp. N°	NaOH %	Molar Ratio NaOH/Benzamide	T, °C	Reaction time, h	% area carboxylic acid	% area benzamide + MW 258 Da	% area others
9	10	40	Reflux	8	64,4	28,6	7,0
11	10	40	Reflux	5	58,5	30,7	9,8
				6	64,0	28,4	7,6
				8	59,8	33,4	6,8
12	10	40	Reflux	2	42,1	51,1	6,8
		+20		4	64,3	27,9	7,8
		+20		6	67,1	24,6	8,3
13	30	40	Reflux	5	14,2	77,1	8,7
	10	+20		10	7,0	83,3	9,7
	4	+20		12	5,1	82,0	12,9

[0250] The results attained with the HPLC method which separated the benzamide from the MW 258 by-product are in Table 4.

Table 4: HPLC results (@270nm) of benzamide hydrolysis using EtOH as solvent

Exp. N°	NaOH %	Molar Ratio NaOH/Benzamide	T, °C	Reaction time, h	% area carboxylic acid	% area benzamide	% area MW 258 Da	% area others
17	5	40	Reflux	6	62,4	15,1	13,0	9,5
				10	64,4	1,8	28,3	7,5
18	10	40	50	27	68,3	16,4	9,4	3,9
				46	73,3	3,1	21,3	2,3

[0251] In some embodiments, milder hydrolytic conditions (lower temperatures/ Exp. N°18) are used.

[0252] When the carboxylic acid was isolated from the reaction mixture, the crude product was contaminated by significant quantities of the MW 258 Da by-product, as shown in Table 5.

Table 5: HPLC analyses (@270nm) of crude carboxylic acid

Exp. N°	% area carboxylic acid	% area benzamide	% area MW 258 Da	% area others
12	66,8	1,4	28,7	3,1
13	11,7	-	74,7	13,6
17	78,1	4,1	20,9	4,9
18	72,7	4,0	20,7	2,6

[0253] As the by-product formation seemed to be related to the use of ethanol, more sterically hindered alcohols as iPrOH and tBuOH were used as solvents, with the aim of reducing its reactivity.

[0254] The use of iPrOH generated a by-product with 272 Da as nominal mass (in Figure 14 the hypothesized structures), but only in moderate amounts. In the isolated crude carboxylic acid, the MW 272 Da by-product was limited to 2-4%, as shown in Table 6.

Table 6: HPLC results (@270nm) of benzamide hydrolysis using iPrOH as solvent

Exp. N°	NaOH* %	T, °C	Reaction time, h	% area carboxylic acid	% area benzamide	% area MW 272	% area others
19	5	50	22	20,6	79,2	-	0,2
		70	70	91,1	5,0	1,8	2,3
isolated product				95,4	-	1,9	2,7
20	5	60	47	88,9	4,2	3,4	3,5
isolated product				90,2	0,2	4,0	0,6
22	5	70	66	90,6	3,4	3,2	2,8
isolated product				95,5	-	3,7	0,8
24	5	65	92	91,7	3,1	3,3	1,9
isolated product				95,0	0,1	4,2	0,7
29	5	65	66	88,8	6,5	2,6	2,1
isolated product				95,6	0,1	2,3	2,0
32	3,5	70	22	91,4	5,4	1,5	1,7
isolated product				97,4	-	1,7	0,9
33	3,5	70	42	93,1	3,2	1,9	1,8
isolated product				97,8	-	1,8	0,4
34	2	70	28	85,0	4,9	1,5	6,6
isolated product				97,1	0,2	1,9	0,8

*Molar Ratio NaOH/Benzamide: 40

[0255] It was confirmed again that the use of more diluted NaOH solution (Exp. N° 32, 33, 34) resulted in a slightly more selective reaction. The reaction yield was between 82% and 89%. Even though the content of the MW 272 Da by-product in the carboxylic acid intermediate had been contained, its purification by flash chromatography on silica gel was studied in order to completely remove the by-product, which would make it easier to purify the final CT101.

[0256] The flash chromatography on silica gel was carried out using mixtures of DCM/AcOEt first and AcOEt/MeOH then. Acetic acid as additive during the purification was tested too. A partial decomposition of the carboxylic acid intermediate was observed both during the dissolution attempts of the crude product (before the purification) and while concentrating the eluted fraction containing the purified product from the flash chromatography. At the end, the HPLC purity of the purified sample was 94.9% (Table 7).

Table 7: HPLC analyses (@270nm) of purified carboxylic acid

Exp. N°	% area carboxylic acid	% area benzamide	% area MW 272 Da	% area MW 476 Da	% area others
37	Crude compound	77,9	1,7	3,2	17,2
	Purified compound	94,9	0,3	0,6	3,9

[0257] When the hydrolysis reaction was carried out using tBuOH as solvent, with 40 equivalents of NaOH, at 70°C, a more diluted NaOH solution was used to obtain a homogenous reaction mixture (1.4%). Neither the MW 272 Da by-product, nor the MW 258 Da by-product, specific respectively to iPrOH and EtOH, formed. No impurity related to the use of tBuOH was detected, as confirmed by HPLC-MS.

[0258] The carboxylic acid intermediate isolated using tBuOH as solvent showed no major impurity (Table 8). The reaction yield was 85-86%.

Table 8: HPLC results (@270nm) of benzamide hydrolysis using tBuOH as solvent

Exp. N°	NaOH* %	T, °C	Reaction time, h	% area carboxylic acid	% area benzamide	% area others
36	1,4	70	36	85,5	7,1	7,4
Isolated product				98,4	0,3	1,3
38	1,4	70	72	87,8	5,2	7,0
Isolated product				98,7	0,1	1,2
44	1,4	70	66	88,2	4,4	7,4
Isolated product				98,7	0,1	1,2

* Molar Ratio NaOH/Benzamide: 40

Step 2: Carboxylic acid intermediate amidation

[0259] The preliminary amidation reactions were carried out using the crude carboxylic acid intermediate isolated from the hydrolysis reactions in EtOH, containing the MW 258 Da byproduct.

[0260] The goals of these trials were: identification of amidation conditions and verification of the fate of the MW 258 Da by-product and of its possible derivative, as well as its effect on the CT101 purification.

[0261] The reactions were carried out in DCM as solvent at 25°C, using 1 equivalent of mPEG2000-NH₂, in the presence of TBTU/TEA or WSC as coupling agent.

[0262] The formation of CT101 was observed with both the coupling agents, but reactions with TBTU proceeded more selectively, so it was chosen for the next amidation reactions.

[0263] Another pegylated compound (RRT 0.976 by-product), likely derived from the MW 258 Da byproduct, formed, which was only partially resolved from the CT101 peak in HPLC. Crude CT101 was purified first by flash chromatography on silica gel, using mixtures of dichloromethane and methanol as eluent phases, then by reversed phase flash chromatography using water and acetonitrile as eluent phases.

[0264] The separation of CT101 from the pegylated impurity appeared quite challenging, as the purified CT101 was obtained with a low HPLC purity (72.1% @ 270 nm).

[0265] Amidation reactions carried out using the crude carboxylic acid intermediate isolated from the hydrolysis reactions in iPrOH, containing the MW 272 Da by-product, resulted in the formation of the CT101 as well as of another pegylated product (RRT 1.014 by-product), likely derived, as before, from the MW 272 Da by-product.

[0266] The reactions were carried out in DCM as solvent at 25°C, using 1.5 equivalents of TEA and 2.5 equivalents of TBTU as coupling agent, varying the excess of mPEG2000-NH₂ from 1.25 to 2 equivalents.

[0267] The use of 1.35 equivalents of mPEG₂₀₀₀-NH₂ appeared as the best compromise between the carboxylic acid conversion and the mPEG₂₀₀₀-NH₂ excess (Exp. N°27/Table 9).

[0268] The HPLC results shown in Table 9 refer to the isolated crude CT101. It should be pointed out that the HPLC analysis doesn't reveal possible residual amounts of mPEG₂₀₀₀-NH₂ in the crude CT101, which is checked through NMR analysis.

Table 9: HPLC results (@270 nm) of carboxylic acid amidation

Exp. N°	Starting carboxylic acid HPLC purity, %	Molar Ratio mPEG ₂₀₀₀ -NH ₂ /carboxylic acid	% area CT101	% area carboxylic acid intermediate	% area RRT 1.014 by-product	% area others
21	95.4	1+0.9	86.9	3.2	2.5	7.4
23	90.2	2	95.5	0.2	3.9	0.4
25	88.7	1.5	93.6	1.1	3.4	1.9
26	95.5	1.25	89.4	1.9	2.8	5.9
27	95.5	1.35	94.4	1.0	3.3	1.3
40	94.9*	1.35	91.6	0.6	0.7	7.1

*Starting carboxylic acid isolated from flash chromatography.

[0269] All the tested conditions generated crude CT101 samples with good HPLC purity, nevertheless the purification by flash chromatography on silica gel first and then by reversed phase flash chromatography didn't succeed in removing the RRT 1.014 by-product, so that the HPLC purities of the final CT101 samples ranged from 96% to 98% (when the purified carboxylic acid was used).

[0270] Amidation reactions carried out using the crude carboxylic acid intermediate isolated from the hydrolysis reactions in tBuOH resulted in crude CT101 samples with good HPLC purity (Exp. N°42, 47/ Table 10). No main impurity was detected.

[0271] The HPLC results shown in Table 9 above refer to the isolated crude CT101.

Table 10: HPLC results (@270 nm) of carboxylic acid amidation

Exp. N°	Starting carboxylic acid HPLC purity, %	% area CT101	% area carboxylic acid intermediate	% area others
42	98.6	98.3	0.4	1.3
47	98.7	98.1	0.2	1.7

[0272] These crude CT101 samples, being free from main other impurities, were only purified by reversed phase flash chromatography using water and acetonitrile as eluent phases, avoiding the purification by flash chromatography on silica gel.

[0273] The HPLC purities of the final CT101 samples were $\geq 99.6\%$.

Experimental procedure for CT101 synthesis

[0274] The selected synthetic protocol consisted, according to the previously described studies, of:

1. hydrolysis reaction in tBuOH, with 40 equivalents of 1.4% NaOH aqueous solution, at 70°C;
2. amidation reaction in DCM, with 1.35 equivalents of mPEG2000-NH₂, 2.5 equivalents of TBTU and 1.5 equivalents of TEA, at 25°C;
3. purification by reversed phase flash chromatography using water and acetonitrile as eluent phases.

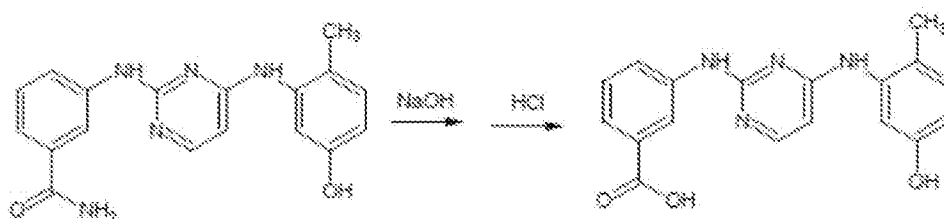
[0275] The developed procedure was verified on gram scale, and representative CT101 samples were synthesized (lot n° 2013RB20/S45, lot n° 2013RB20/S48, lot n° 2013RB20/S49).

Table 11: results of CT101 production

LOT N°	CT101 AMOUNT, g	HPLC PURITY, %	YIELD FROM BENZAMIDE, %
2013RB20/S45	1.50	99.5	41
2013RB20/S48	1.63	99.7	47
2013RB20/S49	0.36	99.7	

[0276] The experimental procedure used for the production of CT101 samples, lot n° 2013RB20/S48 and lot n° 2013RB20/S49, is hereinafter described.

Step 1: benzamide hydrolysis



Material List

Reagents	MW	g	mmol	d	ml	Ratio	
Benzamide	135.13	0.604	4.47			1	molar
1.4% NaOH	40.00	205.8	5.14			40	molar
tBuOH	74.12	62.7		0.775	81	134	ml/g benzamide
Theoretical product							
Carboxylic acid intermediate	316.34	0.606	1.90			1.0	molar

Procedure

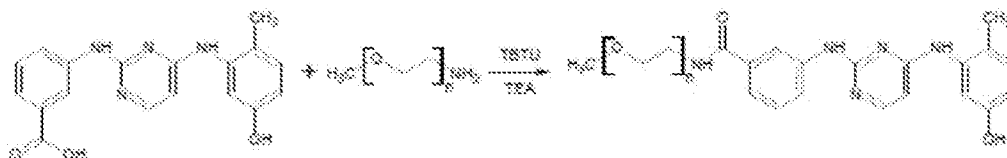
[0277] In a 250 ml four necked round bottom flask, equipped with magnetic stir bar, thermometer and condenser, 0.604 g of benzamide, 81 ml of tBuOH and 206 g of 1.4% NaOH aqueous solution were placed at 25°C. The mixture was heated under stirring to 70°C and aged for 66 h. The reaction progress was monitored by HPLC analyses taking samples during aging time (Figure 15).

[0278] The reaction mixture was cooled to 25°C, diluted with 130 ml of H₂O, and then 131 g of solvent were removed under reduced pressure at 45°C. The residual aqueous solution was extracted with 160 ml of AcOEt. The aqueous phase was diluted with 170 ml of AcOEt and neutralized under stirring with 15% HCl to pH 5.3. The organic phase was dried over sodium sulphate and the solvent was evaporated under reduced

pressure at 40°C. The residual solid was further dried by suspending it in 50 ml of DCM and evaporating the solvent under reduced pressure at 40°C. This drying operation was repeated twice.

[0279] 0.514 g of carboxylic acid intermediate as a yellow solid with 98.7% HPLC purity (@ 270 nm) were obtained (Figure 16). The reaction yield was 84.8%.

Step 2: Carboxylic acid intermediate amidation



Material List

Reagents	MW	g	mmol	d	ml	Ratio	
Carboxylic acid intermediate	386.34	0.51	1.51			1	
mPEG2000-NH2	2015	4.12	2.04			1.35	molar
TEA	101.19	0.38	3.78	0.73	0.52	2.5	molar
TBTU	321.08	0.73	2.27			1.5	molar
DCM	84.93	386		1.327	290	570	ml/g carboxylic acid
Theoretical product							
CT101	2333	3.53	1.51			1	molar

Procedure

[0280] In a 500 ml round bottom flask 0.51 g of carboxylic acid intermediate from the previous step, 4.12 g of mPEG2000-NH2 and 150 ml of DCM were placed at 25°C. The solvent was evaporated under reduced pressure at 30°C. This drying operation was repeated twice.

[0281] The dried solid residue was dissolved in 290 ml of DCM. The solution was placed in a 500 ml three necked round bottom flask, equipped with magnetic stir bar, thermometer and condenser and protected from light by aluminum foil wrapping. 0.38 g of TEA were added at 25°C. The solution was aged under stirring at 25°C for 10 min., then 0.73 g of TBTU were added. The reaction mixture was aged at 25°C for 21 h under nitrogen atmosphere.

[0282] The reaction progress was monitored by HPLC analyses, taking samples during aging time (Figure 17). The final reaction mixture was transferred to a 500 ml separating funnel, and washed with NaCl aqueous saturated solution (2 x 150 ml). The organic phase was dried over sodium sulphate and the solvent was evaporated under reduced pressure at 25°C. 5.3 g of crude CT101 as a yellow solid with 98.1% HPLC purity (@ 270 nm) were obtained (Figure 18).

[0283] Crude CT101 divided into three portions, was purified by reversed-phase flash chromatography using a Biotage Isolera LS System equipped with a Biotage *SNAP KP-C18-HS cartridge* packed with 120 g of KP-C18-HS Silica. The cartridge was equilibrated at 50 ml/min. with 264 ml of acetonitrile/water 24:76 v/v.

[0284] Sample loading was performed by dissolving the crude CT101 sample (1.7-1.8 g per purification) in 10 ml of H₂O and injecting it onto the cartridge through a syringe.

[0285] The *SNAP cartridge* was eluted at 50 ml/min with:

- 264 ml of acetonitrile/water 24:76 v/v;
- 264 ml from acetonitrile/water 24:76 v/v to acetonitrile/water 34:66 v/v;
- 515 ml of acetonitrile/water 34:66 v/v;
- 264 ml of acetonitrile/water 60:40 v/v.

[0286] The first portion of eluate (660 ml) was sent to the waste, then the eluted solvent was collected in fractions of 100 ml each.

[0287] UV profile (@ 270 and 210 nm) of the purification is depicted in Figure 19.

[0288] Collected individual fractions were analyzed by HPLC.

[0289] Fractions with HPLC purity ≥ 99.5 % were combined and concentrated under reduced pressure at 40°C to remove acetonitrile. The residual aqueous solution was extracted with DCM (3 $\times$ 150 ml). NaCl (a spoonful) was added during the first extraction to help phase separation. The combined organic phases were dried over sodium sulphate and evaporated under reduced pressure at 40°C to dryness, affording 2.0 g of a light yellow solid, which was added with 20 ml of diethyl ether and aged for 0.5 h at 5 °C. The precipitated solid was filtered over sintered glass filter (G4), washed with 10 ml of diethyl ether and dried under vacuum at 28°C for 2 h to afford 1.63 g of CT101 as an off white solid (lot n° 2013RB20/S48).

[0290] Purity of CT101, determined by HPLC analysis (method MI CT101 001), was 99.7% (Figure 20). The product was characterized by ¹H-NMR and ESI MS analysis (Figures 21 and 22). Certificate of analysis of the CT101 sample is shown in Figure 23.

[0291] Fractions with HPLC purity ≥ 98.0 % were combined and concentrated under reduced pressure at 40°C to remove acetonitrile. The residual aqueous solution was extracted with DCM (3 $\times$ 60 ml). NaCl (a spoonful) was added during the first extraction to help phase separation. The combined organic phases were dried over sodium sulphate and evaporated under reduced pressure at 40°C to dryness, affording

0.83 g of CT101 as a light yellow solid, which was purified again by reversed-phase flash chromatography, as described above.

[0292] Collected individual fractions were analyzed by HPLC. Fractions with HPLC purity $\geq 99.5\%$ were combined and concentrated under reduced pressure at 40°C to remove acetonitrile. The residual aqueous solution was extracted with DCM (3 x 100 ml). NaCl (a spoonful) was added during the first extraction to help phase separation. The combined organic phases were dried over sodium sulphate and evaporated under reduced pressure at 40°C to dryness, affording 0.45 g of a light yellow solid, which was added with 20 ml of diethyl ether and aged for 0.5 h at 5 °C. The precipitated solid was filtered over sintered glass filter (G4), washed with 10 ml of diethyl ether and dried under vacuum at 28°C for 2 h to afford 0.36 g of CT101 as an off white solid (lot n° 2013RB20/S49).

[0293] Purity of CT101, determined by HPLC analysis (method MI CT101 002), was 99.7% (Figure 24). The product was characterized by ¹H-NMR and ESI MS analysis (Figures 25 and 26).

Analytical Methods

HPLC method MI CT101 001

[0294] The following method has been used both for reaction monitoring and for assessing chemical purity of isolated products.

*Sample preparation*Reaction monitoring

20-50 µl of reaction mixture were evaporated under nitrogen flow and diluted with 0,5-1,0 ml of 10 mM HCOONH₄ pH 4/acetonitrile 1:1 v/v.

Isolated products

1.0 -1.5 mg/ml solutions in a mixture of 10 mM HCOONH₄ pH 4/acetonitrile 1:1 v/v were prepared.

Fractions from reversed phase flash chromatography

100-200 µl were diluted with 10 mM HCOONH₄ pH 4/acetonitrile 1:1 v/v.

Injected volume

5 µl

HPLC

Agilent 1100

Detector

UV 270 nm

Column

Zorbax Eclipse XDB-C18 Solvent Saver - 3 × 150 mm - 5 µm

Temperature

25°C

Flow

0.5 ml/min

Mobile phases

A: 10 mM HCOONH₄ pH 4

B: acetonitrile

Gradient

Time, min.	%A	B %
0	95	5
3	95	5
11	70	30
26	20	80
28	95	5

Benzamide

Retention time 16.6 min. RRT 0.848

Carboxylic acid intermediate

Retention time 18.5 min. RRT 0.934

CT101

Retention time 19.8 min. RRT 1

HPLC method MI CT101 002

[0295] The following method has been used for assessing chemical purity of final CT101 samples.

<i>Sample preparation</i>	1.0-1.5 mg/ml solutions in a mixture of 10 mM HCOONH ₄ pH 4/acetonitrile 1:1 v/v were prepared.
<i>Injected volume</i>	5 μ l
<i>HPLC</i>	Agilent 1100
<i>Detector</i>	UV 270 nm
<i>Column</i>	Zorbax Eclipse XDB-C18 Solvent Saver - 3 x 150 mm - 5 μ m
<i>Temperature</i>	25°C
<i>Flow</i>	0.5 ml/min
<i>Mobile phases</i>	A: 10 mM HCOONH ₄ pH 4 B: acetonitrile

<i>Gradient</i>	Time, min.	%A	B %
	0	90	10
	3	90	10
	23	20	80
	30	20	80
	32	90	10
<i>CT101</i>	Retention time	15.6 min.	

[0296] A certificate of analysis of the CT101 sample from the second synthesis scheme is shown in Figure 27.

Example 3: Profiling study of CT101 and CAS944795-066 against 271 kinases

[0297] CT101 and CAS944795-06-6 (test concentration: 0.2 μ M) were tested against 271 target kinases.

Materials and Methods

Preparation of test compound solution

[0298] The test compound was dissolved in and diluted with dimethylsulfoxide (DMSO) to achieve 100-fold higher concentration. Then the solution was further 25-fold diluted with assay buffer to make the final test compound solution. Reference compounds for assay control were prepared similarly.

Assay reagents and procedures**IMAP Assay**

[0299] 1) The 5 μ L of x4 compound solution, 5 μ L of x4 Substrate/ATP/Metal solution, and 10 μ L of x2 kinase solution were prepared with assay buffer (20 mM HEPES, 0.01% Tween-20, 2 mM DTT, pH7.4) and mixed and incubated in a well of polystyrene 384 well black microplate for 1 hour at room temperature.

[0300] 2) 60 μ L of IMAP binding reagent (IMAP Screening Express kit; Molecular Devices) was added to the well, and incubated for 30 minutes.

[0301] 3) The kinase reaction was evaluated by the fluorescence polarization at 485 nm for excitation and 530 nm for emission of the well.

Off-chip Mobility Shift Assay (MSA)

[0302] 1) The 5 μ L of x4 compound solution. 5 μ L of x4 Substrate/ATP/Metal solution, and 10 μ L of x2 kinase solution were prepared with assay buffer (20 mM HEPES, 0.01% Triton X-100, 2 mM DTT, pH7.5) and mixed and incubated in a well of polypropylene 384 well microplate for 1 or 5 hour(s)* at room temperature. (*; depend on kinase)

[0303] 2) 60 μ L of Termination Buffer (QuickScout Screening Assist MSA; Carna Biosciences) was added to the well.

[0304] 3) The reaction mixture was applied to LabChip3000 system (Caliper Life Science), and the product and substrate peptide peaks were separated and quantitated.

[0305] 4) The kinase reaction was evaluated by the product ratio calculated from peak heights of product(P) and substrate(S) peptides ($P/(P+S)$).

Reaction Conditions

[0306] The reaction conditions are depicted below:

Kinase	Platform	Substrate		ATP (μ M)		Metal		Positive control
		Name	(μ M)	K _m	Assay	Name	(μ M)	
ABL	MSA	ABLtide	1000	16	25	Mg	5	Staurosporine
ALK ¹²	MSA	WASP peptide	1000	97	100	Mg	5	Staurosporine
ALK	MSA	Srcide	1000	57	50	Mg	5	Staurosporine
ARG	MSA	ABLtide	1000	24	25	Mg	5	Staurosporine
ASL	MSA	CNKide	1000	32	50	Mg	5	Staurosporine
BLK	MSA	Srcide	1000	62	75	Mg	5	Staurosporine
BMX	MSA	Srcide	1000	75	75	Mg	5	Staurosporine
BRK ¹²	MSA	Btk/Lytide	1000	250	250	Mg	5	Staurosporine
BTK	MSA	Srcide	1000	72	75	Mg	5	Staurosporine
CEK ¹²	MSA	Srcide	1000	4.8	5	Mg+Mn	5+1	Staurosporine
DDR1 ¹²	MSA	IRS1	1000	94	100	Mg	5	Staurosporine
DDR2 ¹²	MSA	IRS1	1000	38	50	Mg	5	Staurosporine
EGFR	MSA	Srcide	1000	2.7	5	Mg+Mn	5+1	Staurosporine
EPHA1	MSA	Btk/Lytide	1000	22	25	Mg	5	Staurosporine
EPHA2	MSA	Btk/Lytide	1000	67	75	Mg	5	Staurosporine
EPHA3	MSA	Btk/Lytide	1000	170	150	Mg	5	Staurosporine
EPHA4	MSA	Btk/Lytide	1000	52	50	Mg	5	Staurosporine
EPHA5	MSA	Btk/Lytide	1000	56	50	Mg	5	Staurosporine
EPHA6	MSA	Btk/Lytide	1000	27	25	Mg	5	Staurosporine
EPHA7	MSA	Btk/Lytide	1000	58	50	Mg	5	Staurosporine
EPHA8	MSA	Btk/Lytide	1000	69	75	Mg	5	Staurosporine
EPHB1	MSA	Btk/Lytide	1000	29	25	Mg	5	Staurosporine
EPHB2	MSA	Btk/Lytide	1000	86	100	Mg	5	Staurosporine
EPHB3	MSA	Btk/Lytide	1000	49	50	Mg	5	Staurosporine
EPHB4	MSA	Btk/Lytide	1000	56	50	Mg	5	Staurosporine
FAK ¹²	MSA	Btk/Lytide	1000	25	25	Mg	5	Staurosporine
FER	MSA	Srcide	1000	26	25	Mg	5	Staurosporine
FES	MSA	Srcide	1000	43	50	Mg	5	Staurosporine
FCFR1	MSA	CNKide	1000	89	100	Mg	5	Staurosporine
FCFR2	MSA	CNKide	1000	66	75	Mg	5	Staurosporine
FCFR3	MSA	CNKide	1000	43	50	Mg	5	Staurosporine
FCFR4	MSA	CNKide	1000	230	250	Mg	5	Staurosporine
FCR	MSA	Srcide	1000	34	50	Mg	5	Staurosporine
FLT1	MSA	CNKide	1000	140	150	Mg	5	Staurosporine
FLT3	MSA	Srcide	1000	94	100	Mg	5	Staurosporine
FLT3	MSA	CNKide	1000	72	75	Mg	5	Staurosporine
FMS	MSA	Srcide	1000	26	25	Mg	5	Staurosporine
FRK	MSA	Srcide	1000	62	75	Mg	5	Staurosporine
PYK	MSA	Srcide	1000	50	50	Mg	5	Staurosporine
HCK	MSA	Srcide	1000	11	10	Mg	5	Staurosporine
HER2	MSA	Srcide	1000	9.4	10	Mn	5	Staurosporine
HER4	MSA	Srcide	1000	27	25	Mg	5	Staurosporine
ICF1R	MSA	IRS1	1000	63	75	Mg	5	Staurosporine
INSR	MSA	IRS1	1000	58	50	Mg	5	Staurosporine
IKK	MSA	IRS1	1000	64	75	Mg	5	Staurosporine
ITK	MSA	Srcide	1000	6.1	10	Mg	5	Staurosporine

JAK1 ¹⁰⁰	MSA	JAK1 substrate peptide	1000	68	75	Mg	5	Staurosporine
JAK2	MSA	Src tide	1000	13	10	Mg	5	Staurosporine
JAK3	MSA	Src tide	1000	3.3	5	Mg	5	Staurosporine
KDR	MSA	CSK tide	1000	74	73	Mg	5	Staurosporine
KIT ¹⁰¹	MSA	Src tide	1000	370	400	Mg	5	Staurosporine
LCK	MSA	Src tide	1000	14	10	Mg	5	Staurosporine
LYK	MSA	Src tide	1000	49	50	Mg	5	Staurosporine
LYNa	MSA	Src tide	1000	14	10	Mg	5	Staurosporine
LYNb	MSA	Src tide	1000	18	25	Mg	5	Staurosporine
MEK	MSA	CSK tide	1000	36	50	Mg	5	Staurosporine
MET	MSA	Src tide	1000	27	25	Mg	5	Staurosporine
MUSEK ¹⁰²	MSA	CSK tide	1000	14	10	Mg+Mn	5+1	Staurosporine
PDGFPRa	MSA	CSK tide	1000	28	25	Mg	5	Staurosporine
PDGFPRb	MSA	CSK tide	1000	23	25	Mg	5	Staurosporine
PYK2	MSA	Btk/Lyn tide	1000	56	50	Mg	5	Staurosporine
RET	MSA	CSK tide	1000	7.5	10	Mg	5	Staurosporine
RON	MSA	Src tide	1000	27	25	Mg	5	Staurosporine
ROS	MSA	IRS1	1000	37	50	Mg	5	Staurosporine
SRC	MSA	Src tide	1000	31	50	Mg	5	Staurosporine
SRM	MSA	Btk/Lyn tide	1000	38	50	Mg	5	Staurosporine
SYK	MSA	Btk/Lyn tide	1000	26	25	Mg	5	Staurosporine
TEC	MSA	Src tide	1000	55	50	Mg	5	Staurosporine
THE2	MSA	Btk/Lyn tide	1000	94	100	Mg	5	Staurosporine
TNK1 ¹⁰³	MSA	CSK tide	1000	71	75	Mg	5	Staurosporine
TRKA	MSA	CSK tide	1000	65	75	Mg	5	Staurosporine
TRKB	MSA	Src tide	1000	80	75	Mg	5	Staurosporine
TRKC	MSA	Src tide	1000	47	50	Mg	5	Staurosporine
TYK ¹⁰⁴	MSA	Src tide	1000	110	100	Mg	5	Staurosporine
TYK2 ¹⁰⁵	MSA	Src tide	1000	18	25	Mg	5	Staurosporine
TYRO3	MSA	CSK tide	1000	80	75	Mg	5	Staurosporine
YES	MSA	Src tide	1000	13	10	Mg	5	Staurosporine
ZAP70 ¹⁰⁶	MSA	Btk/Lyn tide	1000	1.7	1	Mg+Mn	5+1	Staurosporine
AKT1	MSA	Cross tide	1000	31	50	Mg	5	Staurosporine
AKT2	MSA	Cross tide	1000	110	100	Mg	5	Staurosporine
AKT3	MSA	Cross tide	1000	54	50	Mg	5	Staurosporine
AMPKα1/β1/γ1	MSA	SAMS peptide	1000	130	150	Mg	5	Staurosporine
AMPKα2/β1/γ1	MSA	SAMS peptide	1000	100	100	Mg	5	Staurosporine
AurA	MSA	Kemptide	1000	27	25	Mg	5	Staurosporine
AurA/TPX2 ¹⁰⁷	MSA	Kemptide	1000	1.7	2	Mg	5	Staurosporine
AurB/INCENP	MSA	Kemptide	1000	16	25	Mg	5	Staurosporine
AurC	MSA	Kemptide	1000	24	25	Mg	5	Staurosporine
BRAP_Cascade	MSA	MAP2K1	1	-	1000	Mg	5	ZM336372
		Erk2	2.5					
		Modified Ektide	1000					
BRSK1	MSA	CHRE tide	1000	30	25	Mg	5	Staurosporine
BRSK2	MSA	CHRE tide	1000	31	50	Mg	5	Staurosporine
CaMK1α ¹⁰⁸	MSA	GS peptide	1000	750	1000	Mg	5	Staurosporine
CaMK1β ¹⁰⁹	MSA	Synapsin peptide	1000	11	10	Mg	5	Staurosporine
CaMK2α ¹¹⁰	MSA	GS peptide	1000	33	50	Mg	5	Staurosporine
CaMK2β ¹¹¹	MSA	GS peptide	1000	19	25	Mg	5	Staurosporine
CaMK2γ ¹¹²	MSA	GS peptide	1000	23	25	Mg	5	Staurosporine

Cdk5 ¹²	MSA	GS peptide	1000	6.3	5	Mg	5	Staurosporine
Cdk5 ¹³	MSA	GS peptide	1000	20	25	Mg	5	Staurosporine
CDK2/CycB1	MSA	Modified Histone H1	1000	34	50	Mg	5	Staurosporine
CDK7/ASK ¹⁴	MSA	MCM2 peptide	1000	2.8	5	Mg	10	Staurosporine
CDK2/CycA2	MSA	Modified Histone H1	1000	27	25	Mg	5	Staurosporine
CDK2/CycE1	MSA	Modified Histone H1	1000	130	150	Mg	5	Staurosporine
CDK3/CycB1	MSA	Modified Histone H1	1000	1000	1000	Mg	5	Staurosporine
CDK4/CycDA ¹⁵	MSA	DYRKtide-F	1000	300	200	Mg	5	Staurosporine
CDK5/p25	MSA	Modified Histone H1	1000	10	10	Mg	5	Staurosporine
CDK6/CycD3 ¹⁶	MSA	DYRKtide-F	1000	330	300	Mg	5	Staurosporine
CDK7/Cycl1MAT1 ¹⁷	MSA	CTD1 peptide	1000	52	50	Mg	5	Staurosporine
CDK9/CycT1 ¹⁸	MSA	CDK9 substrate	1000	9.4	10	Mg	5	Staurosporine
CGK2 ¹⁹	MSA	Kegntide	1000	24	25	Mg	5	Staurosporine
CHK1	MSA	CHKtide	1000	50	50	Mg	5	Staurosporine
CHK2	MSA	CHKtide	1000	51	50	Mg	5	Staurosporine
CK1a ²⁰	MSA	CKtide	1000	4.1	5	Mg	5	5-Iodotubercidin
CK1y1	MSA	CKtide	1000	6.3	5	Mg	5	5-Iodotubercidin
CK1y2	MSA	CKtide	1000	10	10	Mg	5	5-Iodotubercidin
CK1y3	MSA	CKtide	1000	3.2	5	Mg	5	5-Iodotubercidin
CK1δ	MSA	CKtide	1000	7.7	10	Mg	5	5-Iodotubercidin
CK1ε ²¹	MSA	CKtide	1000	16	25	Mg	5	5-Iodotubercidin
CK2α1β	MSA	CK2tide	1000	2.9	5	Mg	5	TBB
CK2α2β	MSA	CK2tide	1000	2.1	5	Mg	5	TBB
CLK1	MSA	DYRKtide-F	1000	11	10	Mg	5	Staurosporine
CLK2	MSA	DYRKtide-F	1000	140	150	Mg	5	Staurosporine
CLK3	MSA	DYRKtide-F	1000	75	75	Mg	5	Staurosporine
COY_Cascade	MSA	MAP2K1	1	-	1000	Mg	5	Staurosporine
		Erk2	2.5					
		Modified Erktide	1000					
CRK ²²	MSA	Histone H3 peptide	1000	7.8	10	Mg	5	Staurosporine
DAPK1	MSA	DAPK1tide	1000	1.1	1	Mg	5	Staurosporine
DCAMKL2 ²³	MSA	GS peptide	1000	120	150	Mg	5	Staurosporine
DLK_Cascade ²⁴	MSA	MAP2K4 / MAP2K7	0.5 / 0.5	-	1000	Mg	5	Staurosporine
		JNK2	50					
		Modified Erktide	1000					
DYRK1A	MSA	DYRKtide-F	1000	16	25	Mg	5	Staurosporine
DYRK1B	MSA	DYRKtide-F	1000	59	50	Mg	5	Staurosporine
DYRK2	MSA	DYRKtide-F	1000	7.7	10	Mg	5	Staurosporine
DYRK3	MSA	DYRKtide-F	1000	6.8	5	Mg	5	Staurosporine
EEF2K ²⁵	MSA	EEF2Ktide	1000	12	10	Mg	5	PH125
Erk3	MSA	Modified Erktide	1000	34	50	Mg	5	5-Iodotubercidin
Erk2	MSA	Modified Erktide	1000	33	50	Mg	5	5-Iodotubercidin
Erk5 ²⁶	MSA	EGFR-derived peptide	1000	450	1000	Mg	5	Staurosporine
GSK3α	MSA	CREBtide-p	1000	12	10	Mg	5	Staurosporine
GSK3β	MSA	CREBtide-p	1000	9.1	10	Mg	5	Staurosporine
Hugin	MSA	Histone H3 peptide	1000	140	150	Mg	5	Staurosporine

HGK	MSA	Moesin-derived peptide	1000	9.4	10	Mg	5	Staurosporine
HHPK1	MSA	DYRKtide-F	1000	4.4	5	Mg	5	Staurosporine
HHPK2	MSA	DYRKtide-F	1000	5.9	5	Mg	5	Staurosporine
HHPK3	MSA	DYRKtide-F	1000	7.3	5	Mg	5	Staurosporine
HHPK4	MSA	DYRKtide-F	1000	7	5	Mg	5	Staurosporine
IKK α	IMAP	I κ B α peptide	100	41	40	Mg	10	Staurosporine
IKK β	MSA	I κ B α -derived peptide	1000	16	25	Mg	5	Staurosporine
IKK γ ¹¹	MSA	I κ B α peptide	1000	9.5	10	Mg	5	Staurosporine
IRAK1	IMAP	SRPKtide	100	27	25	Mg	2.5	Staurosporine
IRAK4 ¹²	MSA	IRAK1 peptide	1000	917	1000	Mg	5	Staurosporine
JNK1	MSA	Modified Erktide	1000	29	100	Mg	5	JNK Inhibitor II
JNK2	MSA	Modified Erktide	1000	21	50	Mg	5	JNK Inhibitor II
JNK3	MSA	Modified Erktide	1000	6.0	25	Mg	5	JNK Inhibitor II
LATS2 ¹³	MSA	SGKtide	1000	380	400	Mg	5	Staurosporine
LOK ¹⁴	MSA	Moesin-derived peptide	1000	100	100	Mg	5	Staurosporine
MAP2K1_Cascade	MSA	Erk2	2.5	-	1000	Mg	5	Staurosporine
		Modified Erktide	1000					
MAP2K2_Cascade	MSA	Erk2	2.5	-	1000	Mg	5	Staurosporine
		Modified Erktide	1000					
MAP2K3_Cascade	MSA	p38 α (9-352)	10	-	1000	Mg	5	Staurosporine
		Modified Erktide	1000					
MAP2K4_Cascade ¹⁵	MSA	JNK2	50	-	1000	Mg	5	Staurosporine
		Modified Erktide	1000					
MAP2K5_Cascade ¹⁶	MSA	Erk5	50	-	1000	Mg	5	Staurosporine
		EEF-2-derived peptide	1000					
MAP2K6_Cascade	MSA	p38 α (9-352)	10	-	1000	Mg	5	Staurosporine
		Modified Erktide	1000					
MAP2K7_Cascade ¹⁷	MSA	JNK2	50	-	1000	Mg	5	Staurosporine
		Modified Erktide	1000					
MAP3K1_Cascade	MSA	MAP2K1	1	-	1000	Mg	5	Staurosporine
		Erk2	2.5					
		Modified Erktide	1000					
MAP3K2_Cascade ¹⁸	MSA	MAP2K4 / MAP2K7	0.5 / 0.5	-	1000	Mg	5	Staurosporine
		JNK2	50					
		Modified Erktide	1000					
MAP3K3_Cascade	MSA	MAP2K6	1	-	1000	Mg	5	Staurosporine
		p38 α (9-352)	10					
		Modified Erktide	1000					
MAP3K4_Cascade	MSA	MAP2K6	1	-	1000	Mg	5	Staurosporine
		p38 α (9-352)	10					
		Modified Erktide	1000					
MAP3K5_Cascade	MSA	MAP2K6	1	-	1000	Mg	5	Staurosporine
		p38 α (9-352)	10					
		Modified Erktide	1000					
MAP4K2	MSA	SGK2 peptide	1000	93	100	Mg	5	Staurosporine
MAPKAPK2	MSA	G5 peptide	1000	3.6	5	Mg	5	Staurosporine
MAPKAPK3	MSA	G8 peptide	1000	15	10	Mg	5	K252b

MAPKAPK5	MSA	GS peptide	1000	12	10	Mg	5	Staurosporine
MARK1	MSA	CHKtide	1000	8	10	Mg	5	Staurosporine
MARK2	MSA	CHKtide	1000	8.8	10	Mg	5	Staurosporine
MARK3	MSA	CHKtide	1000	5	5	Mg	5	Staurosporine
MARK4	MSA	CHKtide	1000	12	10	Mg	5	Staurosporine
MELK ¹⁰	MSA	GS peptide	1000	32	50	Mg	5	Staurosporine
MGC42105	MSA	CHKtide	1000	21	25	Mg	5	Staurosporine
MPK ¹¹	MSA	Modified Erktide	1000	36	50	Mg	5	K252b
MLK1_Cascade	MSA	MAP2K1	1	-	1000	Mg	5	Staurosporine
		Erk2	2.5					
		Modified Erktide	1000					
MLK2_Cascade	MSA	MAP2K1	1	-	1000	Mg	5	Staurosporine
		Erk2	2.5					
		Modified Erktide	1000					
MLK3_Cascade	MSA	MAP2K1	1	-	1000	Mg	5	Staurosporine
		Erk2	2.5					
		Modified Erktide	1000					
MNK1	MSA	RS peptide	1000	460	450	Mg	5	Staurosporine
MNK2	MSA	RS peptide	1000	110	100	Mg	5	Staurosporine
MOR_Cascade	MSA	MAP2K1	1	-	1000	Mg	5	Staurosporine
		Erk2	2.5					
		Modified Erktide	1000					
MRCR ¹²	MSA	DAFKtide	1000	0.45	1	Mg	5	Staurosporine
MRCR ⁹	MSA	DAFKtide	1000	0.67	1	Mg	5	Staurosporine
MSK1	MSA	Crosside	1000	13	10	Mg	5	Staurosporine
MSK2 ¹³	MSA	Crosside	1000	40	50	Mg	5	Staurosporine
MSKK1 ¹⁴	MSA	DYRKtide-F	1000	56	50	Mg	5	Staurosporine
MST1 ¹⁰⁰⁰	MSA	IRS1	1000	50	50	Mg	5	K252b
MST2 ¹⁰⁰¹	MSA	IRS1	1000	60	75	Mg	5	Staurosporine
MST3 ¹⁵	MSA	Moesin-derived peptide	1000	66	75	Mg	5	Staurosporine
MST4 ¹⁶	MSA	Moesin-derived peptide	1000	76	75	Mg	5	Staurosporine
NDK1 ¹⁷	MSA	SGKtide	1000	12	10	Mg	5	Staurosporine
NDK2 ¹⁸	MSA	SGKtide	1000	7.6	10	Mg	5	Staurosporine
NEK1 ¹⁹	MSA	CDK7 peptide	1000	64	75	Mg	5	Staurosporine
NEK2	MSA	CDK7 peptide	1000	65	75	Mg	5	Staurosporine
NEK4	MSA	GS peptide	1000	51	50	Mg	5	Staurosporine
NEK6 ²⁰	MSA	CDK7 peptide	1000	60	75	Mg	5	PKR Inhibitor
NEK7 ²¹	MSA	CDK7 peptide	1000	40	50	Mg	5	PKR Inhibitor
NEK9 ²²	MSA	CDK7 peptide	1000	190	200	Mg	5	Staurosporine
NuaK1	MSA	CHKtide	1000	59	50	Mg	5	Staurosporine
NuaK2	MSA	CHKtide	1000	26	25	Mg	5	Staurosporine
p38 α	MSA	Modified Erktide	1000	150	150	Mg	5	SB202190
p38 β	MSA	Modified Erktide	1000	63	75	Mg	5	SB202190
p38 γ	MSA	Modified Erktide	1000	13	10	Mg	5	Staurosporine
p38 δ	MSA	Modified Erktide	1000	5.8	5	Mg	5	Staurosporine
p70S6K	MSA	S6K2 peptide	1000	14	10	Mg	5	Staurosporine
p70S6K β	MSA	S6K2 peptide	1000	3.3	5	Mg	5	Staurosporine
PAK1	MSA	LMKtide	1000	300	300	Mg	5	Staurosporine
PAK2	MSA	DAFKtide	1000	81	100	Mg	5	Staurosporine
PAK4 ²³	MSA	SGKtide	1000	2.5	5	Mg	5	Staurosporine

PAK5	MSA	DAPK1tide	1000	1.9	1	Mg	5	Staurosporine
PAK6 ⁽¹⁾	MSA	SGE1tide	1000	3.7	5	Mg	5	Staurosporine
PASK ⁽¹⁾	MSA	G5 peptide	1000	9.7	10	Mg	5	Staurosporine
PRK ⁽¹⁾	MSA	Histone H3 peptide	1000	33	50	Mg	5	Staurosporine
PDHK2 ⁽¹⁾	MSA	PDHKtide	1000	28	25	Mg+K	5+3	DCA
PDHK4 ⁽¹⁾	MSA	PDHKtide	1000	19	25	Mg+K	5+25	DCA
PDHK1 ⁽¹⁰⁾	MSA	T308tide	1000	9.6	10	Mg	5	Staurosporine
PEK	IMAP	SRPKtide	100	13	10	Mg	5	Staurosporine
PGK ⁽¹⁰⁾	MSA	Kemptide	1000	8.2	10	Mg	5	Staurosporine
PHKG1 ⁽¹⁾	MSA	G5 peptide	1000	71	75	Mg	5	Staurosporine
PHKG2	MSA	G5 peptide	1000	8.1	10	Mg	5	Staurosporine
PIM1	MSA	S6k2 peptide	1000	640	500	Mg	5	Staurosporine
PIM2 ⁽¹⁾	MSA	S6k2 peptide	1000	4	5	Mg	5	Staurosporine
PIM3	MSA	S6k2 peptide	1000	130	150	Mg	5	Staurosporine
PRACu	MSA	Kemptide	1000	2.6	5	Mg	5	Staurosporine
PRACb	MSA	Kemptide	1000	4.7	5	Mg	5	Staurosporine
PKAc ⁽¹⁾	MSA	Kemptide	1000	4.5	5	Mg	5	Staurosporine
PKC α ⁽⁵⁾	MSA	PKC peptide	1000	36	50	Mg+Ca	5+0.05	Staurosporine
PKC β 1 ⁽¹⁾	MSA	PKC peptide	1000	79	75	Mg+Ca	5+0.05	Staurosporine
PKC β 2 ⁽¹⁾	MSA	PKC peptide	1000	41	50	Mg+Ca	5+0.05	Staurosporine
PKC γ ⁽⁵⁾	MSA	PKC peptide	1000	74	75	Mg+Ca	5+0.05	Staurosporine
PKC δ ⁽⁵⁾	MSA	PKC peptide	1000	26	25	Mg	5	Staurosporine
PKC ϵ ⁽⁵⁾	MSA	PKC peptide	1000	16	25	Mg	5	Staurosporine
PKC ζ ⁽⁵⁾	MSA	PKC peptide	1000	11	10	Mg	5	Staurosporine
PKC η ⁽⁵⁾	MSA	PKC peptide	1000	36	50	Mg	5	Staurosporine
PKC θ ⁽⁵⁾	MSA	PKC peptide	1000	25	25	Mg	5	Staurosporine
PKC ι	MSA	PKC peptide	1000	24	25	Mg	5	Staurosporine
PKD1	MSA	G5 peptide	1000	25	25	Mg	5	Staurosporine
PKD2	MSA	G5 peptide	1000	26	25	Mg	5	Staurosporine
PKD3	MSA	G5 peptide	1000	34	50	Mg	5	Staurosporine
PKN1	IMAP	S6K peptide	100	19	25	Mg	1	Staurosporine
PKB	IMAP	SRPKtide	100	13	10	Mg	5	Staurosporine
PLK1 ⁽¹⁾	MSA	CDC25tide	1000	5.6	5	Mg	5	Staurosporine
PLK2	IMAP	CHK2 peptide	50	30	30	Mg	10	K252b
PLK3	MSA	CDC25tide	1000	6.8	5	Mg	5	K252b
PRKX ⁽¹⁾	MSA	Kemptide	1000	20	25	Mg	5	Staurosporine
QIK	MSA	AMARA peptide	1000	42	50	Mg	5	Staurosporine
RAF1_Cascade	MSA	MAP2K1	1	-	1000	Mg	5	ZM336372
		Erk2	2.5					
		Modified Erk1tide	1000					
RCKE1	MSA	LINKtide	1000	3.1	5	Mg	5	Staurosporine
RCKE2	MSA	LINKtide	1000	7.4	5	Mg	5	Staurosporine
RSK1	MSA	S6K peptide (N-FL)	1000	21	25	Mg	5	Staurosporine
RSK2	MSA	S6K peptide (N-FL)	1000	14	10	Mg	5	Staurosporine
RSK3	MSA	S6K peptide (N-FL)	1000	9.9	10	Mg	5	Staurosporine
RSK4	MSA	S6K peptide (N-FL)	1000	20	25	Mg	5	Staurosporine
SGK	MSA	SGRtide	1000	52	50	Mg	5	Staurosporine
SGK2	MSA	SGRtide	1000	58	50	Mg	5	Staurosporine
SGK3	MSA	SGRtide	1000	17	25	Mg	5	Staurosporine

SLK ¹²	MSA	AMARA peptide	1000	47	50	Mg	5	Staurosporine
MLCK ¹³	MSA	MLCKide	1000	820	1000	Mg	5	Staurosporine
SLK ¹²	MSA	Moesin-derived peptide	1000	36	50	Mg	5	Staurosporine
SRPK1	MSA	SRPKide	100	200	100	Mg	10	Staurosporine
SRPK2 ¹⁴	MSA	DYRK6ide-F	1000	14	10	Mg	5	Staurosporine
TAK1-TAB1_Cascade ¹⁵	MSA	MAP2K4 / MAP2K7	0.5 / 0.5	~	1000	Mg	5	Staurosporine
		INK2	50					
		Modified Erkide	1000					
TAKK2 ^{16a}	MSA	TAKKide	1000	39	50	Mg	5	Staurosporine
TPK1	MSA	CKide	1000	21	25	Mg	5	Staurosporine
TNIK	MSA	Moesin-derived peptide	1000	16	25	Mg	5	Staurosporine
TSSK1	MSA	GR peptide	1000	11	10	Mg	5	Staurosporine
TSSK2 ¹⁷	MSA	GR peptide	1000	8.8	10	Mg	5	Staurosporine
TSSK3 ¹⁸	MSA	GR peptide	1000	45	50	Mg	5	Staurosporine
WNK1 ¹²	MSA	SPAKide	1000	140	150	Mg+Mn	5+3	Staurosporine
WNK2 ¹²	MSA	SPAKide	1000	48	50	Mg+Mn	5+3	Staurosporine
WNK3 ¹²	MSA	SPAKide	1000	48	50	Mg+Mn	5+3	Staurosporine

1) Reaction time is 5 hours.

2) CaCl₂, Calmodulin are added at the final concentration of 1 mM and 10 µg/ml, respectively.

4) cGMP is added at the final concentration of 5 µM.

5) Phosphatidylserine and Diacyl Glycerol are added at the final concentration of 50 µg/ml, and 5 µg/ml, respectively.

6) Sodium orthovanadate is added at the final concentration of 25 µM.

7) Cantharidin is added at the final concentration of 10 µM.

8) PIPide and Cantharidin are added at the final concentration of 2 µM and 20 µM, respectively.

10) TPX2 peptide is added at the final concentration of 200 nM.

11) Cantharidin is added at the final concentration of 20 µM.

Data Analysis

[0307] The readout value of reaction control (complete reaction mixture) was set as a 0% inhibition, and the readout value of background (Enzyme(-)) was set as a 100% inhibition, then the percent inhibition of each test solution was calculated.

Results

[0308] The results are shown in Table 12 below.

Gene	Log Fold Change	
	Control	Control + Dox
ABL	-3.5	66.7
ACK	2.6	34.8
ALK	-1.4	-2.9
ARG	4.0	77.8
AXL	-1.7	-1.3
BLK	-7.9	6.3
BMX	2.4	51.9
BRK	-2.4	61.7
BTK	12.1	24.6
CSK	-0.4	6.2
DDR1	4.9	54.7
DDR2	-0.5	35.3
EGFR	1.3	9.6
EPHA1	1.8	41.5
EPHA2	-3.5	13.5
EPHA3	-8.7	14.0
EPHA4	-1.8	13.8
EPHA5	-0.4	27.2
EPHA6	-11.2	34.8
EPHA7	-2.0	-4.4
EPHA8	-6.7	24.3
EPHB1	-7.3	8.8
EPHB2	-4.3	6.0
EPHB3	0.2	1.1
EPHB4	1.4	17.5
FAK	-5.3	-0.6
FER	0.3	6.1
FES	0.2	1.6
FGFR1	-1.5	14.3
FGFR2	-8.0	17.2
FGFR3	-1.0	10.9
FGFR4	-1.8	8.7
FGR	4.3	71.5
FLT1	3.3	94.2
FLT3	-3.7	19.2
FLT4	1.0	61.9
FMS	-1.0	18.6
FRK	15.6	93.0
FYN	17.2	86.2
HCK	0.1	47.4
HER2	6.4	2.0
HER4	1.4	23.5
IGF1R	-0.2	-0.5
INSR	4.2	3.6
IRR	0.2	-1.2
ITK	-1.0	-1.1
JAK1	-1.2	0.7

JAK2	3,1	18,9
JAK3	1,7	11,1
KDR	5,0	89,7
KIT	-0,4	34,2
LCK	3,6	33,3
LTK	-2,7	0,4
LYNa	6,4	63,2
LYNb	2,9	59,0
MER	-2,3	-2,3
MET	-3,6	-3,4
MUSK	-2,9	0,8
PDGFR α	-0,8	24,1
PDGFR β	2,1	54,2
PYK2	-2,8	-3,1
RET	-0,2	69,5
RON	-2,8	-3,2
ROS	-1,8	12,3
SRC	41,7	80,8
SRM	-10,1	7,0
SYK	-1,3	17,3
TEC	-1,8	36,5
TIE2	-8,6	-6,6
TNK1	1,1	11,6
TRKA	0,9	9,2
TRKB	-9,0	2,7
TRKC	-1,7	7,7
TXK	-1,9	25,9
TYK2	-1,8	-1,4
TYRO3	-2,3	7,9
YES	11,3	66,6
ZAP70	-3,6	-1,4
AKT1	-6,3	-7,6
AKT2	-8,7	-8,2
AKT3	-6,0	-6,6
AMPK α 1/ β 1/ γ 1	-4,0	-4,0
AMPK α 2/ β 1/ γ 1	-3,0	-4,9
AurA	-10,1	30,2
AurA/TPX2	-2,9	29,4
AurB	-4,3	9,7
AurC	-0,2	23,3
BRAF_Cascade	-15,0	-20,1
BRSK1	-1,9	-5,2
BRSK2	-1,0	3,0
CaMK1 α	-3,2	-1,7
CaMK1 δ	-3,1	-2,8
CaMK2 α	-8,8	-8,3
CaMK2 β	-7,3	-6,6
CaMK2 γ	-3,7	-6,0
CaMK2 δ	-6,8	-5,7
CaMK4	0,9	-1,9

CDC2/CycB1	-5,1	-2,9
CDC7/ASK	-5,2	-4,0
CDK2/CycA2	-2,2	-2,2
CDK2/CycE1	-10,4	-7,5
CDK3/CycE1	-3,6	-7,1
CDK4/CycD3	-2,4	1,5
CDK5/p25	-15,6	-15,0
CDK6/CycD3	-4,7	-2,4
CDK7/CycH/MAT1	-4,0	-4,3
CDK9/CycT1	-1,5	1,3
CGK2	0,8	-0,4
CHK1	-0,1	-1,8
CHK2	-3,5	1,6
CK1 α	-0,7	-0,5
CK1 γ 1	-2,0	-2,3
CK1 γ 2	-5,9	-2,8
CK1 γ 3	-4,2	-4,3
CK1 δ	-4,5	-6,9
CK1 ϵ	-2,9	-0,8
CK2 α 1/ β	-6,1	-5,6
CK2 α 2/ β	-8,2	-5,0
CLK1	-5,2	8,7
CLK2	-7,6	-2,1
CLK3	-0,9	-1,9
COT_Cascade	9,7	-2,5
CRK	-5,6	-5,1
DAPK1	-6,4	0,2
DCAMK12	-4,7	-2,5
DLK_Cascade	-11,6	0,2
DYRK1A	-1,6	3,5
DYRK1B	-4,4	-7,5
DYRK2	-10,4	-0,1
DYRK3	-1,4	-1,9
EEF2K	-3,7	0,0
Erk1	-7,9	-8,3
Erk2	-7,9	-8,1
Erk5	-4,0	-4,3
GSK3 α	-1,6	2,1
GSK3 β	-14,9	-8,1
Haspin	-1,1	1,7
HGK	-8,5	2,0
HIPK1	1,4	3,7
HIPK2	-3,9	-1,8
HIPK3	-2,4	-1,6
HIPK4	-1,3	8,8
IKK α	-7,0	-6,6
IKK β	-3,3	-4,9
IKK γ	-2,2	-4,4
IRAK1	5,1	9,3
IRAK4	-1,0	-1,6

JNK1	-5.0	-3.3
JNK2	-5.3	-2.7
JNK3	-7.0	-3.3
LATS2	-0.7	3.0
LOK	-13.2	2.3
MAP2K1_Cascade	-5.3	-1.0
MAP2K2_Cascade	-0.8	-27.0
MAP2K3_Cascade	-14.1	-11.9
MAP2K4_Cascade	-15.2	-9.9
MAP2K5_Cascade	-8.0	-8.1
MAP2K6_Cascade	-13.0	0.8
MAP2K7_Cascade	-0.8	-0.9
MAP3K1_Cascade	-16.5	-5.6
MAP3K2_Cascade	-10.9	-3.9
MAP3K3_Cascade	-9.6	-2.7
MAP3K4_Cascade	-13.1	-0.3
MAP3K5_Cascade	-5.8	-0.4
MAP4K2	-3.6	-1.2
MAPKAPK2	-6.5	-10.9
MAPKAPK3	-15.7	-9.9
MAPKAPK5	-1.2	-8.3
MARK1	-3.0	0.6
MARK2	-8.5	-15.5
MARK3	-1.8	-7.5
MARK4	-7.6	-10.6
MELK	0.2	0.0
MGC42105	-6.0	-10.9
MINK	-2.8	-0.8
MLK1_Cascade	-5.0	7.6
MLK2_Cascade	-2.7	5.7
MLK3_Cascade	-14.3	-8.8
MNK1	-7.6	-5.1
MNK2	-3.2	-2.8
MOS_Cascade	-16.6	-10.5
MRCK α	-1.6	1.6
MRCK β	-7.0	-0.8
MSK1	-4.1	-2.3
MSK2	-2.5	-4.9
MSSK1	-1.2	0.2
MST1	-2.6	-0.9
MST2	-4.4	-5.6
MST3	-2.8	12.5
MST4	-3.5	-4.6
NDR1	-3.0	-0.4
NDR2	-4.4	-3.6
NEK1	-0.7	-1.3
NEK2	-3.9	-2.6
NEK4	-5.2	-5.8
NEK6	-1.1	-1.4
NEK7	-2.6	-2.7

NEK9	-4.1	-2.2
NuaK1	-5.6	20.7
NuaK2	-7.4	-2.1
p38 α	-4.0	-2.4
p38 β	-0.8	1.5
p38 γ	-2.4	-1.9
p38 δ	-3.9	-4.0
p70S6K	10.1	-11.1
p70S6K β	-0.8	-3.3
PAK1	-2.4	-2.8
PAK2	-1.9	5.4
PAK4	0.0	2.4
PAK5	-1.0	0.4
PAK6	-6.3	0.4
PASK	-5.0	-4.3
PBK	2.4	-5.1
PDHK2	-0.4	-2.5
PDHK4	-0.7	-1.2
PDK1	-2.7	-2.9
PEK	-4.2	5.8
PGK	-4.0	-5.4
PHKG1	-3.8	-2.6
PHKG2	-5.0	-5.7
PIM1	-13.9	-13.9
PIM2	-1.5	-1.1
PIM3	-2.1	-2.5
PKAC α	-2.4	-7.7
PKAC β	1.2	-5.6
PKAC γ	-3.0	-3.0
PKC α	-5.2	-2.0
PKC β 1	-9.2	5.0
PKC β 2	-4.6	-0.6
PKC γ	-8.0	13.3
PKC δ	0.5	0.8
PKC ϵ	22.2	0.4
PKC ζ	2.3	5.7
PKC η	-4.7	1.5
PKC θ	-1.7	1.0
PKC ι	11.7	16.0
PKD1	-7.7	-8.6
PKD2	-5.4	-5.4
PKD3	-6.7	-4.9
PKN1	2.2	3.7
PKR	-4.2	-1.6
PLK1	-4.9	-4.0
PLK2	-2.4	-1.9
PLK3	7.0	4.2
PRKX	-3.3	-1.7
QIK	-5.7	14.6
RAF1_Cascade	-10.3	-14.7

ROCK1	-5.1	-6.0
ROCK2	-7.9	-3.0
RSK1	-5.1	-3.0
RSK2	-2.9	-4.0
RSK3	-3.8	-3.1
RSK4	-1.1	-1.1
SGK	-3.5	-2.1
SGK2	-3.4	-1.1
SGK3	-3.1	-1.0
SIK	-1.8	28.2
SMMLCK	-6.2	-4.3
SLK	-0.3	5.2
SRPK1	-4.0	0.1
SRPK2	-0.4	0.8
TAK1-TAB1_Cascade	-12.6	-11.2
TAOK2	1.0	2.3
TBK1	-1.3	-2.7
TNIK	-7.2	12.3
TSSK1	1.8	-0.8
TSSK2	-2.6	-1.7
TSSK3	2.5	-0.5
WNK1	-2.9	-2.3
WNK2	-1.4	-1.8
WNK3	-1.0	-0.4

Example 4: IC₅₀ Determination study of CT101 against 4 kinases

[0309] CT101 inhibition of BTK, FYN, and PKC ϵ were examined at the following test concentrations: 50, 15, 5, 1.5, 0.5, 0.15, 0.05, 0.015, 0.005, 0.0015 μ M.

[0310] CT101 inhibition of SRC was examined at the following test concentrations: 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001 μ M.

Materials and Methods

Preparation of test compound solution

[0311] The test compound was dissolved in and diluted with dimethylsulfoxide (DMSO) to achieve 100-fold higher concentration. Then the solution was further 25-fold diluted with assay buffer to make the final test compound solution. Reference compounds for assay control were prepared similarly.

Assay reagents and procedures

Off-chip Mobility Shift Assay (MSA)

[0312] 1) The 5 μ L of x4 compound solution. 5 μ L of x4 Substrate/ATP/Metal solution, and 10 μ L of x2 kinase solution were prepared with assay buffer (20 mM HEPES, 0.01% Triton X-100, 2 mM DTT, pH7.5) and mixed and incubated in a well of polypropylene 384 well microplate for 1 hour at room temperature.

[0313] 2) 60 μ L of Termination Buffer (QuickScout Screening Assist MSA; Carna Biosciences) was added to the well.

[0314] 3) The reaction mixture was applied to LabChip3000 system (Caliper Life Science), and the product and substrate peptide peaks were separated and quantitated.

[0315] 4) The kinase reaction was evaluated by the product ratio calculated from peak heights of product(P) and substrate(S) peptides (P/(P+S)).

Reaction Conditions

[0316] The reaction conditions are depicted below:

Kinase	Platform	Substrate		ATP (μ M)		Metal		Positive control
		Name	(μ M)	K _m	Assay	Name	(μ M)	
RTK	MSA	Scotide	1000	72	75	Mg	5	Staurosporine
PYN	MSA	Scotide	1000	50	50	Mg	5	Staurosporine
SRG	MSA	Scotide	1000	31	50	Mg	5	Staurosporine
PKC α ²³	MSA	PKC peptide	1000	16	25	Mg	5	Staurosporine

23: Phosphatidylcholine and Diacyl Glycerol are added at the final concentration of 30 μ g/mL and 5 μ g/mL, respectively.

Data Analysis

[0317] The readout value of reaction control (complete reaction mixture) was set as a 0% inhibition, and the readout value of background (Enzyme(-)) was set as a 100% inhibition. then the percent inhibition of each test solution was calculated.

[0318] IC₅₀ value was calculated from concentration vs. %Inhibition curves by fitting to a four parameter logistic curve.

Results

[0319] The results are shown in Table 13 below.

Table 13: IC50 Determination

Kinase	IC50 (M)	
	CT101	Staurosporine
BTK	1,05E-05	2,68E-08
FYN	7,93E-07	2,45E-09
SRC	2,90E-07	4,31E-09
PKC ϵ	> 5,00E-05	1,27E-09

**Example 5: Pharmacokinetic Study of a Single Dose of
Compound CT101 in CD-1 Mice**

[0320] This study aims at selecting an appropriate vehicle formulation for topical application of CT101 onto the skin (Part A), validating a pharmacokinetic (PK) analysis method (Part B) and evaluating the PK of CT101 (Part C).

Methodology

Compounds and Reagents

[0321] CT101 was initially a solid compound that was diluted in vehicle (sodium chloride 0.9%, saline for intra-venous administrations and that determined in Part A for epicutaneous applications)

Experimental outline

Part A: Vehicle Assessment

[0322] Adult male CD-1 mice were randomly allocated to experimental groups (two mice per group). On Day 0 and Day 1, a vehicle formulation was administered twice daily by epicutaneous application to the ears (20 μ L per ear). On Day 2, the vehicle formulations were administered once. Topical application was performed in non-anaesthetised but restrained animals. Animals were monitored immediately after each vehicle application and one hour after the first daily application for signs of inflammation to include redness and swelling.

- Vehicle 1 was 25% Transcutol P and 75% Propylene Glycol
- Vehicle 2 was 10% Propylene Glycol, 40% DMSO and 50% distilled water
- Vehicle 3 was 25% Transcutol P, 10% Propylene Glycol and 65% distilled water

Group	Formulation	Route, volume	Frequency
A1	Vehicle 1	Epicutaneous 20 μ L/ear	Day 0-Day 1: Twice daily (BID) Day 2: Once daily (SID)
A2	Vehicle 2		
A3	Vehicle 3		

Part B: Method Optimization

Samples

[0323] Terminal blood samples from adult male CD-1 mice were collected into K2EDTA-coated tubes. Blood samples were processed to isolate plasma. Samples were pooled and stored at -80°C until further analysis.

CT101

[0324] CT101 was weighed out and reconstituted in methanol to give a stock solution at 1 mg/mL. All chemicals used for chromatography were of HPLC grade (Fisher Scientific Ltd., Loughborough, UK).

Liquid Chromatography (LC) conditions

[0325] Samples were analysed on a Waters Alliance 2695 High Pressure LC separations module in combination with a Waters Diode Array Detector and Waters Micromass Quattro Ultima triple quadrupole mass spectrometer. The samples were analysed by reversed phase liquid chromatography employing a gradient separation. Mobile phase A (MPA) consisted of 90% dH₂O: 10% methanol containing 0.1% formic acid. Mobile phase B (MPB) consisted of 90% methanol: 10% dH₂O containing 0.1% formic acid. The column was a Phenomenex Luna C18 (5 μ m, 250 x 2.00 mm).

[0326] The gradient conditions started at 70% MPB and rose linearly to 100% MPB at 6 minutes post injection. Conditions were held at 100% MPB for a further 4 minutes (10 minutes post injection), before returning to the initial conditions. The column was allowed to re-equilibrate before the next injection. Flow rate was 0.4 mL/min and total run time per injection was twenty minutes.

Mass Spectrometry (MS) conditions

[0327] The MS was used in electrospray positive mode, with a capillary voltage of 3.4 kV and a cone voltage of 20 V. Source and desolvation temperatures and gas flows settings were standard for the system. SIR channels were created to measure CT101 at mass-to-charge ratio (m/z) 584.2, 778.6 and 1167.5.

Sample preparation and analysis

[0328] Non-biological standards were prepared by spiking CT101 into 10% methanol: 90% water. Biological standards were prepared by spiking CT101 into blank murine plasma. The calibration curves were prepared with ten non-blank standards each at a volume of 50 µL (individual calibrant concentrations were 0.1, 0.2, 0.5, 0.8, 1, 5, 10, 20, 50 and 100 µg/mL).

[0329] Plasma samples and standards were extracted by adding three volumes of ice-cold methanol to precipitate the plasma proteins. After methanol addition, the sample was briefly vortexed to mix, then centrifuged at 10,000 g for five minutes (4°C). The supernatant was transferred to a labelled LC vial for analysis. Ten microliters of supernatant were injected into the LC-MS system. Calibration standards were analysed first (low to high concentration).

[0330] Samples were kept on ice, in a refrigerator or in cooled equipment wherever possible. After analysis sample vials were transferred to -80°C storage.

Part C: PK analysis

[0331] Adult male CD-1 mice were randomly allocated to experimental groups and allowed to acclimatise for one week. On Day 0, CT101 was administered either by epicutaneous application to the ears (50 mg/kg dose in 20 µL) or by intravenous administration (5 mg/kg dose). Topical application was performed in non-anaesthetised but restrained animals. The average surface of a mouse ear is 155 square millimetre. Test compound was allowed to spread passively onto the surface of the ears. Absorption of the test compound was not promoted by spreading. Animals were housed individually to prevent interferences with the application site. Blood samples were collected into K2EDTA-coated tubes according to the schedule below. Non-terminal blood samples were taken from a superficial vein and terminal blood samples were taken from a cardiac bleed. Blood samples were processed to isolate plasma. Samples were stored at -80°C for seven weeks until further analysis.

[0332] Plasma samples were analysed by LC-MS.

Treatment Groups and Dosages

- All Groups were n=4
- Vehicle for intravenous administrations was a 0.9% sodium chloride solution (saline).
- Vehicle for epicutaneous applications was determined in Part A: 25% Transcutol P and 75% Propylene Glycol
- Administration volume for topical application was 20 µL per ear.

- Administration volume for intravenous injection was 5 mL/kg.

Group	Treatment, Dose, Route, Frequency	Non-terminal Bleeds	Terminal Bleeds
1	CT101, 50 mg/kg, Epicutaneous, Once	0*	16 hours
2		¼ hours	4 hours
3		½ hours	8 hours
4		1 hours	24 hours
5		2 hours	n/a
6	CT101, 5 mg/kg, IV, Once	10 minutes	2 hours
7		20 minutes	4 hours
8		½ hours	8 hours
9		1 hours	24 hours

n/a: not applicable, *prior to compound administration, IV: intra-venous administration

Readouts

Vehicle assessment

[0333] Part A: Three vehicle formulations were assessed by epicutaneous application onto mice ears for up to three days. Animals were monitored for signs of inflammation.

PK analysis

[0334] Part B: Method validation was performed on plasma prepared from blood collected then pooled from strain-, gender- and age-matched mice. The following parameters were tested: selectivity, linearity of standard calibration curve, accuracy and precision, lower detection/quantitation limit's (LLOQ) accuracy and precision and extraction efficacy. The stability of the compound in plasma at -80°C was tested by determining the recovery following a freeze/thaw and a one week storage at -80°C.

[0335] Part C: Study samples were then processed by HPLC for quantitative analysis of CT101 in plasma.

Results

Vehicle assessment

[0336] On Day 0, vehicles were administered by epicutaneous applications of twenty microliters to each ear on un-anaesthetised animals. Animals were observed immediately after the epicutaneous applications and one hour after.

[0337] For all three vehicles, the volume used was sufficient to apply the solution to the entire surface of the ear. The administration volume could be reduced to ten to fifteen microliters per ear.

[0338] Vehicle 1 (25% Transcutol P and 75% Propylene Glycol) was absorbed and left an oily residue at the surface of the ear. The residue was still observed for up to one hour after the epicutaneous application.

[0339] Vehicle 2 (10% Propylene Glycol, 40% DMSO and 50% distilled water) was spread with difficulty and was not absorbed and remained at the surface of the ear. The solution was easily shaken off and/or groomed away by the animals when returned to their cages.

[0340] Vehicle 3 (25% Transcutol P, 10% Propylene Glycol and 65% distilled water) was absorbed and left an oily residue at the surface of the ear. The residue was still observed for up to one hour after the epicutaneous application.

[0341] All animals were seen grooming their ears shortly after epicutaneous application of the vehicles.

[0342] The three vehicles tested did not induce any sign of inflammation such as redness or swelling immediately after epicutaneous application or one hour after.

[0343] On Day 1, identical observations were made.

[0344] On Day 2 (morning), epicutaneous applications were performed on anaesthetised animals. Anaesthesia was induced in an anaesthetic chamber using isoflurane. Animals were maintained under isoflurane anaesthesia using a nose cone for five minutes. Vehicle 1 and vehicle 3 left an oily residue at the surface of the ear. Vehicle 2 was not absorbed. Animals were not seen grooming when returned to their cages.

[0345] Also on Day 2 (afternoon), the epicutaneous application volumes were reduced from twenty to ten microliters. Ten microliters were sufficient to cover the entire surface of the ear. No sign of inflammation were observed.

[0346] For the remaining of the study (Part C), animals were not anaesthetised and the administration volume was not reduced to ten microliters.

Pharmacokinetic analysis

Method validation

[0347] Plasma calibration standards were prepared from CD-1 mouse plasma blank plasma samples and the calibration standards were initially run with UV detection. However, this method was found to have poor separation, with a much lower limit of detection than the MS detection. As a consequence, the UV detection method was abandoned. Analysis of the UV data from the calibration curve showed that the LOQ by UV was 10 µg/mL.

[0348] Figure 28 (A) shows UV (280 nm) traces for, from bottom to top: blank calibration plasma, 10 µg/mL calibration plasma, 100 µg/mL calibration plasma, sample 21 (1/2 hour epicutaneous administration, animal 3.4), and sample 58 (1/2 hour intravenous administration, animal 8.3). Figure 28 (B) shows the MS TIC traces for the same injections. The shaded areas in all traces indicate the CT101 peak. There was co-eluting interference seen in the plasma samples at 280 nm (indicated by the arrow), making baseline to baseline integration difficult. The width of the peak detected at 280 nm is approximately one minute wide (horizontal lines); a reflection on the polymeric nature of the PEGylated compound. In comparison, due to the selective nature of the MS method, there are no co-eluting interferences detected, and the peak monitored at the chosen masses was much narrower.

[0349] Plasma calibration standards were prepared from CD-1 mouse plasma blank plasma samples. The calibration range was 0.1 to 100 µg/mL.

[0350] A 50 µL volume of calibrant was extracted in 150 µL ice-cold methanol. A 10 µL aliquot of supernatant was injected into the LC-MS system.

[0351] The reproducibility of the injection, tested by injecting ten supernatant samples, was calculated at -2.7% CV.

[0352] The limit of detection (LOD) was 10 µg/mL with the UV method and 0.2 µg/mL with the MS method. The limit of quantitation (LOQ) was greater than 10 µg/mL with the UV method and 0.5 µg/mL with the MS method.

[0353] Due to the time constraints for this project, LOQ & LOD were assessed from the plasma calibration curve (range 0.1 to 100 µg/mL CT101). CT101 at 0.1 µg/mL was not detectable. CT101 at 0.2 µg/mL was detectable, but the peak was too small to accurately quantify, so this point was set as the LOD. At 0.5 µg/mL CT101 was detected, with the peak signal at least three times the background signal (a standard LC reference test), so this point was set as the LOQ.

[0354] Figure 29 depicts chromatograms of a CT101 plasma standard extract (100 µg/mL) detected using SIR (TIC, upper) and UV (lower). Figure 30 depicts method validation. Figure 31 depicts individual chromatograms used for the analysis of CT101.

Quantitative analysis of CT101 in plasma

[0355] CT101 was applied topically once at 50 mg/kg or administered once by intravenous injection at 5 mg/kg.

[0356] Plasma samples were analysed by LC-MS method.

[0357] Plasma levels of CT101 in animals treated by topical application were below the detection limit of the assay (0.2 µg/mL) for all but one sample.

[0358] Plasma levels of CT101 in animals treated by intravenous administration were detectable for up to two hours after the injection and were then below the detection limit of the assay. Figure 32 depicts mouse plasma concentrations of CT101.

[0359] Following intra-venous administration, the following parameters were determined using SigmaPlot V8.02:

- C_0 = 53.4 µg/mL at 0 minutes post administration (extrapolated value)
- C_{max} = 22.6 µg/mL at 10 minutes post administration,
- AUC_{last} = 14.6 µg/mL/hour and
- $t_{1/2\alpha}$ = 0.093 hours and $t_{1/2\beta}$ = 0.6 hours (bi-exponential equation)
- V_D = 2.0 mL
- Clearance = 0.143 mL/minute

[0360] Following epicutaneous administration, only one animal had detectable plasmatic levels of CT101 and PK parameters for this group could not be determined due to the high inter-individual variability. Figure 33 depicts representative chromatograms showing CT101 in extracted murine plasma following intra-venous administration. Figure 34 depicts representative chromatograms showing CT101 in extracted murine plasma following epicutaneous administration.

Conclusions

Part A

[0361] The vehicles, when administered by epicutaneous application to the ears twice daily for three days, did not induce any sign of inflammation.

[0362] Vehicle 2 (10% propylene glycol, 40% DMSO and 50% distilled water) is not appropriate for epicutaneous applications.

[0363] Vehicle 1 (25% Transcutol P and 75% Propylene Glycol) was used for Part B and Part C of this study.

[0364] An administration volume of ten microliters would be sufficient to cover the entire surface of the ear. This was not implemented for Part B and Part C.

[0365] Performing the epicutaneous applications under isoflurane anaesthesia is recommended to prevent the animals from grooming the solutions away and/or ingesting the solutions. This was not implemented for Part B and Part C.

Part B

[0366] A suitable method for the analysis of CT101 in murine plasma by LC-MS was developed. The method is reproducible and has a good limit of detection (0.2 µg/mL) when compared to the UV method. This method was used to analyze samples from mice administered with CT101 by epicutaneous application and intra-venous injection.

Part C

[0367] CT101 concentrations were measured in mouse plasma samples after epicutaneous or intravenous administration. The administration dose for epicutaneous administration was ten times higher than when using the intravenous administration route. Plasma levels after epicutaneous application of CT101 were below the detection limit of the assay except for one animal (animal 4) on two time points (15 and 30 minutes). Plasma levels after intra- venous administration were measurable up to two hours after the injection and the following PK parameters were calculated: C_{max} = 22.6 µg/mL at 10 minutes post administration, AUC_{last} = 14.6 µg/mL/hour and Terminal $t_{1/2}$ = 0.6 hours.

Example 6: Evaluation of the ability of CT101 to inhibit conventional NF-κB

signaling

[0368] This study tested the ability of CT101 to inhibit inflammatory cell signalling by assessing its ability to inhibit conventional NF-κB signalling using an NF-κB-inducible Luciferase reporter construct.

Methodology

Compounds and Reagents

- Jurkat-Dual cells (ISG-NF-κB) cells (Invivogen jktd-isnf)

- THP-1-Lucia NF- κ B cells (Invivogen thpl-nfkb)
- HEK-Dual TNF α (Invivogen hkd-tnfa) Pam3CSK4
- Staurosporine (Iris Biotech, lot. 090801)
- TNF- α
- Concanavalin A
- CT101 lot n. 2013RB20/S45

Experimental outline

[0369] Three cell lines were selected based on their expression of the target src family kinases (the human monocyte cell line THP-1, the human T cell line Jurkat, the human fibroblast cell line HEK293). Cell lines were purchased that stably express an NF- κ B reporter construct (a secreted luciferase reporter gene, driven by an IFN- β minimal promoter fused to five copies of the NF- κ B consensus transcriptional response element and three copies of the c-Rel binding site). Use of a stably transfected cell line eliminates the variation associated with transient transfection of the luciferase reporter into cell lines.

[0370] Cell lines were pre-treated with compound CT101, before stimulation with a ligand suitable for the cell type; THP-1 cells were stimulated with the TLR1/2 ligand Pam3CSK4 (100 ng/mL), Jurkat cells were stimulated with Concanavalin A (ConA, 50 μ g/mL) and HEK293 cells with TNF- α (10 ng/mL). Cells were then incubated for a further six hours or twenty-four hours before assessment of luciferase activity in the cultures as a measurement of NF- κ B transcriptional activity.

[0371] a) To allow optimisation of the assay, initial experiments were performed with CT101 pre-treatment for 6 hours, 18 hours and 24 hours. The optimum pre-treatment time, based upon when maximum inhibition of NF- κ B activity was observed, was determined.

[0372] b) Following selection of the optimum pre-treatment time for each compound and cell type, separate experiments were performed over a range of compound concentrations to allow an IC₅₀ for each compound, based on inhibition of NF- κ B activity, to be determined.

[0373] c) To determine that any effect of the test compound on NF- κ B transcriptional activity was due to specific inhibition of signalling pathways and not due to any effect on cell viability, for each of the tested concentrations; cultures were tested for the presence of lactate dehydrogenase (LDH), which is released into the media from damaged cells and is a biomarker for cellular cytotoxicity and cytolysis.

Selection of appropriate time frames and cell lines

[0374] CT101 was tested at three concentrations on the three different cell types (100 μ M, 30 μ M, and 10 μ M). Each condition was tested in sextuplicate. Concentrations may be lowered (if cell toxicity is observed) or increased up to mM ranges (if effect is not observed).

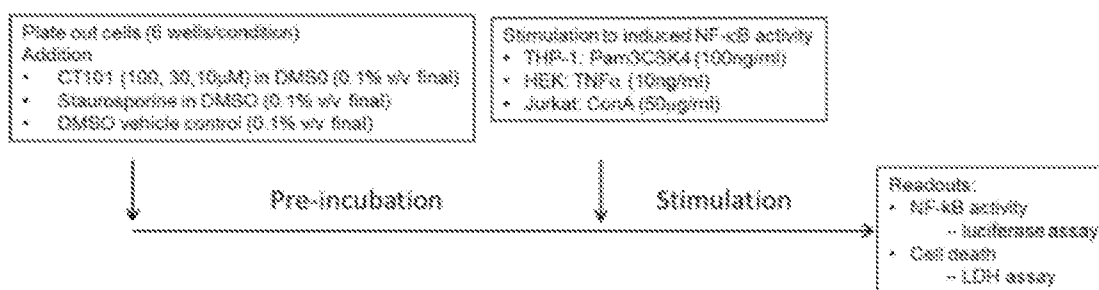
[0375] Following incubation with stimulus cells were assayed for luciferase activity and an LDH assay performed to test for cell viability.

[0376] Table 14 shows the experimental conditions tested for each of the reporter cell lines (HEK, THP-1, Jurkat).

Table 14

Concentration of CT101	Pre-incubation time	Incubation with stimulus
100 μ M	6h	24h
30 μ M	6h	24h
10 μ M	6h	24h
100 μ M	18h	24h
30 μ M	18h	24h
10 μ M	18h	24h
100 μ M	24h	24h
30 μ M	24h	24h
10 μ M	24h	24h
0 (DMSO control)	24h	24h
100 μ M	6h	6h
30 μ M	6h	6h
10 μ M	6h	6h
100 μ M	18h	6h
30 μ M	18h	6h
10 μ M	18h	6h
100 μ M	24h	6h
30 μ M	24h	6h
10 μ M	24h	6h
0 (DMSO control)	24h	6h

[0377] The experimental design is as follows:



IC50 calculation of CT101 inhibition of NF-κB activity

[0378] Following the selection of optimised conditions for CT101 on each cell line, IC50 values were not calculated for CT101 inhibition of NF-κB activity as there was no consistent inhibition observed in cultures conducted in the presence of CT101 when compared to the vehicle control.

[0379] To determine that any effect of the test compound on NF-κB transcriptional activity was due to specific inhibition of signalling pathways and not due to any effect on cell viability, for each of the tested concentrations cultures were tested for the presence of lactose dehydrogenase (LDH), which is released into the media from damaged cells and is a biomarker for cellular cytotoxicity and cytolysis.

Readouts

[0380] Luciferase activity in reporter cell lines was assayed using a Promega luminometer.

[0381] LDH assays were assayed using a colorimetric system and absorbance read on an omega plate reader.

Results

Selection of appropriate time frames and cell lines

[0382] HEK, Jurkat and THP-1 reporter cell lines were pre-incubated with CT101 (100 μM, 30 μM, 10 μM) or DMSO vehicle (0.1% v/v) control for 6 hours, before stimulation for a further 24 hours with 10 ng/mL TNF-α (HEK cells), 100 ng/mL Pam3CSK4 (THP-1 cells) or 50 μg/mL ConA (HEK293 cells). Control cells were not stimulated (non-stimulated) to provide background levels of luciferase activity present in cell lines. As a positive control some cells were incubated with 200 nM of staurosporine. Supernatants were then harvested and assayed for luciferase activity using the Invivogen Quanti-Luc assay solution. The fold induction of luciferase (NF-κB activity) by stimulation with ligands and the ability of CT101 or Staurosporine to inhibit induction were calculated by subtracting the mean media control luminescence value from the

mean sample luminescence value and calculating the fold increase in luminescence above non-stimulated cells.

[0383] The Jurkat cell line did not grow well in the conditions recommended by the supplier therefore in some experiments where cell number was limiting; this cell line was tested in triplicate or quadruplicate for each condition. The Jurkat cell line was not tested for the 6h pre-incubation, 6h stimulation or 24h pre- incubation, 6h stimulation experimental conditions.

[0384] Each experimental condition was performed once, using sextuplicate wells. Data was analysed by two-way ANOVA comparing each cell mean to the control DMSO treated cell mean for that cell line, using Dunnett's multiple comparisons test. Values less than 0.05 were considered significant. These p values represent intra-experimental significance and are a measurement of reproducibility between wells within a single experiment, they do not indicate any differences between different drug treatments, for this to be tested each experimental condition would need to be repeated on multiple occasions (Table 15).

Table 15. Conditions under which the luminescence of treated cells were significantly different from DMSO treated cells (intra-assay statistics). Analysis was carried out using a two-way ANOVA with Dunnett's multiple comparisons tests.

Cell line	Pre-incubation	Stimulation	Significantly different to DMSO (p<0.05)
HEK	6 h	24 h	100 μ M CT101
HEK	6 h	24 h	10 μ M CT101
HEK	6 h	24 h	200 nM Staurosporine
HEK	18 h	24 h	100 μ M CT101
HEK	18 h	24 h	200 nM Staurosporine
HEK	24 h	24 h	200 nM Staurosporine
HEK	18 h	6 h	200 nM Staurosporine
HEK	6 h	6 h	200 nM Staurosporine

Pre-incubation time: 6 hours, stimulation time: 24 hours

[0385] Figure 35 depicts raw luminescence values for a CT101 pre-incubation time of 6 hours and a stimulation time of 24 hours. Each symbol represents an individual well. Each condition was tested in sextuplicate. Figure 36 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 6 hours and a stimulation time of 24 hours.

Pre-incubation time: 18 hours, stimulation time: 24 hours

[0386] Figure 37 depicts raw luminescence values for a CT101 pre-incubation time of 18 hours and a stimulation time of 24 hours. Each condition was tested in sextuplicate except for Jurkat cells where conditions were tested in triplicate. Figure 38 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 18 hours and a stimulation time of 24 hours.

Pre-incubation time: 24 hours, stimulation time: 24 hours

[0387] Figure 39 depicts raw luminescence values for a CT101 pre-incubation time of 24 hours and a stimulation time of 24 hours. Each condition was tested in sextuplicate except for Jurkat cells where conditions were tested in triplicate. Figure 40 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 24 hours and a stimulation time of 24 hours.

Pre-incubation time: 6 hours, stimulation time: 6 hours

[0388] Figure 41 depicts raw luminescence values for a CT101 pre-incubation time of 6 hours and a stimulation time of 6 hours. Each condition was tested in sextuplicate. Figure 42 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 6 hours and a stimulation time of 6 hours.

Pre-incubation time: 18 hours, stimulation time: 6 hours

[0389] Figure 43 depicts raw luminescence values for a CT101 pre-incubation time of 18 hours and a stimulation time of 6 hours. Each condition was tested in sextuplicate except for Jurkat cells where conditions were tested in triplicate. Figure 44 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 18 hours and a stimulation time of 6 hours.

Pre-incubation time: 24 hours, stimulation time: 6 hours

[0390] Figure 45 depicts raw luminescence values for a CT101 pre-incubation time of 24 hours and a stimulation time of 6 hours. Each condition was tested

in sextuplicate. Figure 46 depicts fold induction of Luciferase above non-stimulated cells for a CT101 pre-incubation time of 24 hours and a stimulation time of 6 hours.

Cell viability: LDH Assay

[0391] To test for the effect of compounds on cell viability supernatants were harvested and assayed for LDH activity levels. Some cells were lysed to give maximal levels of LDH when all cells were dead. Graphs show the percentage cytotoxicity of each culture condition (mean of six wells/condition, for Jurkat cells, the mean of 3 wells/condition). Percentage cytotoxicity was calculated as (compound treated LDH release / maximum LDH release control) x 100.

[0392] The percentage cytotoxicity could not be calculated for 18 hour pre-incubation and 6 hour stimulation because the maximum lysis wells did not lyse.

[0393] Data was analysed by two-way ANOVA comparing each cell mean to the control DMSO treated cell mean for that cell line, using Dunnett's multiple comparisons test. Values less than 0.05 were considered significant. These p values represent intra-experimental significance and are a measurement of reproducibility between wells within a single experiment, they do not indicate any differences between different drug treatments, for this to be tested each experimental condition would need to be repeated on multiple occasions.

[0394] Figure 47 depicts percentage cytotoxicity for a CT101 pre-incubation time of 6 hours and a stimulation time of 24 hours. Figure 48 depicts percentage cytotoxicity for a CT101 pre-incubation time of 18 hours and a stimulation time of 24 hours. Figure 49 depicts percentage cytotoxicity for a CT101 pre-incubation time of 24 hours and a stimulation time of 24 hours. Figure 50 depicts percentage cytotoxicity for a CT101 pre-incubation time of 24 hours and a stimulation time of 6 hours. Figure 51 depicts percentage cytotoxicity for a CT101 pre-incubation time of 6 hours and a stimulation time of 6 hours.

[0395] Significant differences were found under various conditions with all cell lines, these are summarized in Table 16.

Table 16: Conditions under which LDH release by treated cells were significantly different from DMSO treated cells (intra-assay statistics). 2 way anova, Dunnetts multiple comparisons test.

Cell line	Pre-incubation	Stimulation	Significantly different to DMSO (p<0.05)
HEK	6hr	24hr	100µM CT101*
HEK	6hr	24hr	30µM CT101*
HEK	6hr	24hr	10µM CT101*
Jurkat	6hr	6hr	Staurosporine [§]
Jurkat	6hr	24hr	Staurosporine [§]
THP-1	6hr	6hr	Staurosporine [§]
THP-1	6hr	24hr	Staurosporine [§]
HEK	18hr	6hr	Staurosporine [§]
HEK	18hr	24hr	Staurosporine [§]
Jurkat	18hr	6hr	30µM CT101 [§]
Jurkat	18hr	6hr	Staurosporine [§]
Jurkat	18hr	24hr	Staurosporine [§]
THP-1	18hr	6hr	Staurosporine [§]
THP-1	18hr	24hr	Staurosporine [§]
Jurkat	24hr	6hr	Staurosporine [§]
Jurkat	24hr	24hr	Staurosporine [§]
THP-1	24hr	6hr	10µM CT101 [§]
THP-1	24hr	6hr	Staurosporine [§]
THP-1	24hr	24hr	Staurosporine [§]

*There was lower cell viability in DMSO-treated cells than those cultured with test compounds, leading to statistically significant differences

[§] Lower viability in Staurosporine/compound-treated cells when compared to the DMSO control

[0396] The following observations were made based on the statistical analyses of the data by two-way ANOVA followed by Dunnett's post-test for multiple comparisons between experimental groups.

[0397] Incubation of HEK cells with TNF-alpha for 6 hours or 24 hours induced a significant increase of luciferase activity in the DMSO-treated cells when compared to the unstimulated cells.

[0398] Incubation of Jurkat cells with Con A for 6 hours or 24 hours induced a significant increase of luciferase activity in the DMSO-treated cells when compared to the unstimulated cells.

[0399] Incubation of THP-1 cells with Pam3CSK4 for 6 hours or 24 hours induced a significant increase of luciferase activity in the DMSO-treated cells when compared to the unstimulated cells.

[0400] Staurosporin significantly reduced the stimulus-induced luciferase activity:

- Regardless of the pre-incubation time followed by 24 hour-stimulation in HEK cells
- Only after 6 or 18 hours pre-incubation followed by 6 hour-stimulation in HEK cells
- At all time-points tested in Jurkat cells
- At all time-points tested in THP-1 cells

[0401] The LDH assay shows that the reduction of luciferase activity in HEK cells is not caused by a reduction of the number of cells.

[0402] The LDH assay suggests that, in Jurkat and THP-1 cells, the reduction of the luciferase activity may in part be associated with a reduction of the number of cells.

[0403] The best experimental conditions to assess the efficacy of compound CT101 in this assay were: HEK cells stimulated for 24 hours.

[0404] In those experimental conditions, the pre-incubation of HEK cells with CT101 at 100 μ M for 6 hours or 18 hours (but not 24 hours) significantly reduced the luciferase activity. This effect was not associated with a reduction of the number of cells.

Conclusions

[0405] 24-hour stimulation induced the highest levels of luciferase and therefore NF- κ B activity in the HEK and THP1 reporter cell lines. 6 hour-stimulation induced lower levels of NF- κ B activity. Jurkat cells produced very low levels of luciferase in response to stimulation with ConA.

[0406] HEK cells had the highest levels of NF- κ B activity.

[0407] CT101 proved to inhibit ligand induced NF- κ B activity in a statistically significant manner at the top tested dose (100 μ M) after 6 and 18 hours of pre-incubation in HEK cells, that proved to be the best performing of the three tested cell lines.

[0408] The control protein kinase inhibitor drug Staurosporine did inhibit NF- κ B activity, under each condition tested for the HEK reporter cell line, the percentage inhibition of NF- κ B induction was dependent on culture conditions and the cell line.

[0409] The percentage cytotoxicity as measured by LDH activity in culture supernatants in HEK cultures was below 15%, and in THP-1 levels were below 25% (except for 6h pre-incubation, 6h stimulation where levels were higher).

[0410] The percentage cytotoxicity in HEK-1 cells was fairly consistent across all culture conditions; cell death was not increased by stimulation, the inhibitory effect of Staurosporine on NF- κ B activity was not due to a loss of cell viability and CT101 did not result in loss of cell viability at the concentrations tested.

[0411] LDH activity in all Jurkat samples was high indicating a high level of cell death in these cultures, independent of stimulation or treatment with CT101, this was consistent with the poor growth of this cell line.

Example 7: CT101 Efficacy in a Mouse Model of Oxazolone-induced Contact Dermatitis

[0412] This study used a mouse model of 4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (Oxazolone)-induced contact dermatitis to test the efficacy of CT101.

Methodology

Compounds and Reagents

[0413] Betamethasone 17-valerate 0.1% (Manx Pharma)

[0414] Propylene Glycol (P4347)

[0415] Transcutol P (Gattefosse, lot. 450931007)

[0416] CT101 was provided as a solid compound that was solved in vehicle (25% Transcutol P and 75% Propylene Glycol for epicutaneous applications)

Experimental outline

[0417] Female BALB/c mice were randomly allocated to experimental groups and allowed to acclimatise for one week. On Day 0, animals were sensitised to oxazolone by epicutaneous application of 50 μ L of a 1.5% solution in acetone: olive oil (4:1) to the clipped abdomen. On Days 7, 10 and 13, animals were challenged by

epicutaneous application of 10 μ L per surface of a 1.0% oxazolone solution, or vehicle, to both surfaces of both ears. Oxazolone and treatments were given according to the administration schedule below. Ear swelling was measured 24 hours after each oxazolone challenge using a digital calliper. At termination ears were collected, cut in two halves and stored for optional histopathology and optional tissue cytokine analysis.

Treatment Groups and Dosages

[0418] All Groups are n=10.

[0419] Vehicle for epicutaneous administrations is 25% Transcutol P, 75% propylene glycol.

[0420] Administration volume for treatment epicutaneous applications was 20 μ L per ear per administration (two administrations per day). Only one ear per animal was treated with the active ingredients (left ear), while the contra-lateral ear was treated with the vehicle each time (right ear).

[0421] The groups of mice were treated as follows:

Group	Treatment	Dose	Route	Frequency	Disease Induction
1	Vehicle	n/a	Epicutaneous	BID, Day 0- Day 13 SID, Day 14	Day 0: Sensitization Day 7, 10, 13: Challenge
2	Betamethasone valerate 0.1%	0.1%	Epicutaneous		
3	CT101	10%	Epicutaneous		
4	CT101	20%	Epicutaneous		

n/a: not applicable, BID: twice daily, SID: once daily

On Day 7, Day 10 and Day 13, treatments were administered one hour prior to the oxazolone challenge and seven hours after the oxazolone challenge

On Day 14, treatments were administered two hours prior to the measurements

Readouts

Bodyweights

[0422] From Day 0, animals were weighed three times a week. Data were graphed (Mean $\pm$ SEM).

Non-specific clinical observations

[0423] From Day 0 until the end of the experiment, animals were checked daily for non-specific clinical signs to include abnormal posture (hunched), abnormal coat condition (piloerection) and abnormal activity levels (reduced or increased activity).

Ear Swelling

[0424] On Day -1 and then twenty-four hours after each oxazolone challenge, ear thickness was measured using digital callipers. Swelling was calculated as the difference in thickness between the pre-challenge thickness (Day -1) and the daily values taken on Day 8, Day 11 and Day 14.

Macroscopic Scores

[0425] From Day 7, animals were monitored daily for signs of inflammation to include erythema (redness), swelling of the ears, dry skin and/or presence of wet lesions. A semi-quantitative system was used in order to allow comparisons between groups.

Score	Description
0	Normal
1	Erythema
2	Erythema and mild swelling
3	Erythema and moderate swelling
4	Erythema and severe swelling
D	Presence of dry skin*
W	Presence of wet lesions*

*These criteria may be followed by a number (1-4) corresponding to the number of ear surfaces affect

Tissue Cytokine Analysis

[0426] At termination ears were dissected, cut in two halves and one half stored for cytokine analysis. To each half ear, extraction buffer was added and the ears homogenised and centrifuged. After centrifugation, cytokine analysis was performed on the supernatant to determine the concentration of IL-1 β , IL-4, IL-10, IFN- γ and TNF- α in each sample. Samples were analysed using multiplex xMAP bead technology, which utilises microspheres as a solid support for sandwich immunoassays and determination of numerous cytokines in the same sample. A standard curve of quantified recombinant cytokines was used to convert detected PE fluorescence values to cytokine concentrations (pg/ml).

Results

Bodyweights

[0427] Bodyweight data were analyzed by two-way ANOVA followed by Dunnett's post-test for multiple comparisons between experimental days in the vehicle-treated group then for multiple comparisons between experimental groups. As shown in Figure 52, bodyweights in the vehicle-treated group increased prior to the oxazolone challenge and Day 6 bodyweights were significantly higher than bodyweights recorded on Day 0 ($p < 0.001$). Oxazolone challenges induced a decrease in bodyweights. Day 11 bodyweights were significantly lower than bodyweights recorded on Day 0 ($p < 0.05$).

[0428] Betamethasone 0.1% induced a significant decrease of the bodyweights when compared to the vehicle-treated group from Day 4 until the end of the experiment on Day 14 ($p < 0.0001$).

[0429] CT101 administered at 10% did not prevent the oxazolone challenges-induced bodyweight loss. The bodyweights in the CT101_ 10%-treated group was significantly lower than in the vehicle-treated group on Day 6 ($p < 0.001$), on Day 8 ($p < 0.05$), Day 11 ($p < 0.05$) and Day 14 ($p < 0.01$).

[0430] CT101 administered at 20% did not prevent the oxazolone challenges-induced bodyweight loss but did not induce any further bodyweight loss when compared to the vehicle-treated group.

Non-Specific Clinical Observations

[0431] From Day 0 until the end of the experiment, animals did not show any nonspecific clinical signs to include abnormal posture (hunched), abnormal coat condition (piloerection) and abnormal activity levels (reduced or increased activity).

Ear Swelling

[0432] Ear swelling data were analysed by two-way ANOVA followed by Dunnett's post-test for multiple comparisons between experimental days in the vehicle-treated group then for multiple comparisons between experimental groups. As depicted in Figure 53, Oxazolone challenges induced a significant increase of ear swelling in the vehicle-treated group on Day 8, Day 11 and Day 14 when compared to Day 0 ($p < 0.0001$).

[0433] CT101 administered at 10% induced a significant reduction of ear swelling when compared to the vehicle-treated group on Day 11 ($p < 0.01$) and Day 14 ($p < 0.0001$).

[0434] CT101 administered at 20% induced a significant reduction of ear swelling when compared to the vehicle-treated group on Day 14 ($p < 0.001$).

Macroscopic Scores

[0435] Macroscopic score data were analysed by two-way ANOVA followed by Dunnett's post-test for multiple comparisons between experimental days in the vehicle-treated group then for multiple comparisons between experimental groups. As depicted in Figure 54, Oxazolone induced a significant increase of the macroscopic scores in the vehicle-treated group from Day 7 until the end of the experiment on Day 14 when compared to Day 0 ($p < 0.0001$).

[0436] Betamethasone 0.1 % induced a significant reduction of the macroscopic scores when compared to the vehicle-treated group from Day 7 until the end of the experiment on Day 14 ($p < 0.0001$).

[0437] CT101 administered at 10% induced a significant decrease of the macroscopic scores when compared to the vehicle-treated group on Day 13 ($p < 0.001$) and Day 14 ($p < 0.0001$).

[0438] CT101 administered at 20% induced a significant reduction of the macroscopic scores when compared to the vehicle-treated group on Day 14 ($p < 0.0001$).

Tissue Cytokine Analysis

[0439] Contact dermatitis presents an immune mediated reaction known to involve both T helper 1 cell (Th1) and Th2 response. Therefore, a mixture of proinflammatory cytokines is expected to be present in this model.

[0440] Snap frozen ear samples were homogenised and measured by Luminex. Cytokine levels between the left (treated with the active ingredients) and the right ear (treated with the Vehicle) within each experimental group were analysed by two-way ANOVA followed by Sidak's post-test for multiple comparison. Further, one-way ANOVA was used to compare cytokine levels measured in left ear only (treated with the active ingredients) in different experimental groups and the Vehicle-treated group.

[0441] Levels of IL-1 β and IFN- γ in the left ear were significantly lower after Betamethasone treatment when compared to those measured in the Vehicle group (Figure 55). Similarly, levels of IL-1 β in the actively treated ear were lower in CT101 10% group when compared to the Vehicle group. This indicates that these compounds lower exacerbated levels of pro-inflammatory cytokines in the model.

[0442] Within the groups, Betamethasone induced significant decrease in IL-1 β and IFN- γ . In contrast, levels IL-4 in the left ear were slightly increased when

compared to the right ear. Levels of IL-4 were higher in the right when compared to the left ear within CT101 20% group. This suggests that an active treatment at the site of inflammation was therefore able to reduce some of the cytokine levels in this model.

[0443] Levels of TNF- α were below detection limit and as such were not plotted in the report. It is possible that the model has led to the transient increase in the levels of this cytokine which has passed by the time of termination selected in this study.

Conclusions

[0444] As expected in this model of chronic dermatitis, the following pathological changes were observed following the topical application of oxazolone: ear swelling as soon as Day 7 and increasing until Day 14 and macroscopic scores including erythema. Betamethasone 0.1% reduced ear swelling and skin erythema. Betamethasone 0.1% caused bodyweight loss, a known side effect of steroids administered to rodents.

[0445] CT101 administered at 10% and 20% partially prevented the development of pathological changes to the skin (swelling and erythema), inducing a statistically significant reduction of ear swelling and of the macroscopic clinical scores

[0446] Cytokines in the present model mostly were present at detectable levels in ear homogenates, with the exclusion of TNF- α which was present at levels below limit of detection.

[0447] Betamethasone induced decrease in pro-inflammatory cytokines, namely IL-1 β and IFN- γ , while CT101 10% decreased IL-1 β levels when compared to the Vehicle-treated group. Within the groups, Betamethasone induced a decrease in IL-1 β in the actively treated ear when compared to the vehicle-treated ear, and the same was the case with IL-4 in CT101 20% group. These findings are in keeping with the clinical measurements of ear swelling and confirm the anti-inflammatory effects of the compounds.

Example 8: Evaluation of CT101 Efficacy in a Mouse Model of Ovalbumin-induced Atopic Dermatitis

[0448] This study used a mouse model of ovalbumin-induced atopic dermatitis to test the efficacy of CT101.

Methodology

Compounds and Reagents

- Albumin from chicken egg white, Ovalbumin (A5503, Sigma)
- Imject Alum (PN77161 Thermo Scientific Pierce)

- Sodium chloride 0.9% solution, saline (NaCl 0.9%, S8776, Sigma)
- Betamethasone 17-valerate 0.1% (Manx Pharma)
- Propylene Glycol (P4347)
- Transcutol P (Gattefossè, lot. 450931007)
- CT101 was provided as a solid compound and was diluted in vehicle (25% Transcutol P and 75% Propylene Glycol for epicutaneous applications)

Experimental outline

[0449] Adult male CD-1 mice were randomly allocated to experimental groups and allowed to acclimatise for one week. On Day -13, animals were administered with 200 µL of an ovalbumin and Alum emulsion by intra-peritoneal injection under gas (isoflurane) anaesthesia. The emulsion was prepared by mixing one volume of a 40 µg/ml ovalbumin solution in distilled water and one volume of Alum Imject. Prior to Day 0, ear thickness was measured using digital callipers. On Day 0, animals were challenged with 20 µL of a 0.5 mg/ml solution of ovalbumin in sodium chloride 0.9% (saline) by intradermal injection into the left ear. An equivalent volume of saline was injected into the contralateral ear. One group of animals (Group 1, Control) received an intradermal injection of saline in each ear. Treatments were administered according to the schedule below. On Day 0, at ¼ hour, ½ hour and one hour after the ovalbumin challenge and on Day 1, twenty-five and twenty-nine hours after the ovalbumin challenge, ears were observed for clinical signs of skin inflammation to include erythema, scaling and skin thickening. Ear thickness was measured using digital callipers. Twenty-eight hours after ovalbumin challenge, animals were culled and ears dissected out. One half of each ear was stored at room temperature in tissue fixative until further optional histopathology analysis.

Treatment Groups and Dosages

[0450] All Groups were n=10

[0451] Vehicle for epicutaneous administrations is 25% Transcutol P and 75% Propylene Glycol. Administration volume for epicutaneous applications was 20 µL per ear. Treatment groups were as follows:

Group	Treatment	Dose	Route Frequency	Sensitization Day -13	Challenge Day 0
1	Control	n/a	Route: EC BID: Day -5 to Day 0* SID: Day 1	Alum/OVA IP	LE: 20 μ L saline, ID RE: 20 μ L saline, ID
2	Betamethasone- 17-Valerate	0.1%			LE: 20 μ L OVA, ID RE: 20 μ L saline, ID
3	Vehicle	n/a			
4	CT101	5%			
5	CT101	10%			
6	CT101	20%			

n/a: not applicable, EC: epicutaneous application on left ears, IP: intra-peritoneal injection, ID: intra-dermal injection, BID: twice per day, SID: Once per day, LE: Left ear, RE: Right ear, *One hour prior to the challenge and seven hours after the challenge

Readouts

Clinical Observations

[0452] Animals were weighed at the start of the study (Day -13), once per week and on Day 0. All animals were observed for signs of ill health daily throughout the study. Bodyweight data were graphed (Mean $\pm$ SEM for each experimental group).

Skin Inflammation

[0453] Prior to the intra-dermal challenge, one hour after the challenge (Day 0) and one hour and four hours after epicutaneous applications (Day 1), ears were observed for signs of skin inflammation to include erythema, scaling and skin thickening. Each criterion was scored on a point-scale where (0) is absent, (1) is mild, (2) is moderate and (3) is severe. A total score was calculated for each ear by adding the scores from each individual criterion. Data were graphed (Mean $\pm$ SEM for each experimental group).

Ear Swelling

[0454] Prior to the intra-dermal challenge, ¼ hour, ½ hour and one hour after the challenge (Day 0) and one hour and four hours after epicutaneous applications (Day

1), ear thickness was measured under gas (isoflurane) anaesthesia using digital callipers. Data were graphed (Mean $\pm$ SEM for each experimental group).

Histopathology

[0455] At termination ears were dissected, cut in two halves and one half stored in tissue fixative until further analysis. Samples were embedded in paraffin wax, sectioned, mounted on a cooled water bath to prevent splitting and stained with haematoxylin and eosin (H&E). *Optional:* Epidermal and dermal layer thickening may be quantified by taking five representative micrometre measurements for each layer using the linear measurement tool in the Leica Application Suite programme. Skin layer measurements are not relevant to this model (but to our models of skin scleroderma) and were not performed. *Optional:* cellular infiltrate may be characterised and or quantified by counting inflammatory cells on three to five H&E-stained sections. Cellular characterisation and quantification were performed.

[0456] Sections were scored for epidermal hyperplasia (0: absent, 1: present), dermal oedema (0: absent, 1: mild, 2: moderate and 3: severe) and dermal inflammation (0: less than 5 inflammatory cells, 1: 5-10 cells, 2: 10-25 cells and 3: more than 25 cells). A total score was calculated by adding the individual parameter scores for a maximum possible score of 7. Sections were evaluated in blinded fashion.

Results

Non-Specific Clinical Observations

[0457] Animals did not show any non-specific clinical signs such as piloerection, hunched posture, altered breathing rate or altered mobility for the duration of the study.

Bodyweights

[0458] Bodyweight data were analyzed by two-way ANOVA followed by Dunnett's post-test for multiple comparisons between experimental groups. Betamethasone 17-valerate administered twice daily by topical application onto the ears from Day -5 induced a significant reduction of the bodyweight measured on Day -3 ($p < 0.05$) and Day 0 ($p < 0.01$) when compared to the vehicle-treated group (Figure 56). This is a known side effect of corticoids. CT101 administered at 5%, 10% or 20% twice daily by topical application onto the ears from Day -5 did not induce any bodyweight reduction.

Ear Swelling

[0459] Measurement of ear swelling is the primary readout in this model.

[0460] Ear swelling data, expressed as the difference in millimetres between the left (ovalbumin-challenged) and right (saline-injected) ears, were analysed by two-way ANOVA followed by Dunnett's post-test for multiple comparisons between experimental groups.

[0461] Ovalbumin induced a significant increase of ear thickness in the vehicle-treated group fifteen minutes ($p < 0.01$), twenty-five hours ($p < 0.05$) and twenty-nine hours ($p < 0.01$) after the challenge when compared to baseline (0 hours) values (Figure 57).

[0462] Ovalbumin induced a significant increase of ear thickness in the vehicle-treated group fifteen minutes ($p < 0.01$), thirty minutes ($p < 0.05$), twenty-five hours ($p < 0.01$) and twenty-nine hours ($p < 0.001$) after the challenge when compared to the Control group. Table 17 below summarizes the ear swelling data.

Table 17: Ear Swelling (Left-Right ear, in millimeters)

Group	Time post-challenge (Hours)					
	0	0.25	0.5	1	25	29
Control	0.04 ± 0.01	$0.05 \pm 0.01^{***}$	$0.03 \pm 0.01^*$	0.07 ± 0.01	$0.04 \pm 0.04^{**}$	$0.03 \pm 0.01^{***}$
Betamethasone 0.1%	0.09 ± 0.01	$0.04 \pm 0.01^{***}$	$0.02 \pm 0.00^*$	0.06 ± 0.01	$0.04 \pm 0.01^{**}$	$0.03 \pm 0.01^{***}$
Vehicle	0.00 ± 0.01	0.24 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.19 ± 0.02	0.21 ± 0.02
CT101 5%	0.05 ± 0.01	$0.36 \pm 0.02^*$	0.27 ± 0.02	0.20 ± 0.01	0.17 ± 0.02	0.22 ± 0.02
CT101 10%	0.05 ± 0.01	$0.08 \pm 0.01^{**}$	0.15 ± 0.01	0.16 ± 0.01	0.20 ± 0.01	0.23 ± 0.02
CT101 20%	0.05 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.20 ± 0.01	0.21 ± 0.01

Statistical significances: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ when compared to the vehicle-treated group

[0463] Ear swelling data recorded at peak disease (corresponding to fifteen minutes after the challenge) were further analysed by one-way ANOVA followed by Dunnett's post-test for multiple comparisons to the vehicle-treated group.

[0464] Ovalbumin challenge induced a significant increase of the ear swelling measured in the vehicle-treated group when compared to the Control group ($p < 0.01$).

[0465] Betamethasone 0.1% induced a significant reduction of the ovalbumin-induced ear swelling when compared to the vehicle-treated group ($p < 0.001$).

[0466] CT101, administered at 5%, did not reduce the ovalbumin-induced ear swelling (Figure 58).

[0467] CT101, administered at 10% and 20%, reduced the ovalbumin-induced ear swelling. The reduction was found to be statistically significant when CT101 was administered at 10% ($p < 0.01$).

Skin inflammation

[0468] Scaling and skin thickening are not expected in this model. Erythema is expected to occur, but tends to be relatively mild compared to that seen in other skin inflammation models, and should be viewed as a secondary readout.

[0469] Erythema was scored on a point-scale where (0) is absent, (1) is mild, (2) is moderate and (3) is severe. Erythema score data were analysed by two-way ANOVA followed by Dunnett's post-test for multiple comparisons between experimental times or between experimental groups.

[0470] The ovalbumin challenge (intra-dermal administration on Day 0) induced a significant increase in erythema scores observed in the vehicle-treated group at all experimental times when compared to the scores observed prior to the challenge.

[0471] Betamethasone 17-valerate induced a reduction of the erythema scores when compared to the vehicle-treated group. The reduction was statistically significant thirty minutes after the challenge ($p < 0.01$), twenty-five hours after the challenge ($p < 0.0001$) and twenty-nine hours after the challenge ($p < 0.0001$).

[0472] CT101 administered at 5% reduced the erythema scores from thirty minutes after the challenge when compared to the vehicle-treated group but the reduction was not statistically significant.

[0473] CT101 administered at 10% reduced the erythema scores from one hour after the challenge when compared to the vehicle-treated group but the reduction was not statistically significant. Fifteen minutes after the challenge, the erythema scores in the CT101_10%-treated group was significantly higher than in the vehicle-treated group ($p < 0.05$).

[0474] CT101 administered at 20% reduced the erythema scores from thirty minutes after the challenge when compared to the vehicle-treated group and the reduction was statistically significant twenty-nine hours after the challenge ($p < 0.05$).

[0475] Erythema scores were occasionally observed on the right (saline-injected) ears, especially on Day 1, twenty-five and twenty-nine hours after the challenge.

[0476] Figure 59 and Figure 60 depict erythema scores (challenged ears) at different times post-challenge.

Histopathology

[0477] The pathological changes were as expected in this model of atopic dermatitis: epidermal hyperplasia, dermal oedema and dermal inflammation. The inflammation is dominated by eosinophils and also variably includes neutrophils, macrophages and mast cells. All right ears and Group 1 left ears appeared essentially

normal. The remaining groups all showed pathological change to the left ears, which was maximum in Group 3 and then progressively less in Group 2, Groups 4 and 5, and then Group 6. Group 6 also has the fewest animals affected by left ear pathology ($n = 7$). Right and left ear scores were analysed by two-way ANOVA followed by Tukey's post-test for multiple comparisons between experimental groups. There was no significant difference between the right and left ears in the Control group. There was a significant increase of the histopathology scores for the left ears when compared to the right ears in all other experimental groups ($p < 0.0001$ except CT101_20% where $p < 0.01$). Right ear scores were analysed by Kruskal-Wallis test for non-parametric data followed by Dunn's post-test for multiple comparisons between experimental groups. There was no significant difference between experimental groups (Table 18).

Table 18: Histopathology Scores, Right ears

Group	Epidermal hyperplasia	Dermal oedema	Dermal inflammation	Total score
Control	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
Betamethasone 0.1%	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CT101 5%	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CT101 10%	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
CT101 20%	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1

[0478] Left ear scores were analysed by Kruskal-Wallis test for non-parametric data followed by Dunn's post-test for multiple comparisons between experimental groups.

[0479] The ovalbumin challenge induced a significant increase of the epidermal hyperplasia scores ($p < 0.01$), the dermal oedema scores ($p < 0.01$), the dermal inflammation scores ($p < 0.01$) and the total histopathology scores ($p < 0.001$) in the vehicle-treated group when compared to the control group (Figure 61).

[0480] Betamethasone 0.1% did not significantly reduce the histopathology scores.

[0481] CT101 applied at 5%, 10% or 20% induced a non-significant reduction of the dermal oedema but did not significantly reduce the epidermal hyperplasia, the dermal inflammation or the total histopathology scores.

[0482] Representative pictures in Figure 62 show, from left to right and from top to bottom: Mouse #1.5, normal left ear (score 0) and normal right ear (score 0);

Mouse #2.5 left ear with epidermal hyperplasia and inflammatory infiltration next to the auricular cartilage (score 6) and normal right ear (score 0); Mouse #3.8 left ear with epidermal hyperplasia, dermal oedema and marked eosinophilic infiltration of the dermis next to the auricular cartilage (score 6) and normal right ear (score 0).

[0483] Representative pictures in Figure 63 show, from left to right and from top to bottom: Mouse #4.9 left ear with epidermal hyperplasia and eosinophilic infiltration of the dermis close to the auricular cartilage (score 4) and normal right ear (score 0); Mouse #5.1 left ear with epidermal hyperplasia and mild eosinophilic infiltration of the dermis (score 3) and normal right ear with focal neutrophilic inflammation (score 1); Mouse #6.1 left ear with eosinophilic infiltration (score 1) and normal right ear (score 0).

CONCLUSIONS

[0484] Ovalbumin challenge induced erythema and ear swelling as expected in this mouse model of atopic dermatitis. Maximum response was seen fifteen minutes after the ovalbumin challenge. Ovalbumin also induced histopathological change: epidermal hyperplasia, dermal oedema and dermal inflammation, as expected in this model.

[0485] Betamethasone 0.1% applied topically reduced the ovalbumin-induced erythema and swelling but did not reduce the histopathology changes.

[0486] CT101 administered at 5%, 10% or 20% did not reduce the erythema scores at peak disease, but the 20% dose elicited a statistically significant erythema reduction at 29 h after the ovalbumin challenge.

[0487] CT101 administered at 10% or 20% reduced the ear swelling at peak disease. The reduction was statistically significant when CT101 was administered at 10%.

[0488] CT101 administered at 5%, 10% or 20% reduced the dermal oedema but did not significantly reduce the total histopathology scores.

[0489] CT101, administered at 20%, reduced the incidence of histopathological change.

Example 9: BioMAP Platform Analysis of SNA-101

Aim of Study

[0490] The goal of this study was to characterize SNA-101 in the BioMAP Diversity PLUS panel of 12 human primary cell-based systems. These systems are designed to model complex human tissue and disease biology of the vasculature, skin,

lung and inflammatory tissues. Quantitative measurements of biomarker activities across this broad panel, along with comparative analysis of the biological activities of known bioactive agents in the BioMAP reference database are used to predict the safety, efficacy and function of these test agents.

Overview of BioMAP Technology Platform

[0491] BioMAP panels consist of human primary cell-based systems designed to model different aspects of the human body in an in vitro format. The 12 systems in the Diversity PLUS panel allow test agent characterization in an unbiased way across a broad set of systems modeling various human disease states. BioMAP systems are constructed with one or more primary cell types from healthy human donors, with stimuli (such as cytokines or growth factors) added to capture relevant signaling networks that naturally occur in human tissue or pathological conditions. Vascular biology is modeled in both a Th1 (3C system) and a Th2 (4H system) inflammatory environment, as well as in a Th1 inflammatory state specific to arterial smooth muscle cells (CASM3C system). Additional systems recapitulate aspects of the systemic immune response including monocyte-driven Th1 inflammation (LPS system) or T cell stimulation (SAg system), chronic Th1 inflammation driven by macrophage activation (/Mphg system) and the T cell-dependent activation of B cells that occurs in germinal centers (BT system). The BE3C system (Th1) and the BF4T system (Th2) represent airway inflammation of the lung, while the MyoF system models myofibroblast-lung tissue remodeling. Lastly, skin biology is addressed in the KF3CT system modeling Th1 cutaneous inflammation and the HDF3CGF system modeling wound healing.

[0492] Each test agent generates a signature BioMAP profile that is created from the changes in protein biomarker readouts within individual system environments. Biomarker readouts (7 - 17 per system) are selected for therapeutic and biological relevance, are predictive for disease outcomes or specific drug effects and are validated using agents with known mechanism of action (MoA). Each readout is measured quantitatively by immune-based methods that detect protein (e.g., ELISA) or functional assays that measure proliferation and viability. BioMAP readouts are diverse and include cell surface receptors, cytokines, chemokines, matrix molecules and enzymes. In total, the Diversity PLUS panel contains 148 biomarker readouts that capture biological changes that occur within the physiological context of the particular BioMAP system.

[0493] Using custom-designed software containing data mining tools, a BioMAP profile can be compared against a proprietary reference database of > 4,000 BioMAP profiles of bioactive agents (biologics, approved drugs, chemicals and

experimental agents) to classify and identify the most similar profiles. This robust data platform allows rapid evaluation and interpretation of BioMAP profiles by performing the unbiased mathematical identification of similar activities. Specific BioMAP activities have been correlated to in vivo biology, and multiparameter BioMAP profiles have been used to distinguish compounds based on MoA and target selectivity and can provide a predictive signature for in vivo toxicological outcomes (e.g., vascular toxicity, developmental toxicity, etc.) across diverse physiological systems.

Materials and Methods

Test Agent

[0494] SNA-101 was profiled in the BioMAP Diversity PLUS panel at concentrations of 29 μ M, 9.7 μ M, 3.2 μ M, and 1.1 μ M. Apremilast was employed as the benchmark compound.

Methods for Diversity PLUS

[0495] Human primary cells in BioMAP systems are used at early passage (passage 4 or earlier) to minimize adaptation to cell culture conditions and preserve physiological signaling responses. All cells are from a pool of multiple donors ($n = 2$ to 6), commercially purchased and handled according to the recommendations of the manufacturers. Human blood derived CD14+ monocytes are differentiated into macrophages in vitro before being added to the /Mphg system. Abbreviations are used as follows: Human umbilical vein endothelial cells (HUVEC), Peripheral blood mononuclear cells (PBMC), Human neonatal dermal fibroblasts (HDFn), B cell receptor (BCR), T cell receptor (TCR) and Toll-like receptor (TLR).

[0496] Cell types and stimuli used in each system are as follows: 3C system [HUVEC + (IL-1 β , TNF α and IFN γ)], 4H system [HUVEC + (IL-4 and histamine)], LPS system [PBMC and HUVEC + LPS (TLR4 ligand)], SAg system [PBMC and HUVEC + TCR ligands], BT system [CD19+ B cells and PBMC + (α -IgM and TCR ligands)], BF4T system [bronchial epithelial cells and HDFn + (TNF α and IL-4)], BE3C system [bronchial epithelial cells + (IL-1 β , TNF α and IFN γ)], CASM3C system [coronary artery smooth muscle cells + (IL-1 β , TNF α and IFN γ)], HDF3CGF system [HDFn + (IL-1 β , TNF α , IFN γ , EGF, bFGF and PDGF-BB)], KF3CT system [keratinocytes and HDFn + (IL-1 β , TNF α and IFN γ)], MyoF system [differentiated lung myofibroblasts + (TNF α and TGF β)] and /Mphg system [HUVEC and M1 macrophages + Zymosan (TLR2 ligand)].

[0497] Systems are derived from either single cell types or co-culture systems. Adherent cell types are cultured in 96 or 384-well plates until confluence, followed by the addition of PBMC (SAg and LPS systems). The BT system consists of

CD19+ B cells co-cultured with PBMC and stimulated with a BCR activator and low levels of TCR stimulation. Test agents prepared in either DMSO (small molecules; final concentration $\leq 0.1\%$) or PBS (biologics) are added at the indicated concentrations 1-hr before stimulation, and remain in culture for 24-hrs or as otherwise indicated (48-hrs, MyoF system; 72-hrs, BT system (soluble readouts); 168-hrs, BT system (secreted IgG)). Each plate contains drug controls (e.g., legacy control test agent colchicine at $1.1 \mu\text{M}$), negative controls (e.g., non-stimulated conditions) and vehicle controls (e.g., 0.1% DMSO) appropriate for each system. Direct ELISA is used to measure biomarker levels of cell-associated and cell membrane targets. Soluble factors from supernatants are quantified using either HTRF® detection, bead-based multiplex immunoassay or capture ELISA. Overt adverse effects of test agents on cell proliferation and viability (cytotoxicity) are detected by sulforhodamine B (SRB) staining, for adherent cells, and alamarBlue® reduction for cells in suspension. For proliferation assays, individual cell types are cultured at subconfluence and measured at time points optimized for each system (48-hrs: 3C and CASM3C systems; 72-hrs: BT and HDF3CGF systems; 96-hrs: SAg system). Cytotoxicity for adherent cells is measured by SRB (24-hrs: 3C, 4H, LPS, SAg, BF4T, BE3C, CASM3C, HDF3CGF, KF3CT, and IMphg systems; 48-hrs: MyoF system), and by alamarBlue staining for cells in suspension (24-hrs: SAg system; 42-hrs: BT system) at the time points indicated. Additional information can be found in previous descriptions.

Data Analysis

[0498] Biomarker measurements in a test agent-treated sample are divided by the average of control samples (at least 6 vehicle controls from the same plate) to generate a ratio that is then log₁₀ transformed. Significance prediction envelopes are calculated using historical vehicle control data at a 95% confidence interval.

Profile Analysis

[0499] Biomarker activities are annotated when 2 or more consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope and have at least one concentration with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxic conditions are noted when total protein levels decrease by more than 50% ($\log_{10}$ ratio of SRB or alamarBlue levels < -0.3) and are indicated by a thin black arrow above the X-axis. A compound is considered to have broad cytotoxicity when cytotoxicity is detected in 3 or more systems. Concentrations of test agents with detectable broad cytotoxicity are

excluded from biomarker activity annotation and downstream benchmarking, similarity search and cluster analysis. Antiproliferative effects are defined by an SRB or alamarBlue log10 ratio value < -0.1 from cells plated at a lower density and are indicated by grey arrows above the X-axis. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation.

Benchmark Analysis

[0500] Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ in the same direction. Differentiating biomarkers are annotated when one profile has a readout outside of the significance envelope with an effect size $> 20\%$, and the readout for the other profile is either inside the envelope or in the opposite direction. Unless specified, the top non-cytotoxic concentration of both the test agent and benchmark agent are included in the benchmark overlay analysis.

Similarity Analysis

[0501] Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ in the same direction. Concentrations of test agents that have 3 or more detectable systems with cytotoxicity are excluded from similarity analysis. Concentrations of test agents that have 1 – 2 systems with detectable cytotoxicity will be included in the similarity search analysis, along with an overlay of the database match with the top concentration of the test agent. This will be followed by an additional overlay of the next highest concentration of the test agent containing no systems with detectable cytotoxicity and the respective database match. To determine the extent of similarity between BioMAP profiles of compounds run in the Diversity PLUS panel, we have developed a custom similarity metric (BioMAP Z-Standard) that is a combinatorial approach that has improved performance in mechanism classification of reference agents compared to other measures tested (including Pearson's and Spearman's correlation coefficients). This approach more effectively accounts for variations in the number of data points, systems, active biomarker readouts and the amplitude of biomarker readout changes that are characteristic features of BioMAP profiles. A Pearson's correlation coefficient (r) is first generated to measure the linear association between two profiles that is based on the similarity in the direction and magnitude of the relationship. Since the Pearson's correlation can be influenced by the magnitude of any biomarker activity, a per-system weighted average Tanimoto metric is used as a filter to account for underrepresentation of less robust systems. The Tanimoto metric does not consider the amplitude of

biomarker activity, but addresses whether the identity and number of readouts are in common on a weighted, per system basis. A real-value Tanimoto metric is calculated

first by normalizing each profile to the unit vector (e.g., $A = \frac{A}{\|A\|}$) and then applying the

following formula: $\frac{A \cdot B}{\|A\| + \|B\| - A \cdot B}$, where A and B are the 2 profile vectors. Then, it is incorporated into a system weighted-averaged real-value Tanimoto metric in this

calculation: $\frac{\sum W_i \cdot T_i}{\sum W_i}$. The calculation uses the real-value Tanimoto score for each *i*th system (*T_i*) and the weight of each *i*th system (*W_i*). *W_i* is calculated for each system in

the following formula: $\frac{1}{1 + e^{-100 * (lr - 0.09)}}$, where *lr* is the largest absolute value of the ratios from the 2 profiles being compared. Based on the optimal performance of reference compounds, profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient (*r*) ≥ 0.7 . Finally, a Fisher *r*-to-*z*-transformation is used to calculate a *z*-score to convert a short tail distribution into a normal distribution as

follows: $z = 0.5 \log_{10} \frac{1+r}{1-r}$. Then the BioMAP Z-Standard, which adjusts for the number of common readouts (CR), is generated according to the following formula: Z-Standard = $z \sqrt{CR-3}$. A larger BioMAP Z-Standard value corresponds to a higher confidence level, and this is the metric used to rank similarity results.

Cluster Analysis

[0502] Cluster analysis (function similarity map) uses the results of pairwise correlation analysis to project the "proximity" of agent profiles from multi-dimensional space into two dimensions. Functional clustering of the agent profiles generated during this analysis uses Pearson correlation values for pairwise comparisons of the profiles for each agent at each concentration, and then subjects the pairwise correlation data to multidimensional scaling. Profiles that are similar with a Pearson's correlation coefficient (*r*) ≥ 0.7 are connected by lines. Agents that do not cluster with one another are interpreted as mechanistically distinct. This analysis is performed for projects with 3 or more agents tested. Cytotoxic concentrations are excluded from cluster analysis.

Mechanism HeatMAP Analysis

[0503] Mechanism HeatMAP analysis provides a visualization of the test compound and 19 consensus mechanisms allowing comparison of biomarker activities across all compound concentrations and consensus mechanisms. The synthetic consensus profiles used in the Mechanism HeatMAP analysis are representative BioMAP profiles of the average of multiple compounds from structurally distinct chemical classes. Profiles were calculated by averaging the values for each biomarker endpoint for all profiles selected (multiple agents at different concentrations) to build the consensus mechanism profile.[8] Biomarker activities are colored in the heatmap for consensus mechanisms and compounds when they have expression relative to vehicle controls outside of the significance envelope. Red represents increased protein expression, blue represents decreased expression and white indicates levels that were unchanged or within filtering conditions. Darker shades of color represent greater change in biomarker activity relative to vehicle control. The Mechanism HeatMAP was prepared using R and the gplots package for R.

Assay Acceptance Criteria

[0504] A BioMAP assay includes the multi-parameter data sets generated by the BioMAP platform for agents tested in the systems that make up the Diversity PLUS panel. Assays contain drug controls (e.g., legacy control test agent colchicine), negative controls (e.g., non-stimulated conditions), and vehicle controls (e.g., DMSO) appropriate for each system. BioMAP assays are plate-based, and data acceptance criteria depend on both plate performance (% CV of vehicle control wells) and system performance across historical controls for that system. The QA/QC Pearson Test is performed by first establishing the 1% false negative Pearson cutoff from the reference dataset of historical positive controls. The process iterates through every profile of system biomarker readouts in the positive control reference dataset, calculating Pearson values between each profile and the mean of the remaining profiles in the dataset. The overall number of Pearson values used to determine the 1% false negative cutoff is the total number of profiles present in the reference dataset. The Pearson value at the one percentile of all values calculated is the 1% false negative Pearson cutoff. A system will pass if the Pearson value between the experimental plate's negative control or drug control profile and the mean of the historical control profiles in the reference dataset exceeds this 1% false negative Pearson cutoff. Overall assays are accepted when each individual system passes the Pearson test and 95% of all project plates have % CV <20%.

Results

BioMAP Profile

[0505] Figure 64 depicts the BioMAP profile of SNA-101 in the Diversity PLUS Panel. SNA-101 was found to be modestly active with 3 annotated readouts, mediating changes in key biomarker activities listed by biological and disease classifications in Table 19 below. SNA-101 impacted inflammation-related activities (decreased SAA; increased sPGE2) and tissue remodeling activities (increased Collagen I).

[0506] There were no cytotoxic or antiproliferative impacts detected at the concentration range tested.

Table 19: Key Biomarker Activities Impacted by SNA-101

Biological and Disease Relevance Category	Decreased Activity	Increased Activity
Inflammation-related activities	SAA	sPGE2
Tissue remodeling activities		Col-I

Reference Benchmark Overlay

[0507] Figure 65 depicts an overlay of SNA-101 at 29 μ M and the selected reference benchmark apremilast at 10 μ M. Apremilast is a PDE4 inhibitor approved for the treatment of plaque psoriasis and psoriatic arthritis. The two agents did not have any common activities that meet the defined criteria for annotation.

[0508] Differentiating biomarkers (not shown) are defined when one profile has a readout outside of the significance envelope with an effect size > 20% ($|\log_{10} \text{ratio}| > 0.1$), and the readout for the other profile is either inside the envelope or in the opposite direction. There are 12 differentiating activities between the two compounds: LPS (sTNF α), SAg (CD69, E-selectin, MCP-1), BT (sIL-6, sIgG), CASM3C (Prolif), HDF3CGF (Collagen III, PAI-1), MyoF (Collagen I), and IMphg (MIP-1 α , sIL-10).

Top Database Search Result for SNA-101

[0509] In a search for mathematically similar compound profiles from the BioMAP reference database, SNA-101 (29 μ M) was most similar to topiramate (3.3 μ M) (Pearson's correlation, $r = 0.344$). The Pearson's correlation coefficient between these two profiles is below our determined threshold ($r < 0.7$) indicating that the relevance of the similarity is

unknown. Topiramate is an anticonvulsant drug that is used to treat epilepsy. The two agents do not have any common activities that meet the defined criteria for annotation. Figure 66 depicts an overlay of SNA-101 (29 μ M) and topiramate (3.3 μ M).

Top BioSeek Reference Database Matches for SNA-101

[0510] Table 20 depicts the top 3 similarity matches from a search of the BioMAP Reference Database of > 4,000 agents for each concentration of SNA-101. The similarity between agents is determined using a combinatorial approach that accounts for the characteristics of BioMAP profiles by filtering (Tanimoto metric) and ranking (BioMAP Z-Standard) the Pearson's correlation coefficient between two profiles. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 . For profiles with a Pearson's correlation coefficient below our determined threshold ($r < 0.7$), the relevance of the similarity is unknown.

Table 20: Top BioMAP Reference Database Matches for SNA-101

SNE_SNA-101	Database Match	BioMap Z-Standard	Pearson's Score	# of Common Readouts	Database Match (Mechanism Class)
29 μ M	Topiramate, 3.3 μ M	4.216	0.344	141	Antiepileptic Agent
	Bosutinib, 41 nM	4.090	0.327	148	
	Nafcillin Sodium, 1.1 μ M	3.894	0.313	148	Antimicrobial Agent
9.7 μ M	Ritonavir, 3.3 μ M	4.113	0.329	148	HIV Type 1 Protease Inhibitor
	Neomycin Sulfate, 6.3 μ M	4.057	0.325	148	Bacterial 70S Ribosome Inhibitor
	CGS 21680, μ M	3.832	0.371	100	A2A Agonist
3.2 μ M	Cobicistat, 1.1 μ M	4.334	0.345	148	Pharmacokinetic Enhancer
	Ritonavir, 3.3 μ M	4.199	0.335	148	HIV Type 1 Protease Inhibitor
	TAPN-Bombesin, 10 μ M	4.848	0.408	148	BBR1/BBR2/BBR3 Agonist
1.1 μ M	Albendazole, 10 μ M	4.599	0.364	148	Anthelmintic Agent

	Paclitaxel, 30 μ M	4.581	0.363	148	Microtubule Stabilizer
	Albendazole, 30 μ M	4.454	0.354	148	Anthelmintic Agent

Mechanism HeatMAP Analysis of SNA-101

[0511] Figure 67 depicts Mechanism HeatMAP Analysis of SNA-101, with the 148 biomarker readouts within the Diversity PLUS panel compared to 19 consensus mechanism class profiles. This analysis informs on the regulatory mechanisms controlling increases or decreases in each of the biomarker readouts.

Conclusions

[0512] In this study SNA-101 was characterized by profiling in the BioMAP Diversity PLUS panel of human primary cell based assays modeling complex tissue and disease biology of organs (vasculature, immune system, skin, lung) and general tissue biology. The Diversity PLUS panel evaluates the biological impact of test agents in conditions that preserve the complex crosstalk and feedback mechanisms that are relevant to in vivo outcomes. SNA-101 was largely inactive in the Diversity PLUS panel, with no cytotoxic or antiproliferative effects observed at the concentration range tested. A modest increase in sPGE2 and Collagen I expression was observed, as was a modest decrease in serum amyloid A expression. Overall, the profile of SNA-101 was not similar to the benchmark Apremilast.

Example 10: Additional BioMAP Platform Analysis of SNA-101

Aim of Study

[0513] The goal of this study was to repeat the characterization of SNA-101 in the BioMAP Diversity PLUS panel of 12 human primary cell-based systems using higher concentrations of SNA-101 than employed in Example 9.

Materials and Methods

Test Agent

[0514] SNA-101 was profiled in the BioMAP Diversity PLUS panel at concentrations of 300 μ M, 100 μ M, 33 μ M, and 11 μ M. Staurosporine was employed as the benchmark compound.

Methods and Analysis

[0515] BioMAP Platform Analysis was performed as described in Example 9.

Results

BioMAP Profile

[0516] Figure 68 depicts the BioMAP profile of SNA-101 in the Diversity PLUS Panel. SNA-101 was found to be modestly active with 4 annotated readouts, mediating changes in key biomarker activities listed by biological and disease classifications in Table 21 below. SNA-101 impacted inflammation-related activities (decreased sTNF α , IL-8, IL-1 α) and a tissue remodeling activity (decreased MMP-1). SNA-101 is antiproliferative to endothelial cells at the top concentration only (grey arrow of Figure 68). SNA-101 had no cytotoxic effects in the concentration range tested.

Table 21: Key Biomarker Activities Impacted by SNA-101

Biological and Disease Relevance Category	Decreased Activity
Inflammation-related activities	IL8, IL1a, sTNFa
Tissue remodeling activities	MMP1

Reference Benchmark Overlay

[0517] Figure 69 depicts an overlay of SNA-101 at 300 μ M and the selected reference benchmark staurosporine at 10 nM. Staurosporine is a broad-spectrum protein kinase inhibitor that acts on protein kinase C (PKC), protein kinase A (PKA), p60v-src tyrosine protein kinase, and CaM kinase II (CAMKII). Staurosporine is an analog of K252a.

[0518] There are 4 common activities that are annotated within the following systems: 3C (Prolif), BT (sIL-6, sTNF α), and HDF3CGF (PAI-1).

[0519] Differentiating biomarkers (not shown) are defined when one profile has a readout outside of the significance envelope with an effect size > 20% ($|\log_{10} \text{ratio}| > 0.1$).

and the readout for the other profile is either inside the envelope or in the opposite direction. There are 65 differentiating activities within the following systems: 3C (HLA-DR, TF, uPAR), 4H (Eotaxin 3, MCP-1, P-selectin, VEGFR2, uPAR), LPS (CD40, CD69, M-CSF, MCP-1, TF, VCAM-1, sTNF α), SAg (CD40, CD69, E-selectin, IL-8, MCP-1, Prolif), BT (sIL-17A, sIL-17F, sIL-2, sIgG), BF4T (IL-1 α , MCP-1, PAI-1, VCAM-1), BE3C (IL-8, MMP-1, MMP-9, PAI-1, tPA, uPA, uPAR), CASM3C (M-CSF, MCP-1, Prolif, SAA, TF, uPAR), HDF3CGF (MCP-1, MIG, MMP-1, Prolif 72), KF3CT (MCP-1, PAI-1), MyoF (Collagen I, Collagen III, Collagen IV, MMP-1, PAI-1, TIMP-1, VCAM-1), and IMphg (CD40, CD69, E-selectin, IL-1 α , IL-8, M-CSF, MCP-1, MIP-1 α , VCAM-1, sIL-10).

Top Database Search Result for 300 μ M SNA-101

[0520] In a search for mathematically similar compound profiles from the BioMAP reference database, SNA-101 (300 μ M) was most similar to N037 (490 ng/ml) (Pearson's correlation, $r = 0.760$). SNA-101 and N037 share 84 readouts in 8 common BioMAP systems. The Pearson's correlation coefficient between these two profiles is above our determined threshold ($r \geq 0.7$) indicating these compounds share mechanistically relevant similarity. N037 [($\pm$)-Norketamine-D4 HCl] is the most prevalent urinary metabolite of ketamine, a widely used anesthetic sold under trade names, including Ketanest, Ketaset and Ketalar. There is 1 common activity that is annotated within the following system: 3C (Prolif). Figure 70 depicts an overlay of SNA-101 (300 μ M) and N037 (490 ng/ml).

Top Database Search Result for 100 μ M SNA-101

[0521] In a search for mathematically similar compound profiles from the BioMAP reference database, SNA-101 (100 μ M) was most similar to infliximab (30000 ng/ml) (Pearson's correlation, $r = 0.424$). The Pearson's correlation coefficient between these two profiles is below our determined threshold ($r < 0.7$) indicating that the relevance of the similarity is unknown. Remicade (infliximab) is a chimeric monoclonal antibody against TNF alpha approved for the treatment of psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis and ulcerative colitis. There are 4 common activities that are annotated within the following systems: BT (sTNF α) and IMphg (E-selectin, IL-1 α , IL-8). Figure 71 depicts an overlay of SNA-101 (100 μ M) and infliximab (30000 ng/ml).

Top BioSeek Reference Database Matches for SNA-101

[0522] Table 22 depicts the top 3 similarity matches from a search of the BioMAP Reference Database of > 4,000 agents for each concentration of SNA-101. The similarity between agents is determined using a combinatorial approach that accounts for the characteristics of BioMAP profiles by filtering (Tanimoto metric) and ranking (BioMAP Z-Standard) the Pearson's correlation coefficient between two profiles. Profiles are identified

as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 . For profiles with a Pearson's correlation coefficient below our determined threshold ($r < 0.7$), the relevance of the similarity is unknown.

Table 22: Top BioMAP Reference Database Matches for SNA-101

SNE_SNA-101	Database Match	BioMap Z-Standard	Pearson's Score	# of Common Readouts	Database Match (Mechanism Class)
300 μ M	N037, 490 ng/ml	8.956	0.760	84	Anesthetic
	Embelin, 1.1 μ M	8.829	0.630	145	Caspase-9 Activator
	17alpha-Estradiol, 10 μ M	8.239	0.718	86	ERalpha Agonist
100 μ M	Infliximab, 30000 ng/ml	5.218	0.424	148	TNF-alpha Antagonist
	Infliximab, 10000 ng/ml	5.186	0.406	148	TNF-alpha Antagonist
	Troxipide, 10 μ M	4.969	0.391	100	Gastric Cytoprotective Agent
33 μ M	Dabrafenib, 3.3 μ M	5.550	0.431	148	B-RafV600E Antagonist
	Dabrafenib, 10 μ M	5.472	0.429	148	B-RafV600E Antagonist
	Infliximab, 100 ng/ml	5.472	0.426	148	TNF-alpha Antagonist
11 μ M	Ritonavir, 370 nM	5.520	0.429	148	HIV Type 1 Protease Inhibitor
	Meticrane, 1.1 μ M	5.101	0.400	148	Diuretic Agent
	Fluticasone Propionate, 3780 nM	5.013	0.394	148	GR Agent

[0523] The Pearson's correlation coefficient between profiles that is above our determined threshold ($r \geq 0.7$) indicates these compounds share mechanistically

relevant similarity. For profiles with a Pearson's correlation coefficient below our determined threshold ($r < 0.7$), the relevance of the similarity is unknown.

Mechanism HeatMAP Analysis of SNA-101

[0524] Figure 72 depicts Mechanism HeatMAP Analysis of SNA-101, with the 148 biomarker readouts within the Diversity PLUS panel compared to 19 consensus mechanism class profiles. This analysis informs on the regulatory mechanisms controlling increases or decreases in each of the biomarker readouts.

Conclusions

[0525] In this study SNA-101 was characterized by profiling in the BioMAP Diversity PLUS panel of human primary cell based assays modeling complex tissue and disease biology of organs (vasculature, immune system, skin, lung) and general tissue biology. The Diversity PLUS panel evaluates the biological impact of test agents in conditions that preserve the complex crosstalk and feedback mechanisms that are relevant to in vivo outcomes. SNA-101 was modestly active in the Diversity PLUS panel with only 4 annotated readouts that impact inflammation and tissue-remodeling activities. SNA-101 had no cytotoxic effects at the concentration range tested. Antiproliferative effects to endothelial cells were observed at the top tested concentration of 300 μ M, but not at the lower concentrations. SNA-101 was also previously profiled in the Diversity PLUS panel at a lower concentration range (1.1 μ M - 29 μ M, refer to Example 9) where it was minimally active. SNA-101 and the selected reference benchmark staurosporine, a broad-spectrum kinase inhibitor, had only 4 common activities, but 65 differentiating activities. In general, staurosporine at 10 nM was more active than SNA-101 at 300 μ M. The top database search match for SNA-101 was N037, a metabolite of the anesthetic ketamine. However, the only common activity between the two profiles was the antiproliferative effect on endothelial cells, an activity only observed for the top concentration of SNA-101. An overlay with the second concentration of SNA-101 and its top match is also provided, although the Pearson's correlation coefficient did not meet our threshold for significance.

Example 11: Mouse Model of IMQ-induced Psoriasis

Aim of Study

[0526] The objective of this study was to determine the efficacy of SNA-101, SNA-103, and SNA-352 as a therapeutic in the mouse model of IMQ-induced psoriasis.

Methodology

Test groups and experimental timing

[0527] Table 23 depicts the test groups and Figure 73 depicts the timing of the experiments performed in this study.

Table 23: Test Groups

Group Number	Group Size	Group Description	Disease Induction	TI/vehicle Route	TI/vehicle Dose Level	Dosing Route/Regimen
1	N=5	Naïve	NA	NA	NA	NA
2	N=10	IMQ Only	5% IMQ on back and on both ears daily	NA	NA	NA
3	N=10	Clobetasol		Topical	50mg applied to backs, 5 mg applied to each ear	1×daily, 2 hrs post IMQ application
4	N=10	Vehicle		Topical	100 µl applied to back, 10 µl applied to each ear	2×daily, 2 and 8 hrs post IMQ application
5	N=10	1.0% SNA-352				
6	N=10	5.0% SNA-352				
7	N=10	10.0% SNA-352				
8	N=10	5.0% SNA-125				
9	N=10	10.0% SNA-125				
10	N=10	20.0% SNA-101				

Psoriasis Clinical Scoring

[0528] The animals were examined for signs of psoriasis on study day 0. These scores served as a baseline for the psoriasis clinical score parameter. Starting from IMQ cream application on day 0, psoriasis responses were examined daily until termination of the study.

[0529] Psoriasis reactions (erythema and plaques) were scored based on the parameters shown in Table 24 and recorded according to a 0-12 scale. The clinical score is determined by summing the score of each section.

Table 24: Psoriasis Clinical Scoring Parameters

<u>Psoriasis Score</u>					
Plaques	<u>Grade</u>	Erythema	<u>Grade</u>	<u>Punctate redness/scabbing</u>	<u>Grade</u>
Normal	0	Normal	0	No red dots	0
Very few plaques (cover about 5-10% of the back area).	1	Slight	1	Very few (1-10) red dots present on the entire back	1
Several plaques (cover about 20%-30% of the back area).	2	Moderate	2	Red dots are diffuse and cover ~50% of the back (10-25 red dots)	2
Moderate plaques (cover about 50% of the back area).	3	Marked	3	Red dots cover the entire back and are more concentrated (>25 red dots)	3
Spread plaques (cover over 90% of the back area).	4	Extreme	4		
Plaques are no longer detected, however the skin is irritated and no fur is growing on the skin	5				

Results***Psoriasis clinical score***

[0530] The total psoriasis score was determined by summing the plaque score, the erythema score and the punctate redness/scabbing score. As seen in Figure 74, the difference between SNA-125 at 5% and the vehicle is statistically significant on day 8 and 10, while for SNA-125 at 10% is significant from day 8 to day 10. Further, a

statistically significant difference between SNA-352 at 5% and the vehicle on day 10 was found, as well as for SNA-125 at 10% from day 8 to day 10. Further, the difference between SNA-101 at 20% and the vehicle is statistically significant from day 8 to day 10.

Erythema scores

[0531] As shown in Figure 75, the differences between SNA-125 at 5% and 10% and the vehicle are statistically significant from day 8 to day 10. Further, statistically significant differences were found between SNA-352 at 5% and 10% and the vehicle from day 8 to day 10. Additionally, the difference between SNA-101 at 20% and the vehicle is statistically significant from day 8 to day 10.

Plaque scores

[0532] As shown in Figure 76, the difference between SNA-125 at 5% and the vehicle is statistically significant from day 5 to day 8, while for SNA-125 at 10% it is significant at day 8. Additionally, a statistically significant difference between SNA-352 at 10% and the vehicle was observed from day 5 to day 8. Additionally, the difference between SNA-101 at 20% and the vehicle is statistically significant at day 8.

Punctate redness/scabbing scores

[0533] As shown in Figure 77, the difference between SNA-352 at 10% and the vehicle is statistically significant on day 9. Additionally, a statistically significant difference between SNA-101 at 20% and the vehicle was observed on day 9 and day 10.

Spleen weight and ear thickness

[0534] Topical application of the IMQ cream causes the enlargement of spleen and lymph nodes, and increased ear thickness. The commonly used antipsoriatic agent clobetasol almost completely attenuated these IMQ-induced changes. Neither treatments with vehicle or SNA-125, SNA-352, SNA-101 significantly modulated spleen weight (Figure 78A). There was no difference on ear thickness found between the vehicle and the different doses of SNA-125, SNA-352 and SNA-101 (Figure 78B). Figure 78C depicts the daily weight of mice throughout the study.

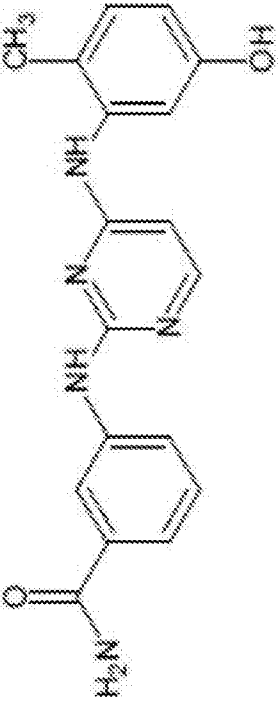
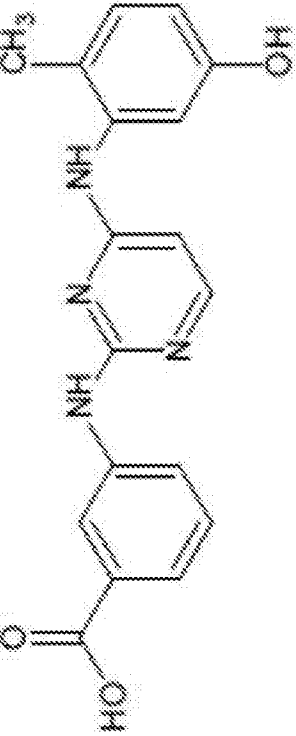
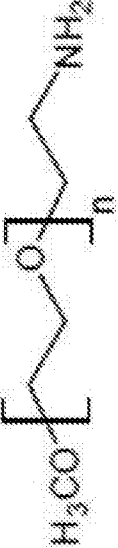
Cytokine analysis

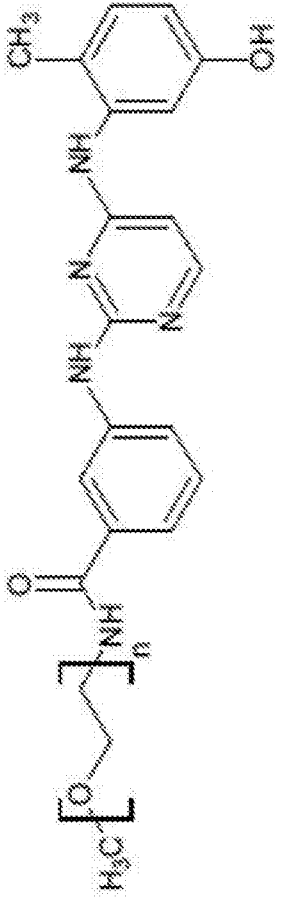
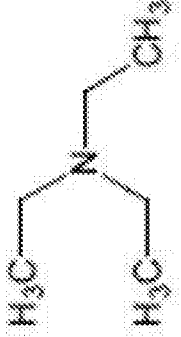
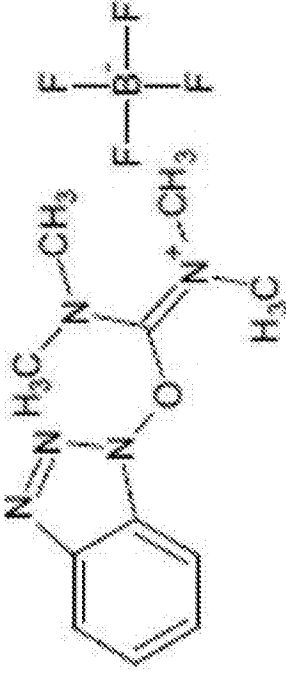
[0535] Left ears were biopunched on day 4 and after tissue homogenization, the levels of cytokines IL-17F, TNF- α , IL-22, and IL-17A in the tissue lysates were measured via multiplex and then normalized with total protein amounts. Mean values for each group are displayed in Figure 79.

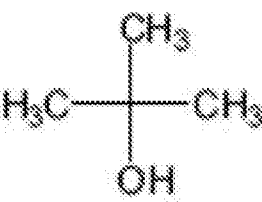
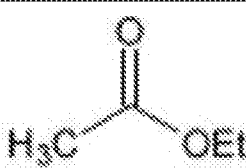
Example 12: Synthesis of SNA-101

1. Abbreviations

[0536] The following abbreviations are used in this example:

IUPAC name (nickname)	Formula	MW
3-({4-[(5-hydroxy-2-methylphenyl)amino]pyrimidin-2-yl}amino)benzamide (Benzamide)		335.36
3-({4-[(5-hydroxy-2-methylphenyl)amino]pyrimidin-2-yl}amino)benzoic acid (Carboxylic acid intermediate)		336.34
α -methoxy- ω -{2-aminoethyl}poly(ethyleneoxy) (mPEG ₂₀₀₀ -NH ₂)		(for n=43) 1969.37 (Mp 1964)

IUPAC name (nickname)	Formula	MW
3-({4-((5-hydroxy-2-methylphenyl)amino)pyrimidin-2-yl}amino)-N-mPEG ₂₀₀₀ benzamide (CT101)		(for n=44) 2287.70 (Mw 2282)
Triethylamine (TEA)		101.19
O-(1H-benzotriazole-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU)		321.08

IUPAC name (nickname)	Formula	MW
Dichloromethane (DCM)	CH_2Cl_2	84.93
2-Methylpropan-2-ol (tBuOH)		74.12
Ethyl acetate (AcOEt)		88.11
Acetonitrile (ACN)	$\text{H}_3\text{C}-\text{C}\equiv\text{N}$	41.05
Diethyl ether		74.12

Introduction

[0537] This example is directed to a scaled-up synthetic procedure for SNA-101 (Figure 80). As depicted in Figure 81, the selected synthetic scheme consists of Benzamide hydrolysis to the carboxylic acid intermediate (Step 1) and amidation with mPEG2000-NH₂ (Step 2).

[0538] Two batches of CT101 were produced: the first batch generated two CT101 samples, 1.2 g with 99.7% HPLC purity and 0.7 g with 97.1% HPLC purity, with 53% overall yield from the starting benzamide; the second batch generated 16.2 g with 57% overall yield from the starting benzamide, and 99.6% HPLC purity.

[0539] CT101 yield has been calculated assigning it 2282 Da as molecular weight (CT101 molecular weight has been calculated according to the following formula: CT101 molecular weight= Benzamide nominal mass + MeO-PEG₂₀₀₀-NH₂ peak molecular weight - 17), as the peak molecular weight of MeO-PEG₂₀₀₀-NH₂ used for its preparation was 1964 Da (The MeO-PEG₂₀₀₀-NH₂ peak molecular weight was obtained from the certificate of analysis provided by the supplier).

2. Experimental procedure for CT101 synthesis

[0540] The synthetic protocol consisted, according to the previously described studies, of:

[0541] 1) hydrolysis reaction in tBuOH, with 40 equivalents of 1.4% NaOH aqueous solution, at 70°C;

[0542] 2) amidation reaction in DCM, with 1.35 equivalents of mPEG₂₀₀₀-NH₂, 2.5 equivalents of TBTU and 1.5 equivalents of TEA, at 25°C;

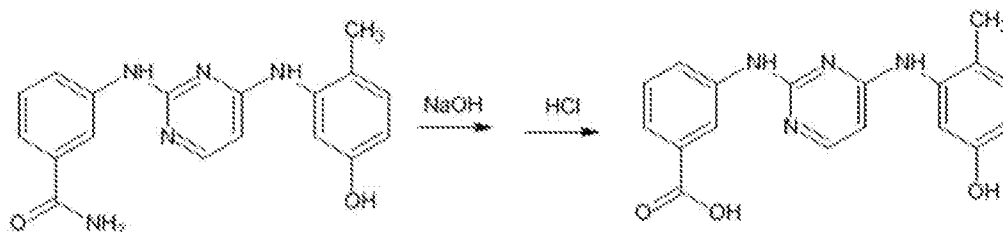
[0543] 3) purification by reversed phase flash chromatography using water and acetonitrile as eluent phases.

[0544] The procedure was first verified on a smaller scale (use test, lot n° 2017CG07/S2) to test starting materials, then a representative CT101 sample was synthesized (lot n° 2017CG07/S4). The results are depicted in Table 25. The experimental procedure used for the production of CT101 sample, lot n° 2017CG07/S4, is hereinafter described.

Table 25: Results of CT101 production

LOT N°	CT101 AMOUNT, g	HPLC PURITY, %	YIELD FROM BENZAMIDE, %
2017CG07/S2A	1,2	99.7	53
2017CG07/S2B	0,7	97.1	
2017CG07/S4	16,2	99.6	57

3.1 Step 1: Benzamide Hydrolysis



Material List

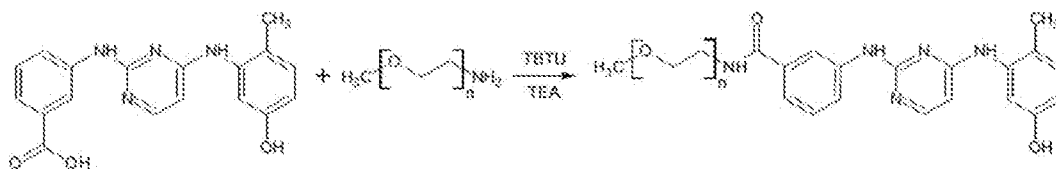
Reagents	MW	g	mmol	d	ml	Ratio	
Benzamide lot n° Z150605	335,35	4,196	12,5			1	molar
1,4% NaOH	40,00	1429,8	500,5		1430	40	molar
tBuOH	74,12	435,8	5879,0	0,775	562	134	ml/g benzamide
<i>Theoretical product</i>							
Carboxylic acid intermediate	336,34	4,208	12,5			1,0	molar

Procedure

[0545] In a 3.0 L four necked jacketed reactor, equipped with mechanical stirring, thermometer and condenser, 4.20 g of benzamide (Figure 82), 562 ml of tBuOH and 1430 g of 1.4% NaOH aqueous solution were placed at 25°C. The mixture was heated under stirring to 70°C and aged for 69 h. The reaction progress was monitored by HPLC analyses taking samples during aging time (Figure 83). The reaction mixture was cooled to 25°C, diluted with 900 ml of H₂O, and then about 810 g of solvent were removed under reduced pressure at 42°C. The residual aqueous solution (1960 g) was extracted with 1 L of AcOEt. The aqueous phase was diluted with 500 ml of AcOEt and neutralized under stirring with 15% HCl to pH 5. The organic phase was concentrated by rotavapor distillation under reduced pressure at 40°C. The residual solid was further dried by suspending it in 200 ml of DCM and evaporating the solvent under reduced pressure at 40°C. This drying operation was repeated twice.

[0546] 3.37 g of carboxylic acid intermediate as a white-yellow solid with 98.7% HPLC purity (@ 270 nm) were obtained (Figure 84). The reaction yield was 79%.

3.2 Step 2: Carboxylic acid intermediate amidation



Material List

Reagents	MW	g	mmol	d	ml	Ratio	
Carboxylic acid intermediate, 98.7%	336.34	3.37	9.89			1	
mPEG ₂₀₀₀ -NH ₂ lot n° 1216167	1964	26.6	13.54			1.37	molar
TEA	101.19	2.6	25.22	0.73	3.50	2.55	molar
TBTU	321.08	4.8	14.96			1.51	molar
DCM	84.93	1275	15007	1.327	960	285	ml/g carboxylic acid
Theoretical product							
CT101	2782.34	22.868	10.02			1	molar

Procedure

[0547] In a 1 L round bottom flask 3.37 g of carboxylic acid intermediate from the previous step, 26.6 g of mPEG₂₀₀₀-NH₂ and 600 ml of DCM were placed at 25°C. The solvent was evaporated under reduced pressure at 40°C. This drying operation was repeated twice.

[0548] The dried solid residue was dissolved in 960 ml of DCM. The solution was placed in a 3 L four necked jacketed reactor, equipped with mechanical stirring, thermometer and condenser and protected from light by aluminum foil wrapping. 3.5 ml of TEA were added at 25°C. The solution was aged under stirring at 25°C for 10 minutes, then 4.8 g of TBTU were added. The reaction mixture was aged at 20°C for 20 h under nitrogen atmosphere.

[0549] The reaction progress was monitored by HPLC analyses, taking samples during aging time (Figure 85). The final reaction mixture was transferred to a 2 L separating funnel, and washed with NaCl aqueous saturated solution (2 x 700 ml). The organic phase was concentrated under reduced pressure at 40°C. 32 g of crude CT101 as a light orange solid with 96% HPLC purity (@ 270 nm) were obtained (Figure 86).

3.2 Step 3: Purification of CT101

[0550] Crude CT101 divided into five portions and was purified by reversed-phase flash chromatography using a Biotage Isolera LS System equipped with a Biotage SNAP KP-C18-HS cartridge packed with 400 g of KP-C18-HS Silica (column volume 510

ml). The cartridge was equilibrated at 100 ml/min. with 1020 ml of acetonitrile/water 24:76 v/v.

[0551] Sample loading was performed by dissolving the crude CT101 sample (about 6.4 g per purification) in 20 ml of H₂O and injecting it onto the cartridge through a syringe.

[0552] The *SNAP cartridge* was eluted at 100 ml/min with:

[0553] 1) 1020 ml of acetonitrile/water 24:76 v/v;

[0554] 2) 1020 ml from acetonitrile/water 24:76 v/v to acetonitrile/water 34:66 v/v;

[0555] 3) 1938 ml of acetonitrile/water 34:66 v/v;

[0556] 4) 1020 ml of acetonitrile/water 60:40 v/v.

[0557] The first portion of eluate (2550 ml) was sent to the waste, then the eluted solvent was collected in fractions of 150 ml each. UV profile (@ 270 and 210 nm) of the purification is depicted in Figure 87. Collected individual fractions were analyzed by HPLC. Fractions from each purification with HPLC purity $\geq 98.0\%$ were combined and concentrated under reduced pressure at 40°C to remove acetonitrile. The residual aqueous solution was extracted with DCM (3 x 300 ml). NaCl (a spoonful) was added during the first extraction to help phase separation. The combined organic phases were evaporated under reduced pressure at 40°C to dryness, affording about 3.6 g of a light yellow solid from each purification. The five samples obtained from purifications were combined (18.8 g), treated with 150 ml of diethyl ether and aged for 0.5 h at 25°C and 1 h at 5°C. The precipitated solid was filtered over sintered glass filter (G3), washed with 10 ml of diethyl ether and dried under vacuum at 25°C for 16 h to afford 16,2 g of CT101 as an off white solid (lot n° 2017CG07/S4).

[0558] Purity of CT101, determined by HPLC analysis (method MI CT101 002), was 99.43% (Figure 88). The product was characterized by ¹H-NMR and ESI MS analyses (Figures 89-91). The Certificate of analysis of the CT101 sample is depicted in Figure 92.

[0559] Following the same procedure, 1.2 g of CT101 as an off white solid (lot n° 2017CG07/S2A) were obtained. Purity of CT101, determined by HPLC analysis (method MI CT101 002), was 99.67% (Figure 93). The product was characterized by ¹H-NMR and ESI MS analyses (Figures 94-96). The certificate of analysis of the CT101 sample is shown in Figure 97.

[0560] The final CT101 samples were stored at -22°C.

4. Analytical methods

4.1 HPLC method MI CT101 001

[0561] The following method has been used both for reaction monitoring and for assessing chemical purity of isolated intermediates.

Sample preparation

Reaction monitoring

20-50 µl of reaction mixture were evaporated under nitrogen flow and diluted with 0.5-1.0 ml of 10 mM HCOONH₄ pH 4/acetonitrile 1:1 v/v.

Isolated products

1.0 -1.5 mg/ml solutions in a mixture of 10 mM HCOONH₄ pH 4/acetonitrile 1:1 v/v were prepared.

Fractions from reversed phase flash chromatography

100-200 µl were diluted with 10 mM HCOONH₄ pH 4/acetonitrile 1:1 v/v.

Injected volume

5 µl

HPLC

Agilent 1100

Detector

UV 270 nm

Column

Zorbax Eclipse XDB-C18 Solvent Saver - 3 x 150 mm - 5 µm

Temperature

25°C

Flow

0.5 ml/min

Mobile phases

A: 10 mM HCOONH₄ pH 4

B: acetonitrile

Gradient

Time, min.	%A	B %
0	95	5
3	95	5
11	70	30
36	20	80
38	95	5

Benzamide

Retention time 17.0 min. RRT 0.85

Carboxylic acid intermediate

Retention time 19.0 min. RRT 0.95

CT101

Retention time 19.9 min. RRT 1

4.2 HPLC method MI CT101 002

[0562] The following method has been used for assessing chemical purity of final CT101 samples.

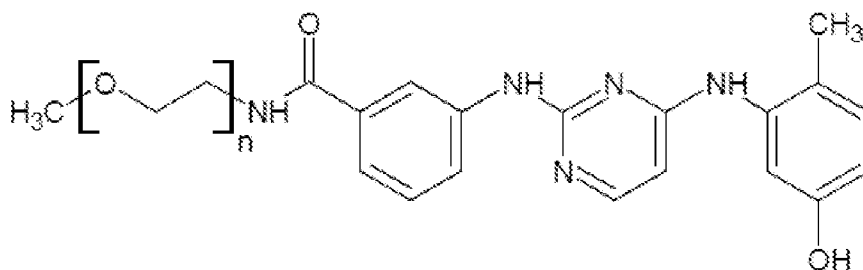
<i>Sample preparation</i>	1.0-1.5 mg/ml solutions in a mixture of 10 mM HCOONH ₄ pH 4/acetonitrile 1:1 v/v were prepared.
<i>Injected volume</i>	5 µl
<i>HPLC</i>	Agilent 1100
<i>Detector</i>	UV 270 nm
<i>Column</i>	Zorbax Eclipse XDB-C18 Solvent Saver - 3 x 150 mm - 5 µm
<i>Temperature</i>	25°C
<i>Flow</i>	0.5 ml/min
<i>Mobile phases</i>	A: 10 mM HCOONH ₄ pH 4 B: acetonitrile

<i>Gradient</i>	Time, min.	%A	B %
	0	90	10
	3	90	10
	23	20	80
	30	20	80
	32	90	10

<i>CT101</i>	Retention time	15.6 min.
--------------	----------------	-----------

WHAT IS CLAIMED IS:

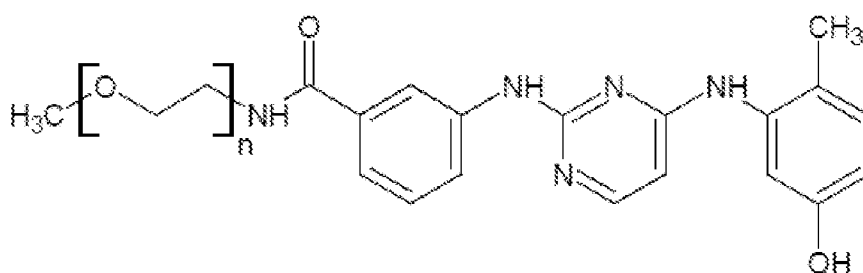
1. A compound having the formula:



wherein n is 4-1140;

and any pharmaceutically acceptable salts thereof.

2. A reduced exposure composition for treating a target site, comprising a conjugate comprising an active entity linked to at least one polymer, wherein the conjugate has reduced exposure at a non-target site as compared to the active entity delivered without the polymer, wherein the non-target site comprises the systemic system, the lymphatic system and/or another non-target tissue site, wherein the conjugate has the formula:



wherein n is 4-1140;

and any pharmaceutically acceptable salts thereof; and

a pharmaceutically acceptable carrier formulated for delivery of the conjugate to the target site.

3. A reduced exposure composition for treating a cell within a target site, comprising a conjugate, the conjugate comprising an active entity linked to at least one polymer, and a pharmaceutically acceptable carrier formulated for delivery of the conjugate to the target site;

wherein the composition has reduced exposure at a non-target site as compared to the active entity delivered without the polymer;

wherein the active entity is an inhibitor, antagonist, or inverse agonist of a cellular kinase;

wherein the active entity is selected from any one of compounds 1-71;

wherein the at least one polymer is polyethylene glycol (PEG) or methoxy-polyethylene glycol (m-PEG); and

wherein the conjugate can traverse the cell membrane and distribute among both lipophilic and hydrophilic cellular compartments within the cell, thereby promoting interactions between the active entity and the cellular kinase.

4. The composition of claim 3, wherein the non-target site includes non-target tissue at which pharmacological activity is not desired and/or not achieved.

5. The composition according to any one of claims 3-4, wherein the active entity comprises compound 1.

6. The composition according to any one of claims 3-5, wherein the composition comprises SNA-101.

7. The composition according to any one of claims 3-6, wherein the cellular kinase is c-Src.

8. The composition according to any one of claims 3-7, wherein the active entity binds to c-Src.

9. The composition according to any one of claims 3-8, wherein the active entity inhibits c-Src.

10. The composition according to any one of claims 3-9, wherein the active entity has one or more carboxyl, hydroxyl, amino and/or sulfhydryl groups.

11. The composition of claim 10, wherein the at least one polymer is conjugated to the active entity at the one or more carboxyl, hydroxyl, amino and/or sulfhydryl groups.

12. The composition of any one of claims 3-11, wherein the conjugate has a longer residence time within the cell compared to the active entity without conjugation to the polymer.

13. The composition of claim 12, wherein the residence time of the conjugate is at least 25% longer as compared to the active entity without conjugation to the polymer.

14. The composition of claim 12, wherein the residence time of the conjugate is at least 2-20 fold longer as compared to the active entity without conjugation to the polymer.

15. The composition of any one of claims 3-14, wherein the conjugate exhibits greater access to the kinase compared to the active entity without conjugation to the polymer.

16. The composition of any one of claims 3-15, wherein the conjugate exhibits a depo effect across cellular compartments, thereby reducing the dose of the active

entity required to inhibit kinase activity compared to the active entity without conjugation to the polymer.

17. The composition of claim 16, wherein the dose of the conjugate needed to achieve a comparable therapeutic effect is 10-90% lower as compared to the active entity without conjugation to the polymer.

18. The composition of any one of claims 3-17, wherein the activity entity has a concentration, activity and/or bioavailability at the target site that is at least 2-20 fold greater than at a non-target site, wherein the non-target site comprises the circulatory system.

19. The composition of any one of claims 3-18, wherein the activity entity has a concentration, activity and/or bioavailability at the target site that is at least 2-20 fold greater than at a non-target site, wherein the non-target site comprises the lymphatic system.

20. The composition of Claim 18 or 19, wherein the reduced concentration, activity and/or bioavailability reduces toxicity.

21. The composition of any one of claims 3-17, wherein the activity entity has a concentration, activity and/or bioavailability at the target site that is at least 2-20 fold greater than at a non-target site, wherein the non-target site comprises bone marrow.

22. The composition of Claim 21, wherein the reduced concentration, activity and/or bioavailability in the bone marrow reduces immunosuppression.

23. The composition of any one of claims 3-22, wherein the conjugate is present at a biologically inactive concentration at a non-target site.

24. The composition of any one of claims 3-23, wherein the conjugate is amphiphilic

25. The composition of any one of claims 3-24, wherein the conjugate is at least 25% more amphiphilic than the active entity without conjugation to the polymer.

26. The composition of any one of claims 3-25, wherein the conjugate is at least 25% more hydrophilic than the active entity without conjugation to the polymer, thus facilitating non-compartmentalization within the cell.

27. The composition of any one of claims 3-26, wherein the conjugate is at least 25% more hydrophilic than the active entity without conjugation to the polymer, thus facilitating access to and activity in both the lipid bilayer and the cytosol of the cell.

28. The composition of any one of claims 3-27, wherein the conjugate is at least 25% more hydrophilic than the active entity without conjugation to the polymer, thus facilitating access to and/or activity in both the lipid bilayer and the cytoplasm of the cell.

29. The composition of any one of claims 3-28, wherein the conjugate is at least 25% more hydrophilic than the active entity without conjugation to the polymer, thus

facilitating access to and/or activity across the lipid bilayer.

30. The composition according to any one of claims 2-29, wherein the composition is formulated for topical administration.

31. The composition according to any one of claims 2-29, wherein the composition is formulated as an inhalant.

32. The composition according to any one of claims 2-29, wherein the composition is formulated as an injectable.

33. The composition according to any one of claims 2-29, wherein the composition is formulated as an eye drop.

34. The composition according to any one of claims 2-29, wherein the composition is formulated for oral administration.

35. The composition according to any one of claims 2-29, wherein the composition is administered via at least two routes of administration, either simultaneously or sequentially.

36. The composition according to any one of claims 2-29, wherein said composition is administered via a topical route to a subject, and wherein the subject further receives an additional agent via a non-topical route to achieve synergetic effects.

37. The composition according to any one of claims 2-36, the composition further comprising one or more additional ingredients from the group consisting of a protective agent, an emollient, an astringent, a humectant, a sun screening agent, a sun tanning agent, a UV absorbing agent, an antibiotic agent, an anti-angiogenesis agent, a preventive or therapeutic agent for inflammatory bowel disease, a physiological cooling agent, an antifungal agent, an antiviral agent, an antiprotozoal agent, an anti-acne agent, an anesthetic agent, a steroidal anti-inflammatory agent, a non-steroidal anti-inflammatory agent, an antipruritic agent, an additional antioxidant agent, a chemotherapeutic agent, an anti-histamine agent, a vitamin or vitamin complex, a hormone, an anti-dandruff agent, an anti-wrinkle agent, an anti-skin atrophy agent, a skin whitening agent, and a cleansing agent.

38. A method for treating an inflammatory condition in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

39. A method for treating an inflammatory skin condition in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

40. A method for treating a wound in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

41. A method for treating a scar in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

42. A method for treating a cancerous or pre-cancerous lesion in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

43. A method for treating a lung in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

44. A method for treating the gastrointestinal system in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

45. A method for treating an autoimmune disorder in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

46. A method for treating an eye in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

47. A method for treating a joint in a subject in need thereof, the method comprising administering to the subject an effective amount of a composition according to any one of claims 2-37.

48. Use of the composition of any one of claims 2-37 for treating non-dermal inflammation in a subject in need thereof.

49. Use of the composition of any one of claims 2-37 for treating an inflammatory skin disease in a subject in need thereof.

50. Use of the composition of any one of claims 2-37 for treating a wound in a subject in need thereof.

51. Use of the composition of any one of claims 2-37 for treating a scar in a subject in need thereof.

52. Use of the composition of any one of claims 2-37 for treating a cancerous or pre-cancerous lesion in a subject in need thereof.

53. Use of the composition of any one of claims 2-37 for treating a lung in a subject in need thereof.

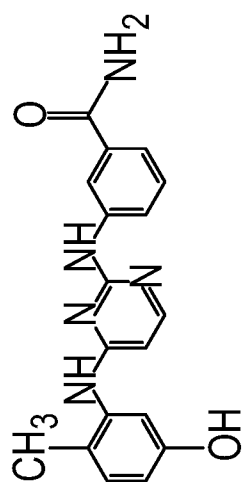
54. Use of the composition of any one of claims 2-37 for treating the gastrointestinal system in a subject in need thereof.

55. Use of the composition of any one of claims 2-37 for treating an autoimmune disorder in a subject in need thereof.

56. Use of the composition of any one of claims 2-37 for treating an eye in a subject in need thereof.

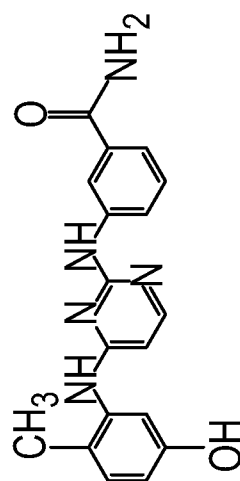
57. Use of the composition of any one of claims 2-37 for treating a joint in a subject in need thereof.

58. Use of the composition of any one of claims 2-37 for treating or preventing one or more of the following conditions: psoriasis, psoriasis guttata, inverse psoriasis, pustular psoriasis, psoriatic erythroderma, acute febrile neutrophilic dermatosis, eczema, xerotic eczema, dyshidrotic eczema, vesicular palmar eczema, acne vulgaris, atopic dermatitis, contact dermatitis, allergic contact dermatitis, dermatomyositis, exfoliative dermatitis, hand eczema, pompholyx, rosacea, rosacea due to sarcoidosis, rosacea due to scleroderma, rosacea due to Sweet syndrome, rosacea due to systemic lupus erythematosus, rosacea due to urticaria, rosacea due to herpetic pain, Sweet's disease, neutrophilic hydrodenitis, sterile pustule, drug rash, seborrheic dermatitis, pityriasis rosea, Kikuchi's disease of the skin, pruritic urticarial papules and plaques of pregnancy, Stevens-Johnson syndrome and toxic epidermal necrolysis, tattoo reaction, Wells syndrome (eosinophilic cellulitis), reactive arthritis (Reiter syndrome), bowel-associated dermatosis-arthritis syndrome, rheumatoid neutrophilic dermatosis, neutrophilic eccrine hidradenitis, neutrophilic skin disease of dorsum of hand, balanitis circumscripta plasmacellularis, balanoposthitis, Behcet's disease, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiforme, granuloma annulare, dermatitis of hand, lichen nitidus, lichen planus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, nummular dermatitis, pyoderma gangrenosum, sarcoidosis, subcorneal pustular dermatosis, urticaria, skin fibrosis, and transient acantholytic dermatosis, alopecia, alopecia areata, androgenic alopecia, and dry eye.

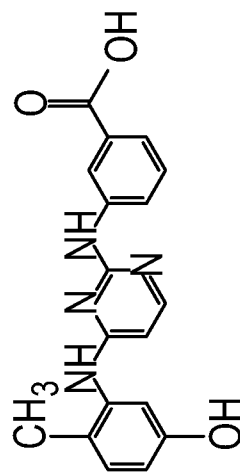


Chembl 249097

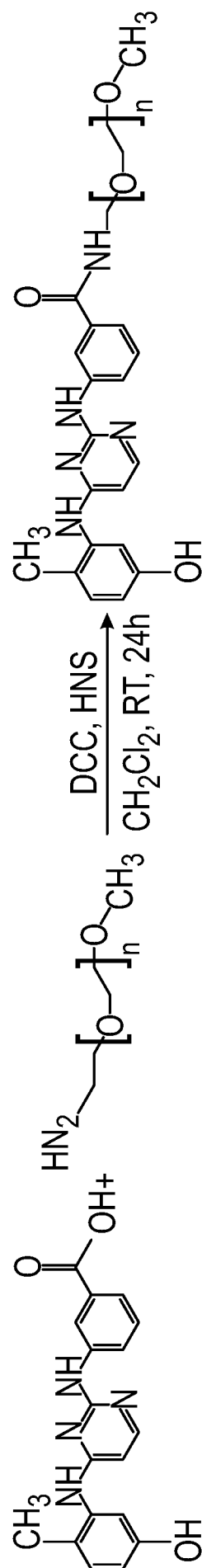
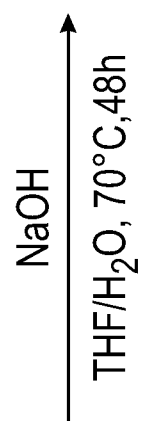
(CAS RN 944795-06-6)

FIG. 1

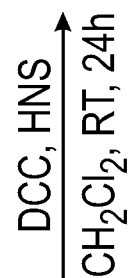
Chembl 249097 (CAS 944795-06-6, Benzamide 1)

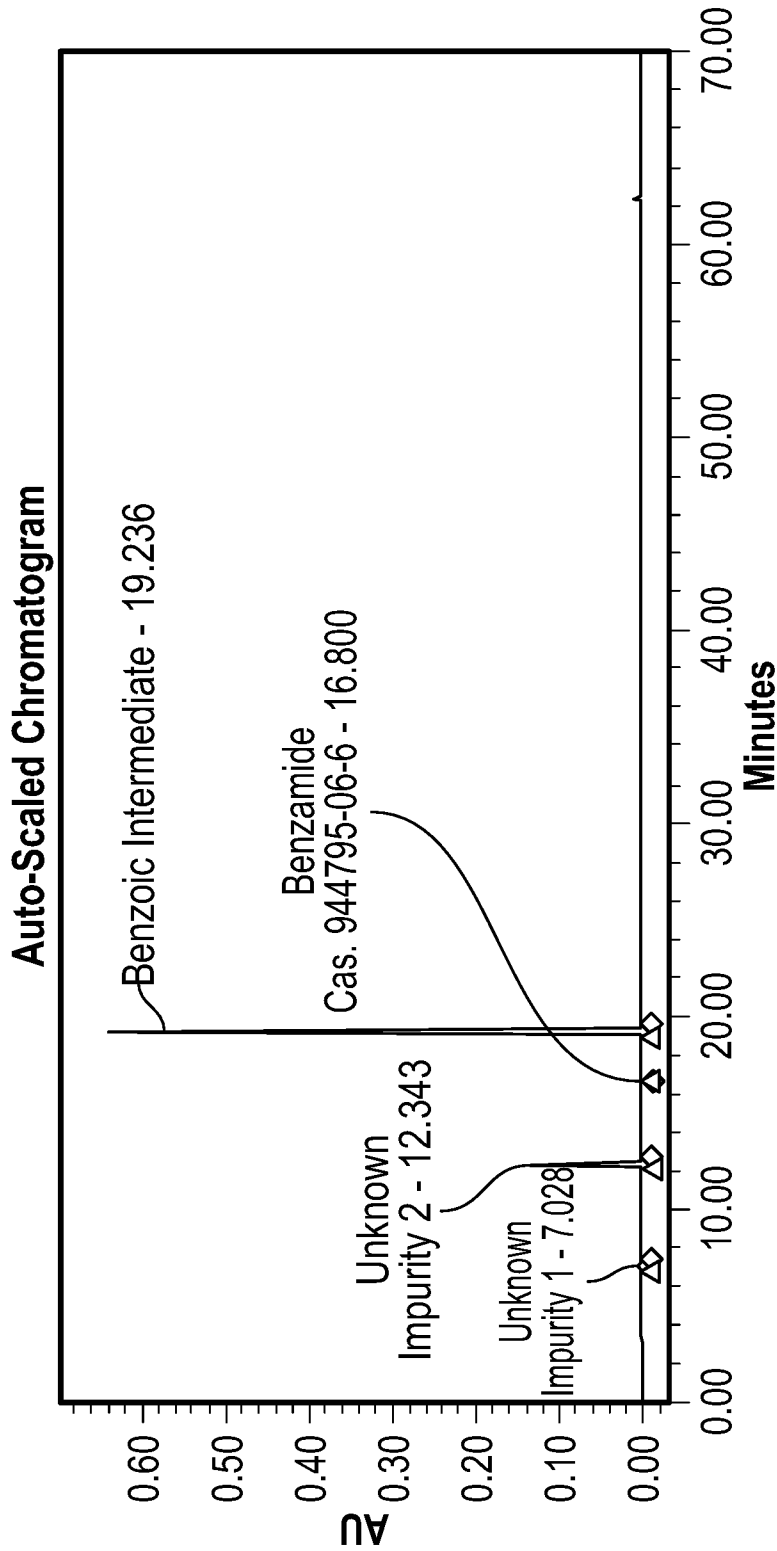


(Carboxylic Intermediate 2)



CT101

**FIG. 2**



Peak Results

	Name	RT	Area	Height	Amount	Units	% Area
1	Unknown Impurity 1	7.028	253643	30763			4.71
2	Unknown Impurity 2	12.343	1036164	160134			19.22
3	Benzamide Cas. 944795-06-6	16.800	24757	4354			0.46
4	Benzoic Intermediate	19.236	4075887	659253			75.61

FIG. 3

Cartridge	SNAP Ultra 10g	Detection Mode	UV1+UV2
Flowrate	12 ml/min	UV1 (Collection)	280 nm
Solvent A	Dichloromethane	UV2 (Collection)	254 nm
Solvent B	Ethyl acetate	Collect All	On
		Slope Mode	Low Slope
Rack Type	16x150 mm Creabillis		
Max Fraction Volume	18 ml		
Dispense Order	S		

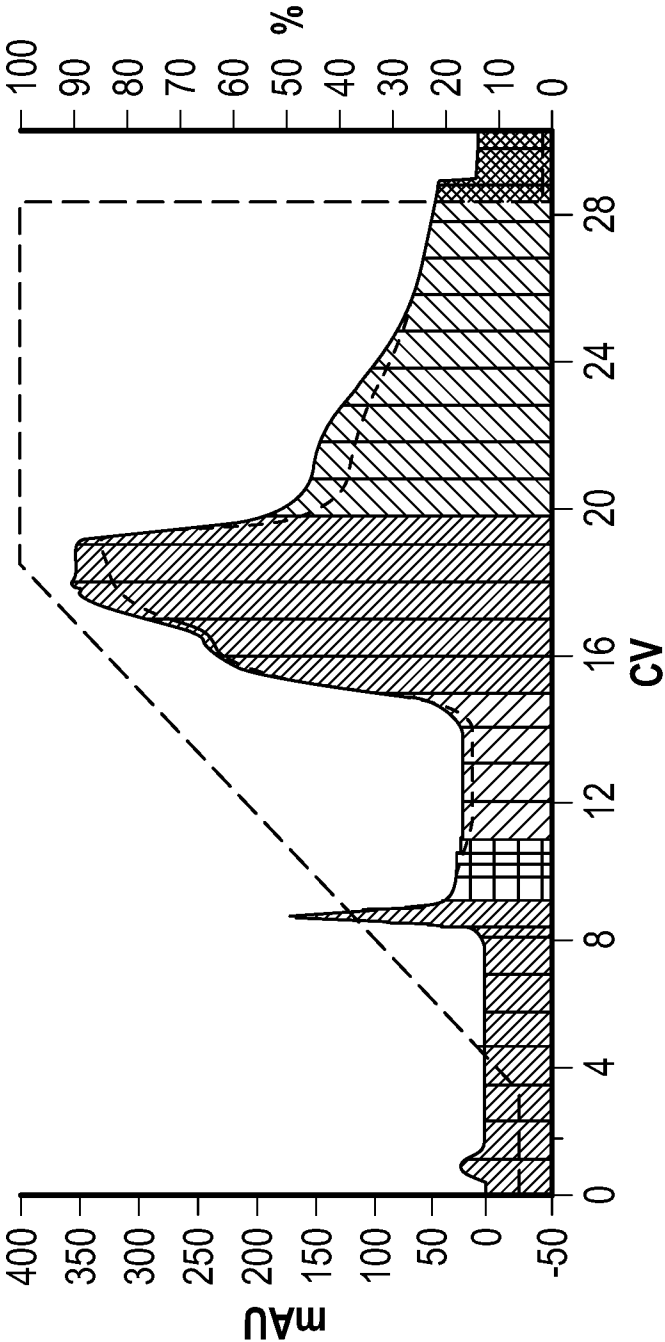


FIG. 4

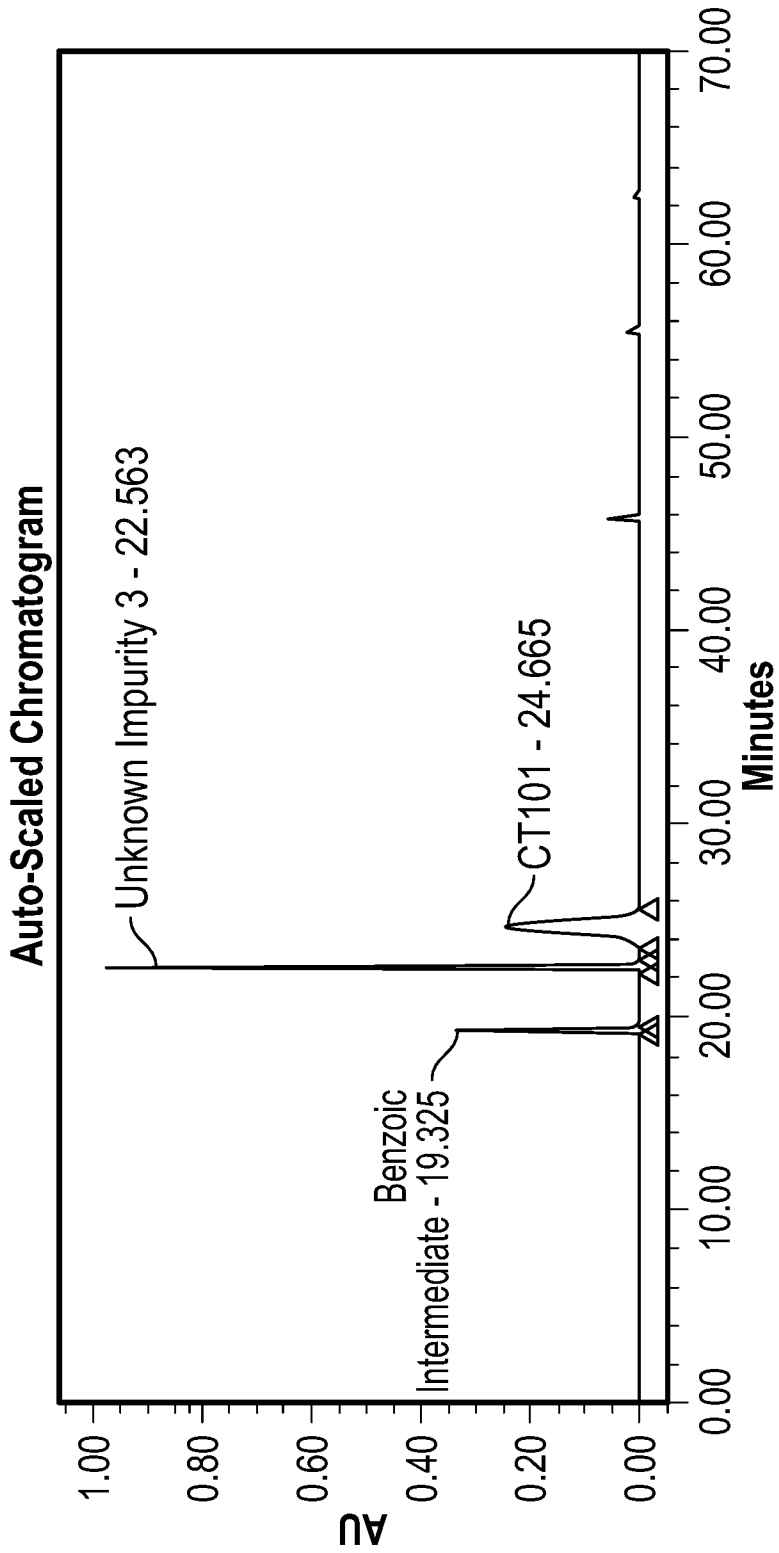
Gradient

	Solvents	Mix	Length (CV)	
Equil.	A/B	5%	3.0	Flowrate 12 ml/min
1	A/B	5%	3.0	
2	A/B	5% - 100%	15.0	
3	A/B	100%	2.0	
4	A/B	100%	4.7	
5	A/B	100%	3.4	Isocratic Hold Enabled
6	A/B	100%	0.2	Isocratic Hold Disabled

Log

Occurred At	Message
24.8 CV	Enabled Isocratic Hold
28.2CV	Disabled Isocratic Hold

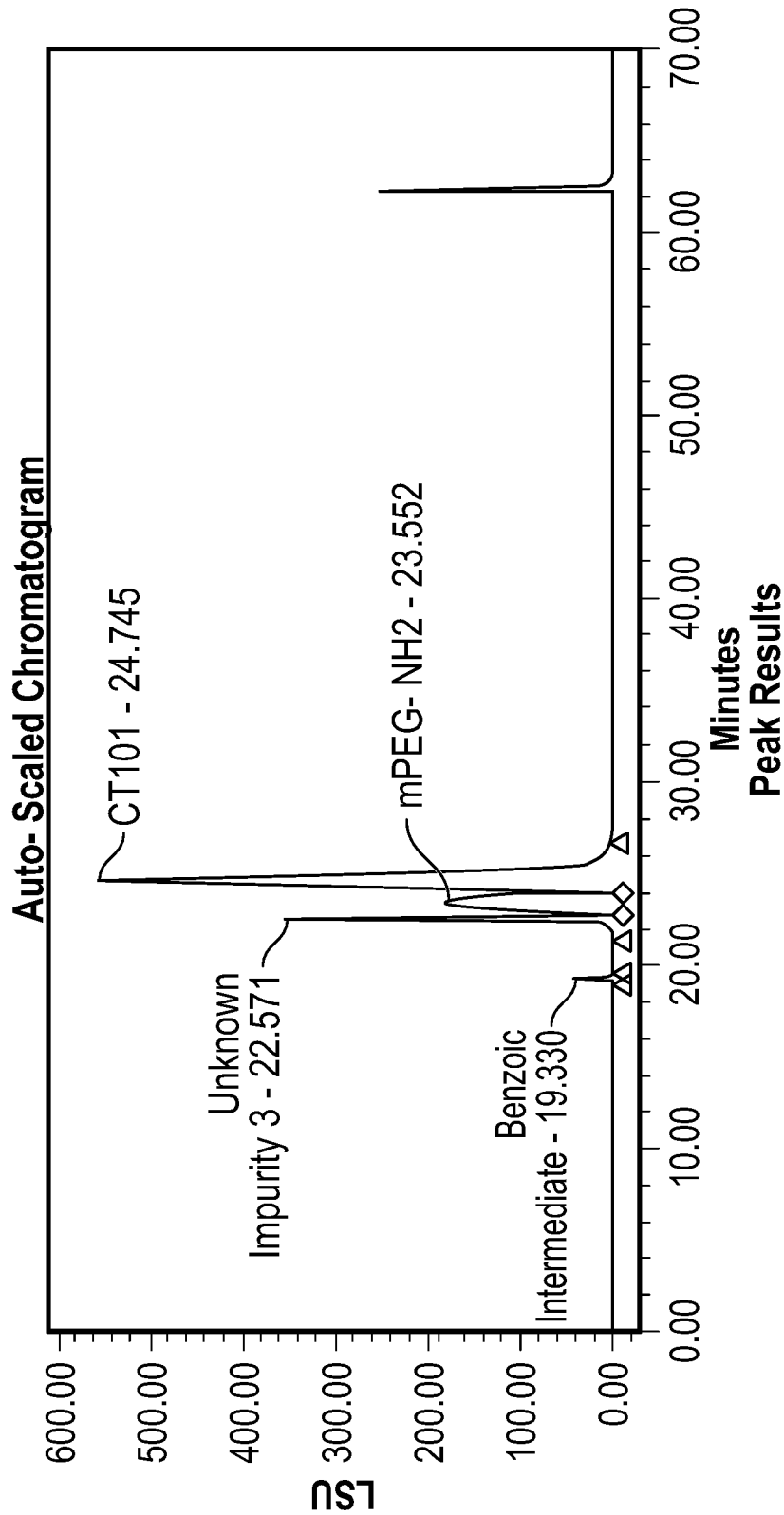
FIG. 4
(Continued)



Peak Results

	Name	RT	Area	Height	Amount	Units	% Area
1	Benzoic Intermediate	19.325	2239687	365214			11.01
2	Unknown Impurity 3	22.563	7223857	1012047			35.53
3	CT 101	24.665	10870521	254381			53.46

FIG. 5



	Name	RT	Area	Height	Amount	Units	% Area
1	Benzoic Intermediate	19.330	449479	61204			1.13
2	Unknown Impurity 3	22.571	4171192	366896			10.52
3	mPEG-NH2	23.552	9361843	185136			23.61
4	CT101	24.745	25662166	574043			64.73

FIG. 6

Cartridge	SNAP Ultra 10g	UV1+UV2
Flowrate	12 ml/min	280 nm
Solvent A	Dichloromethane	214 nm
Solvent B	Methanol	On
Rack Type	16x150 mm Creabillis	Low Slope
Max Fraction Volume	18 ml	
Dispense Order	S	
		Detection Mode
		UV1 (Collection)
		UV2 (Collection)
		Collect All
		Slope Mode

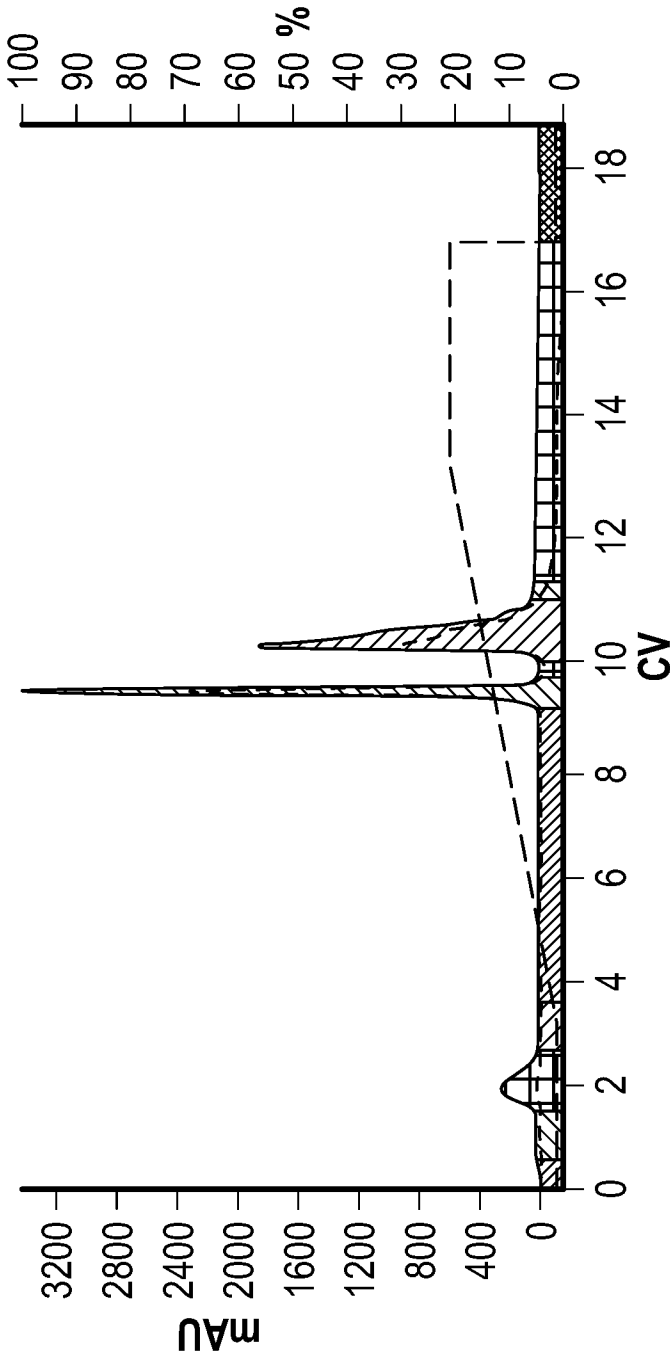


FIG. 7

Gradient			
	Solvents	Mix	Length (CV)
Equil.	A/B	0%	3.0
1	A/B	0%	3.0
2	A/B	0% - 20%	10.0
3	A/B	20%	4.0

FIG. 7
(Continued)

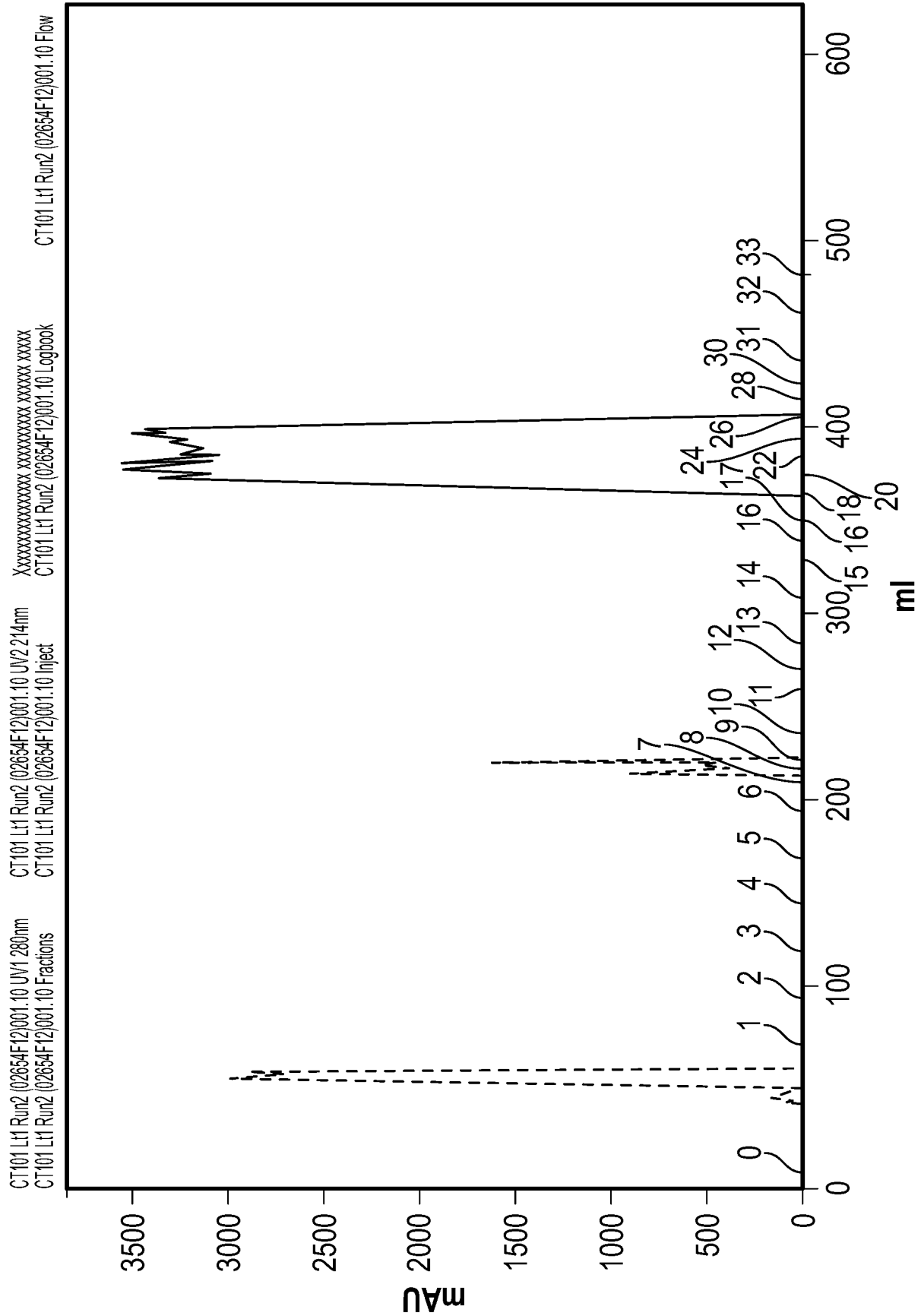


FIG. 8

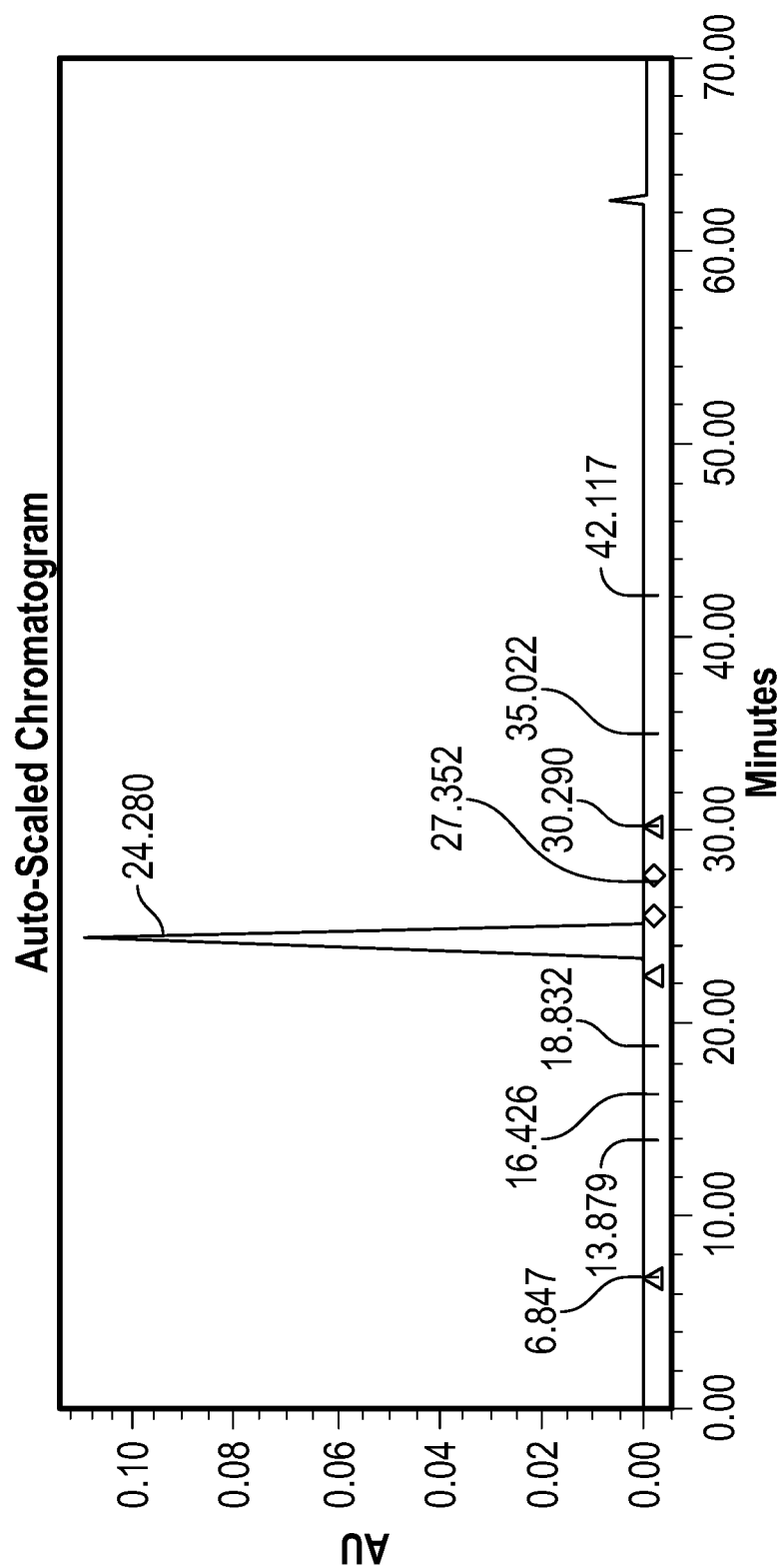


FIG. 9

Peak Results

	Name	RT	Area	Height	Amount	Units	% Area
1		6.847	660	70			0.01
2		13.879	117	26			0.00
3		16.426	137	27			0.00
4		18.832	1536	271			0.03
5		24.280	4513350	109021			99.50
6		27.352	18561	586			0.41
7		30.290	976	117			0.02
8		35.022	478	51			0.01
9		42.117	228	33			0.01

FIG. 9
(Continued)

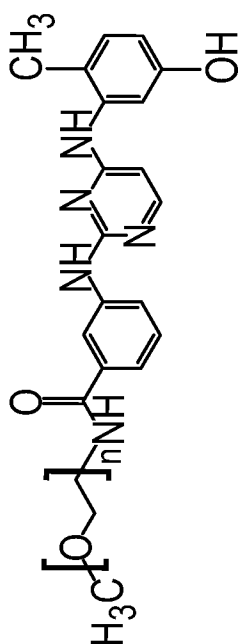
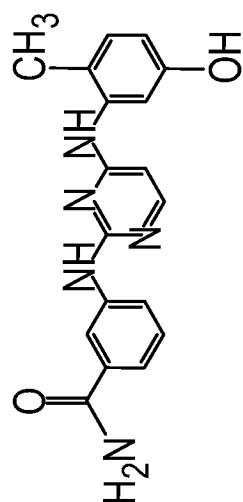
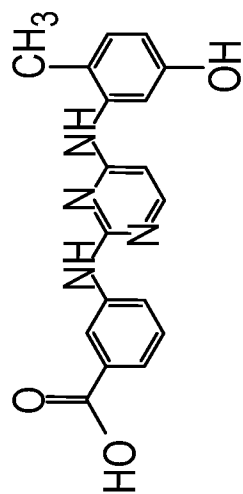


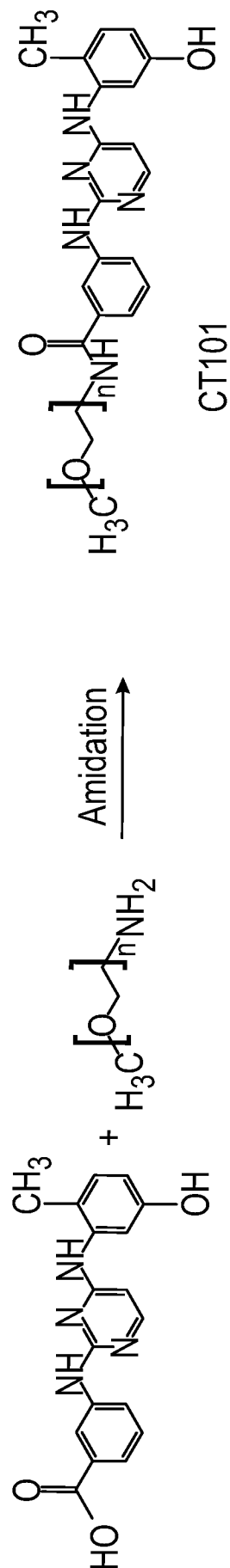
FIG. 10



Benzamide



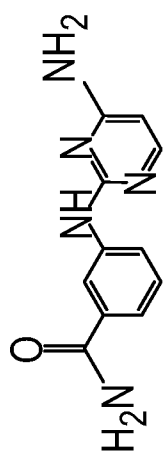
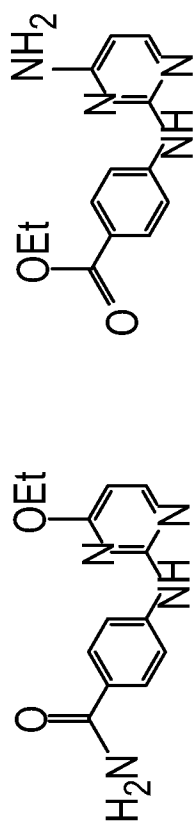
Carboxylic Acid Intermediate



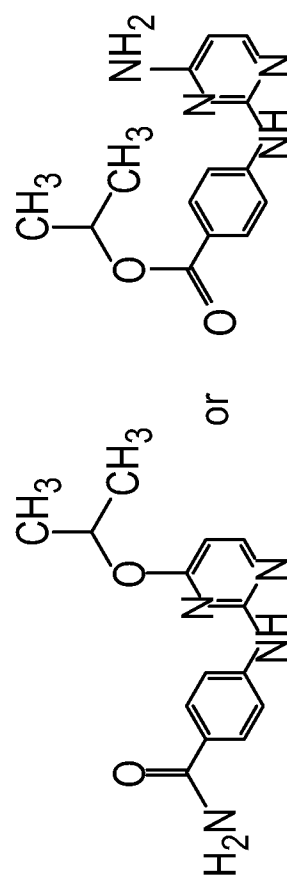
CT101



FIG. 11

**FIG. 12**

Nominal Mass = 258 Da

FIG. 13

or

Nominal Mass = 272 Da

FIG. 14

CT101 Colonna Zorbax Eclipse XDB-C18 150x3.0 mm 5 micron P.N. 993967-302; S.N. USTD002295
AN-HPLC -77

*MWD1 A, Sig=270.4 Ref=450.80 (CT101\14022102.D-CT101\14022101.D)

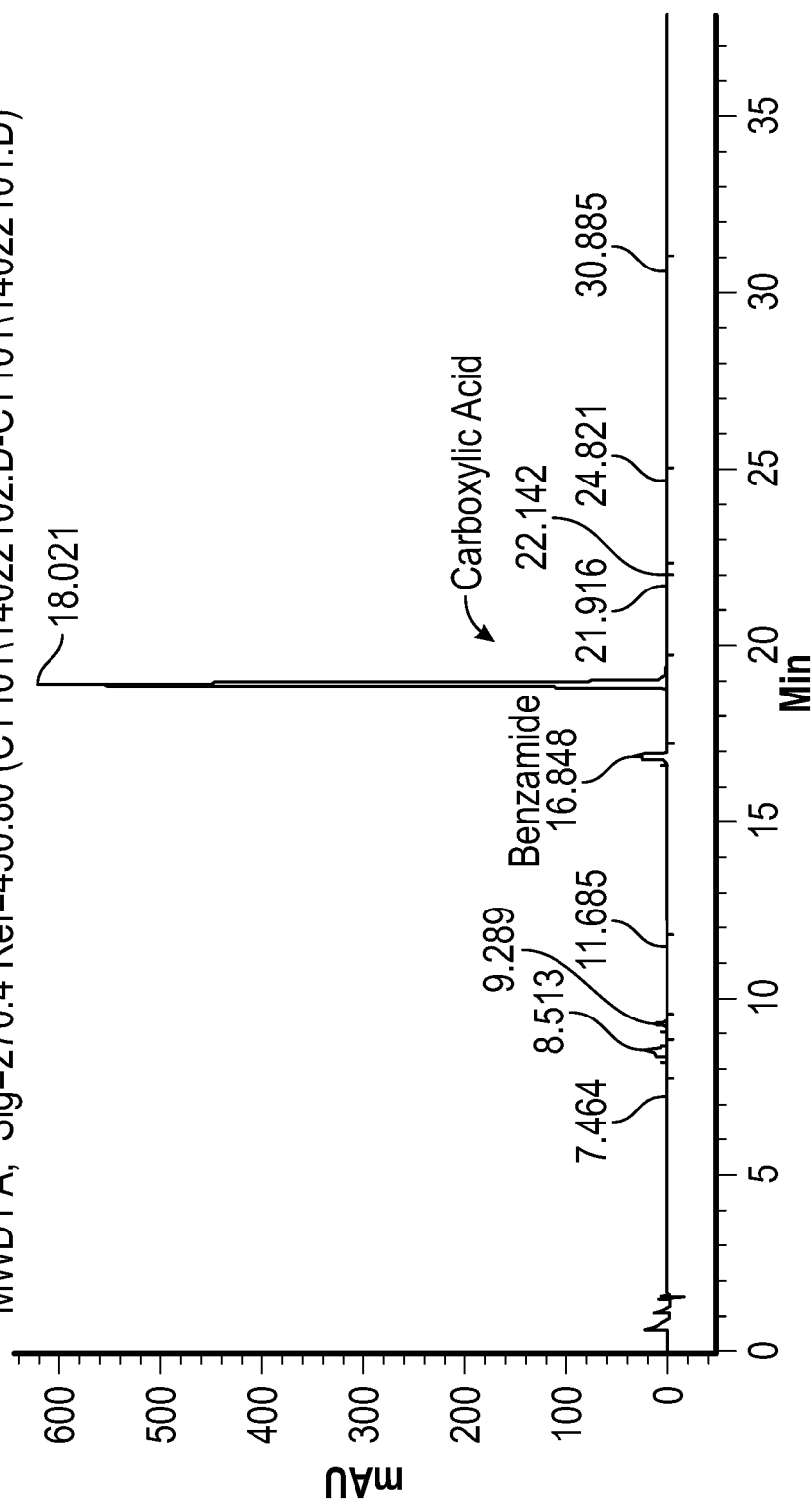


FIG. 15

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=270,4 Ref=450, 80
Signal has been Modified After Loading from Rawdata File!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU* S]	Height [mAU]	Area %
1	7.454	BP	0.1492	14.11138	1.45484	0.2988
2	8.513	BB	0.1758	243.94739	22.26995	5.1656
3	9.289	BB	0.1005	81.07771	11.74654	1.7168
4	11.685	PB	0.0757	3.50853	7.09590e-1	0.0743
5	16.848	VB	0.0939	207.13454	33.60223	4.3861
6	18.921	PB	0.1025	4159.84229	618.27643	88.0846
7	21.916	BV	0.1402	4.12227	4.00075e-1	0.0873
8	22.142	VP	0.1270	2.68067	3.22434e-1	0.0568
9	24.821	BB	0.1204	2.52544	2.99537e-1	0.0535
10	30.855	BP	0.1564	3.60468	3.55264e-1	0.0763

Totals : 4722.55489 689.43689

FIG. 15
(Continued)

CT101 Colonna Zorbax Eclipse XDB-C18 150x3.0 mm 5 micron P.N. 993967-302; S.N. USTD002295
AN-HPLC -77

*MWD1 A, Sig=270.4 Ref=450.80 (CT101\14022404.D-CT101\14022403.D)

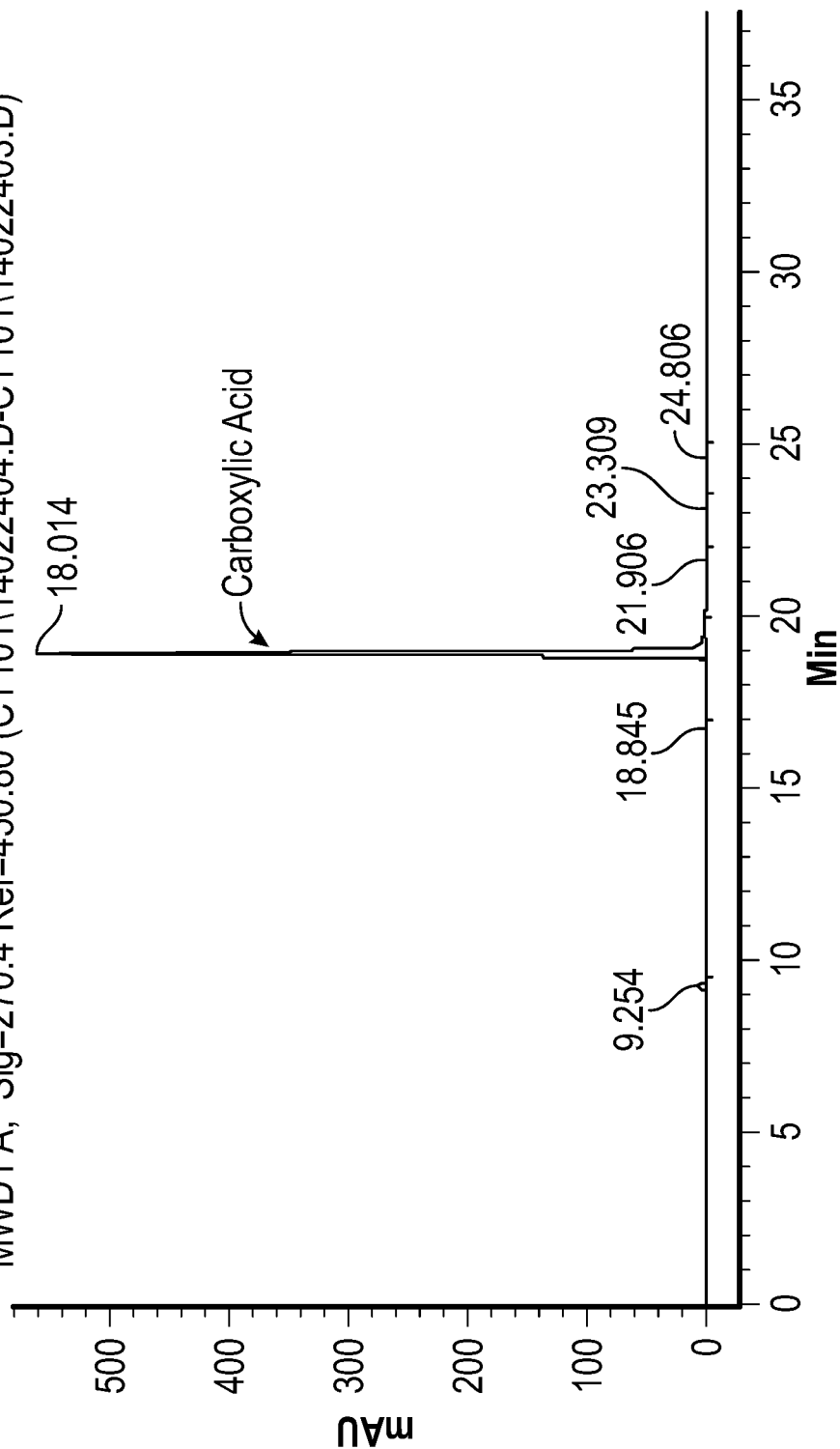


FIG. 16

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=270, 4 Ref=450, 80

Signal has been Modified After Loading from Rawdata File!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU* s]	Height [mAU]	Area %
1	9.254	PB	0.1002	39.40636	6.03579	1.0159
2	16.845	BB	0.0923	3.47067	5.75807e-1	0.0895
3	18.914	PB	0.1065	3827.62476	554.38959	98.6759
4	21.906	PV	0.1383	3.60504	3.44011e-1	0.0929
5	23.309	PB	0.1355	2.67129	2.84736e-1	0.0689
6	24.806	VP	0.1315	2.20674	2.19164e-1	0.0569

Totals : 3878.98486 561.84909

FIG. 16
(Continued)

CT101 Colonna Zorbax Eclipse XDB-C18 150x3.0 mm 5 micron P.N. 993967-302; S.N. USTD002295
AN-HPLC -77

*MWD1 A, Sig=270.4 Ref=450.80 (CT101\14022502.D-CT101\14022501.D)

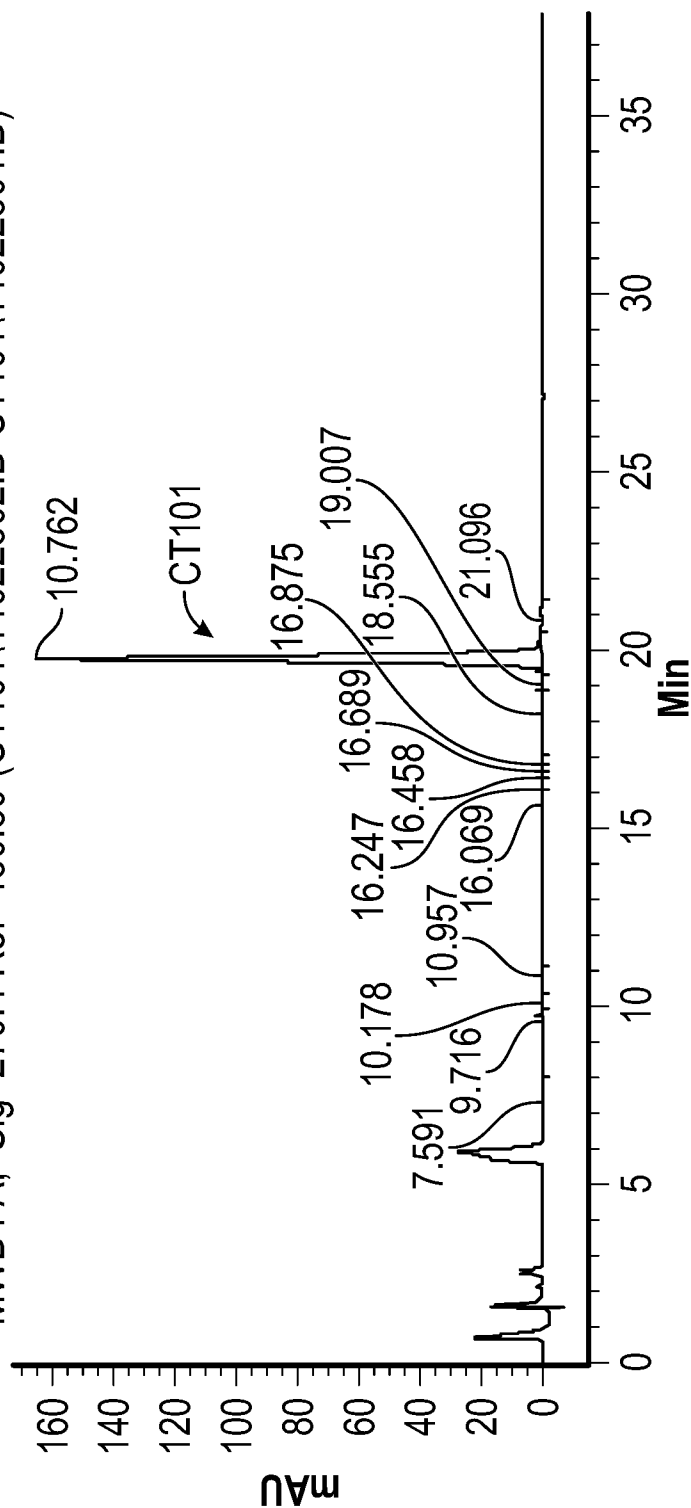


FIG. 17

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=270, 4 Ref=450, 80

Signal has been Modified After Loading from Rawdata File!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU* S]	Height [mAU]	Area %
1	7.591	PB	0.2008	4.82903	2.98481e-1	0.2241
2	9.716	BB	0.0953	16.80132	2.60262	0.7797
3	10.178	BB	0.0890	4.49678	7.83046e-1	0.2087
4	10.957	VB	0.0743	2.09114	4.18147e-1	0.0970
5	16.069	BV	0.2557	8.70382	4.13291e-1	0.4039
6	16.247	VV	0.1810	6.51970	4.90711e-1	0.3026
7	16.458	VV	0.1269	4.25473	4.55956e-1	0.1974
8	16.689	VV	0.0936	1.48897	2.23934e-1	0.0691
9	16.875	VB	0.1230	2.20417	2.54573e-1	0.1023
10	18.555	BV	0.2928	12.04438	5.26624e-1	0.5589
11	19.007	VB	0.1553	7.42675	6.49868e-1	0.3446
12	19.762	PB	0.1947	2078.94385	165.29558	94.4759
13	21.096	PP	0.1780	5.07927	3.94939e-1	0.2357

Totals : 2154.88392 172.80777

FIG. 17
(Continued)

CT101 Colonna Zorbax Eclipse XDB-C18 150x3.0 mm 5 micron P.N. 993967-302; S.N. USTD002295
AN-HPLC -77

*MWD1 A, Sig=270.4 Ref=450.80 (CT101\14022505.D-CT101\14022501.D)

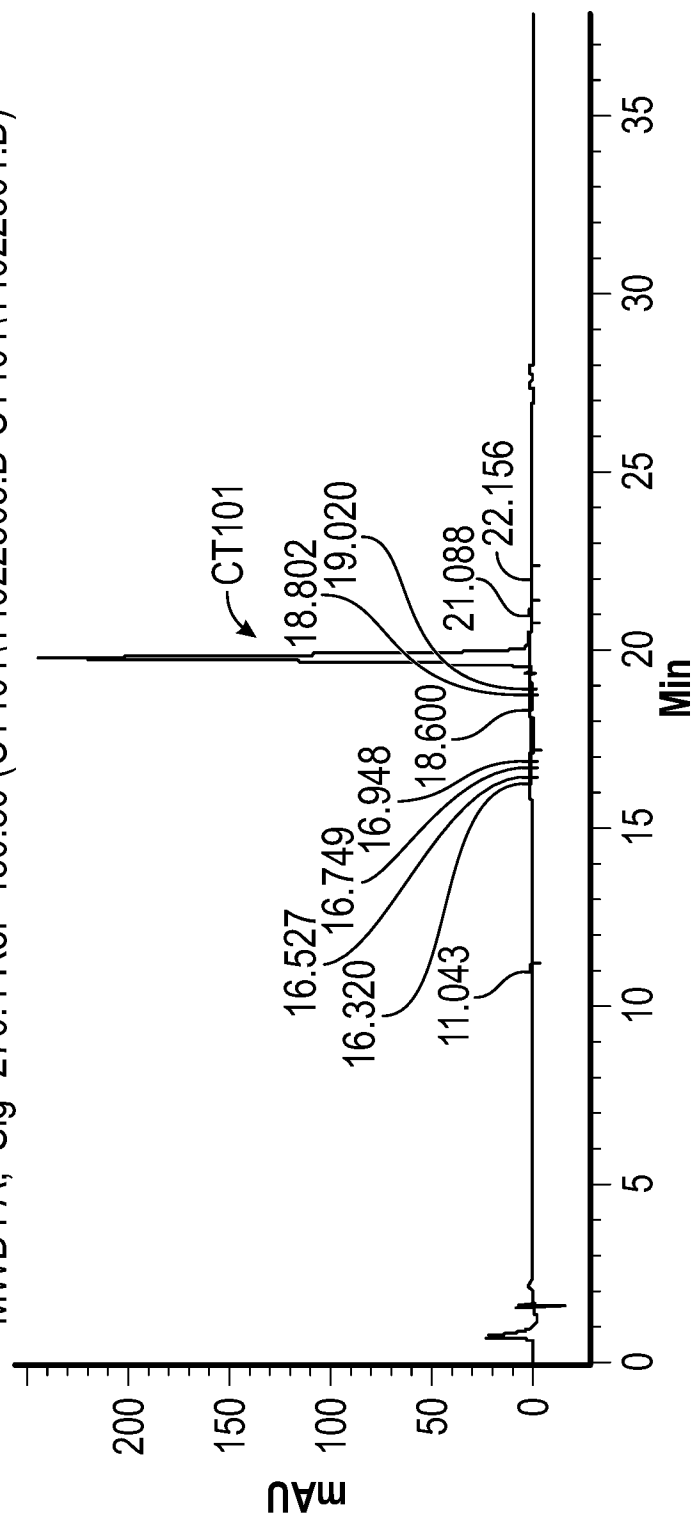


FIG. 18

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=270, 4 Ref=450, 80
Signal has been Modified After Loading from Rawdata File!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU* S]	Height [mAU]	Area %
1	11.043	PB	0.0739	2.67071	5.57061e-1	0.0868
2	16.320	BV	0.1398	4.79952	4.67445e-1	0.1560
3	16.527	VB	0.1244	4.40297	4.92223e-1	0.1431
4	16.749	BP	0.0907	1.84676	2.96421e-1	0.0600
5	16.948	VV	0.1343	4.36751	4.45837e-1	0.1420
6	18.600	BV	0.1981	12.10444	8.30096e-1	0.3935
7	18.802	VV	0.1334	6.28383	6.46618e-1	0.2043
8	19.020	VB	0.1923	11.53971	9.20543e-1	0.3751
9	19.767	PB	0.1896	3017.60986	245.10083	98.0936
10	21.088	BP	0.1839	6.59361	5.42255e-1	0.2143
11	22.155	BV	0.2290	4.03583	2.14985e-1	0.1312

Totals : 3076.25475 250.51432

FIG. 18
(Continued)

Sample Name	CT101 S47P3
Method	SNAP C18 120g CT101
Project	
Comment	
Cartridge	SNAP C18 120g
Flowrate	50 ml/min
Solvent A	Water
Solvent B	Acetonitrile
Rack Type	120 ml
Max Fraction Volume	100 ml
Dispense Order	S
Initial Waste	5CV

Detection Mode	UV1+UV2
UV1 (Collection)	210 nm
UV2 (Collection)	270 nm

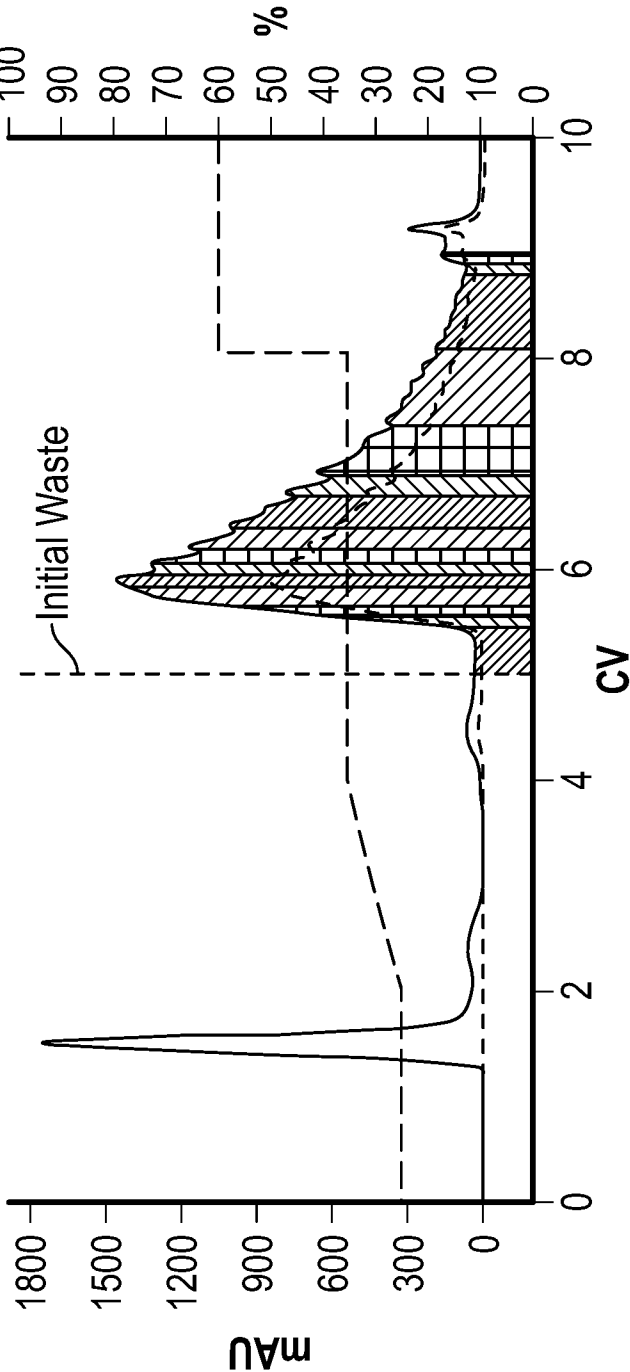


FIG. 19

Gradient

	Solvents	Mix	Length (CV)
Equil.	A/B	24%	2.0
1	A/B	24%	2.0
2	A/B	24% - 34%	2.0
3	A/B	34%	3.3
4	A/B	34%	0.5
5	A/B	34%	0.1
6	A/B	60%	2.0

Flowrate 50 ml/min

Isocratic Hold Enabled

Isocratic Hold Disabled

FIG. 19
(Continued)

Metodo HPLC x CT101
 Zorbax Eclipse XBD-C18 Solvent Saver (3x150 mmx5µm)
 P.N. 993967-302
 S.N. USTD002293

*DAD1 A, Sig=270, 4 Ref=450, 80 (CT101\14031108.D-CT101\14031107.D)

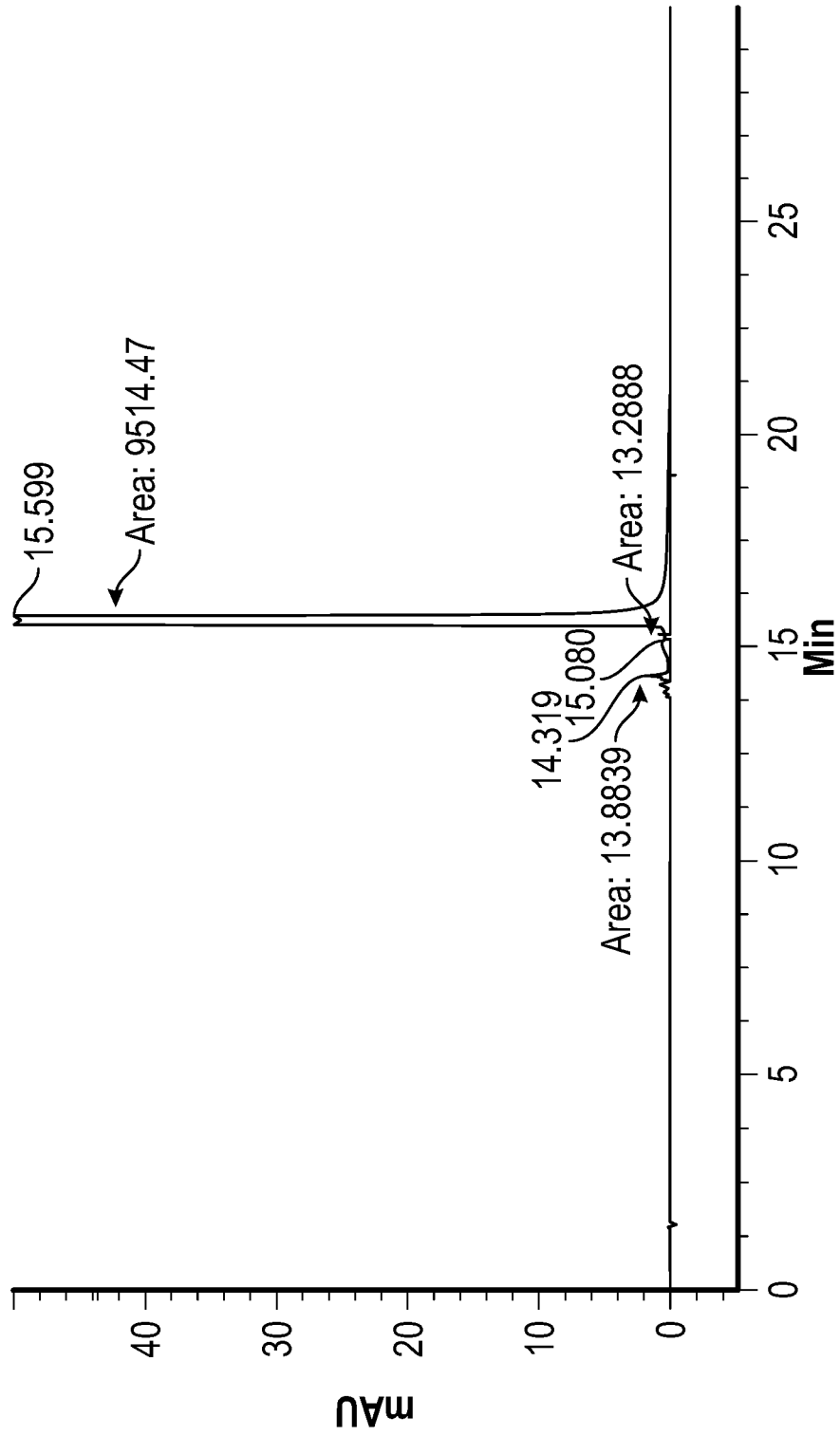


FIG. 20

=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=270, 4 Ref=450, 80
Signal has been Modified After Loading from Rawdata File!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.319	MF	0.1026	13.88386	2.25500	0.1455
2	15.040	MF	0.3163	13.28880	7.00331e-1	0.1393
3	15.599	FM	0.0980	9514.47266	1617.99426	99.7152
Totals :				9541.64532	1620.94959	

FIG. 20
(Continued)

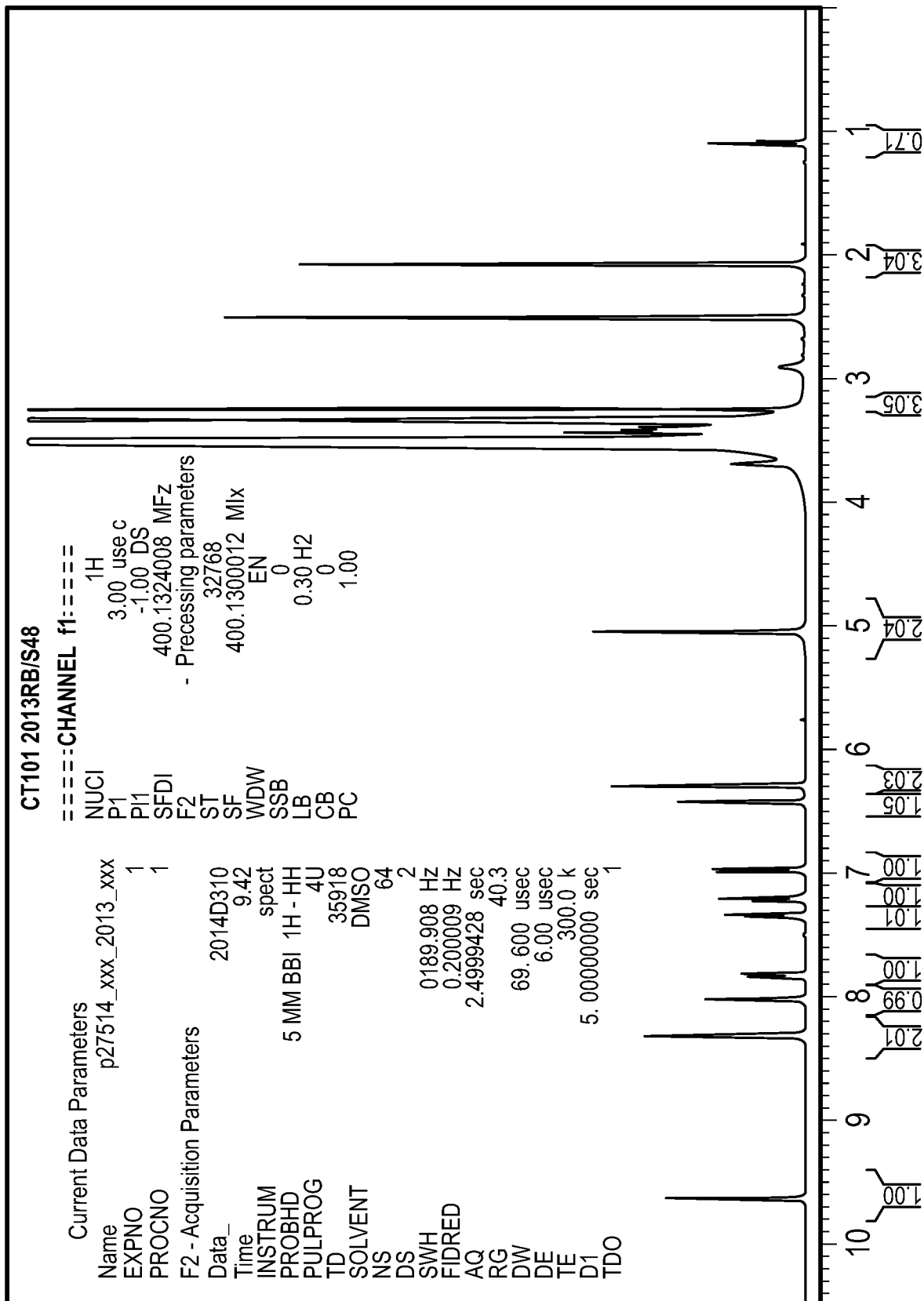


FIG. 21

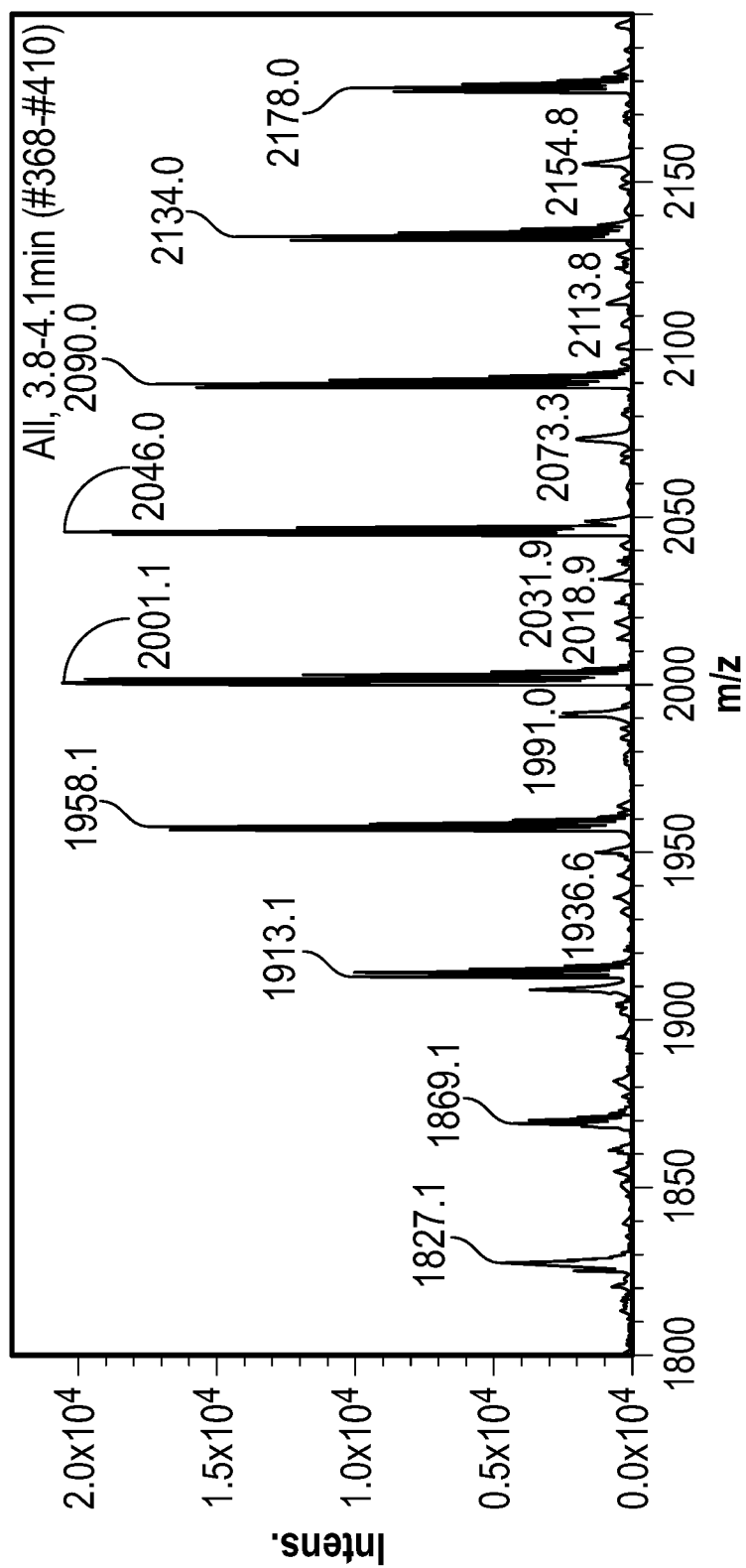


FIG. 22

Test Report n. 2014/021**CT101**

Product : (3-({4-[(5-hydroxy-2-methylphenyl)amino]pyrimidin-2-yl}amino)-N-mPEG₂₀₀₀benzamide)

Formula : $C_{18}H_{16}N_5O_2-[C_2H_4O]_n-CH_3$ Mol. Weight: 2333 (*)

Cas n°: ***** Quantity: 1,50 g

Lot number: 2013RB20/S48

Parameter	Unit	Results	Anal. Meth.
Aspect		Off White Crystalline Powder	Visual
NMR Spectrum		Conform to Structure	
Purity (HPLC,292 nm)	% Area	99,71	MI CT101 002
Impurities (HPLC,292 nm)			MI CT101 002
Unknown Rrt 0,92	% Area	0,14	
Unknown Rrt 0,96	% Area	0,14	

(*) Based on $M_w = 2015$ Da of MeO-PEG-NH₂

Note: Purity Chromatogram Enclosed

FIG. 23

Metodo HPLC x CT101
 Zorbax Eclipse XBD-C18 Solvent Saver (3 x 150 mm x 5µm)
 P.N. 993967-302
 S.N. USTD002293

*DAD1 A, Sig=270, 4 Ref=450, 80 (CT101\14031110.D-CT101\14031107.D)

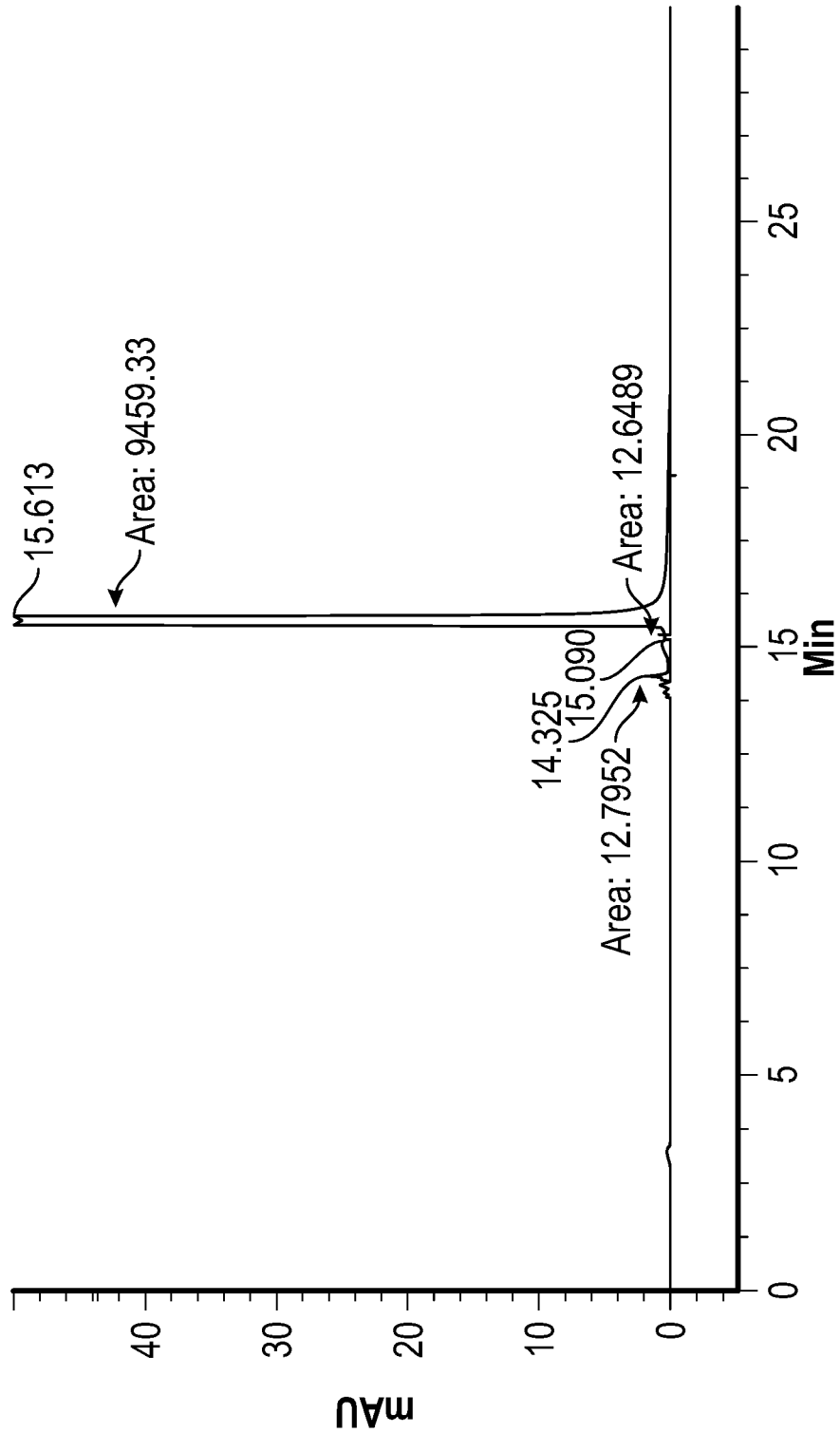


FIG. 24

=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=270, 4 Ref=450, 80
Signal has been Modified After Loading from Rawdata File!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.325	MF	0.1087	12.79521	1.96140	0.1349
2	15.090	MF	0.2866	12.64890	7.35464e-1	0.1334
3	15.613	FM	0.0985	9459.32715	1600.37048	99.7317
Totals :				9484.77126	1603.06735	

FIG. 24
(Continued)

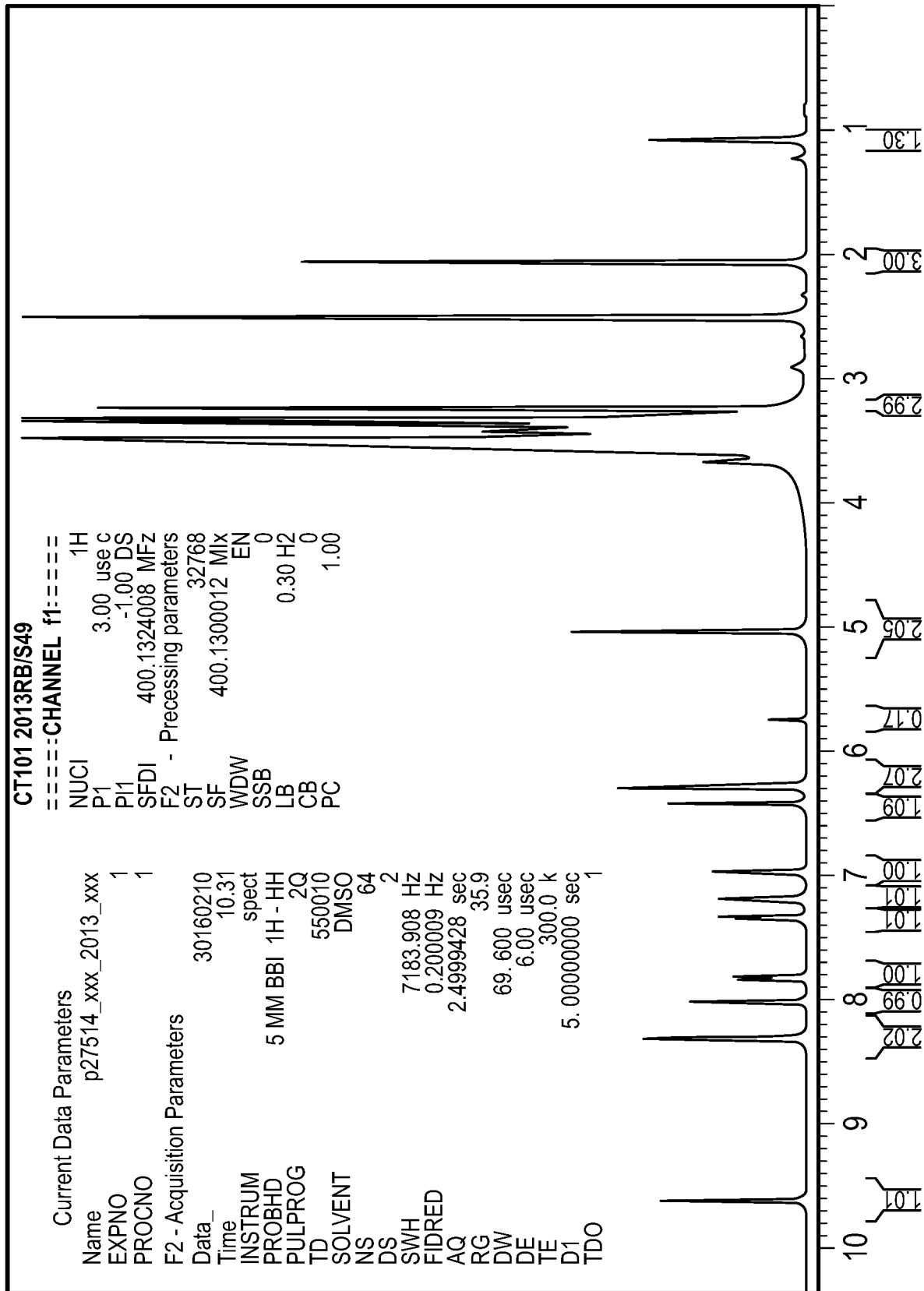


FIG. 25

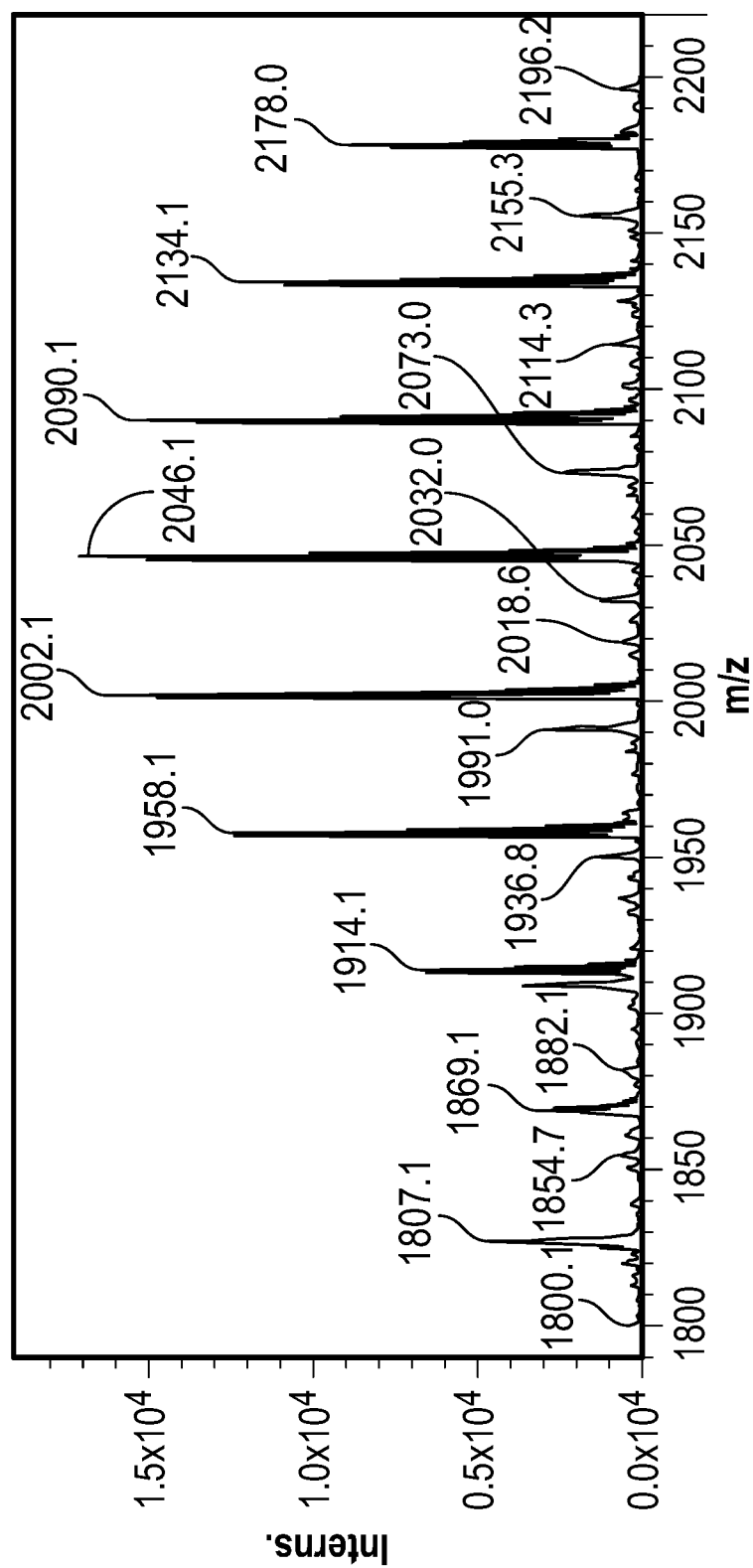


FIG. 26

Test Report n. 2014/022**CT101**

Product : (3-({4-[(5-hydroxy-2-methylphenyl)amino]pyrimidin-2-yl}amino)-N-mPEG₂₀₀₀benzamide)

Formula : $C_{18}H_{16}N_5O_2-[C_2H_4O]_n-CH_3$ Mol. Weight: 2333 (*)

Cas n°: ***** Quantity: 0,30 g

Lot number: 2013RB20/S49

Parameter	Unit	Results	Anal. Meth.
Aspect		Off White Crystalline Powder	Visual
NMR Spectrum		Conform to Structure	
Purity (HPLC,292 nm)	% Area	99,73	MI CT101 002
Impurities (HPLC,292 nm)			MI CT101 002
Unknown Rrt 0,92	% Area	0,13	
Unknown Rrt 0,96	% Area	0,13	

(*) Based on $M_w = 2015$ Da of MeO-PEG-NH₂

Note: Purity Chromatogram Enclosed

FIG. 27

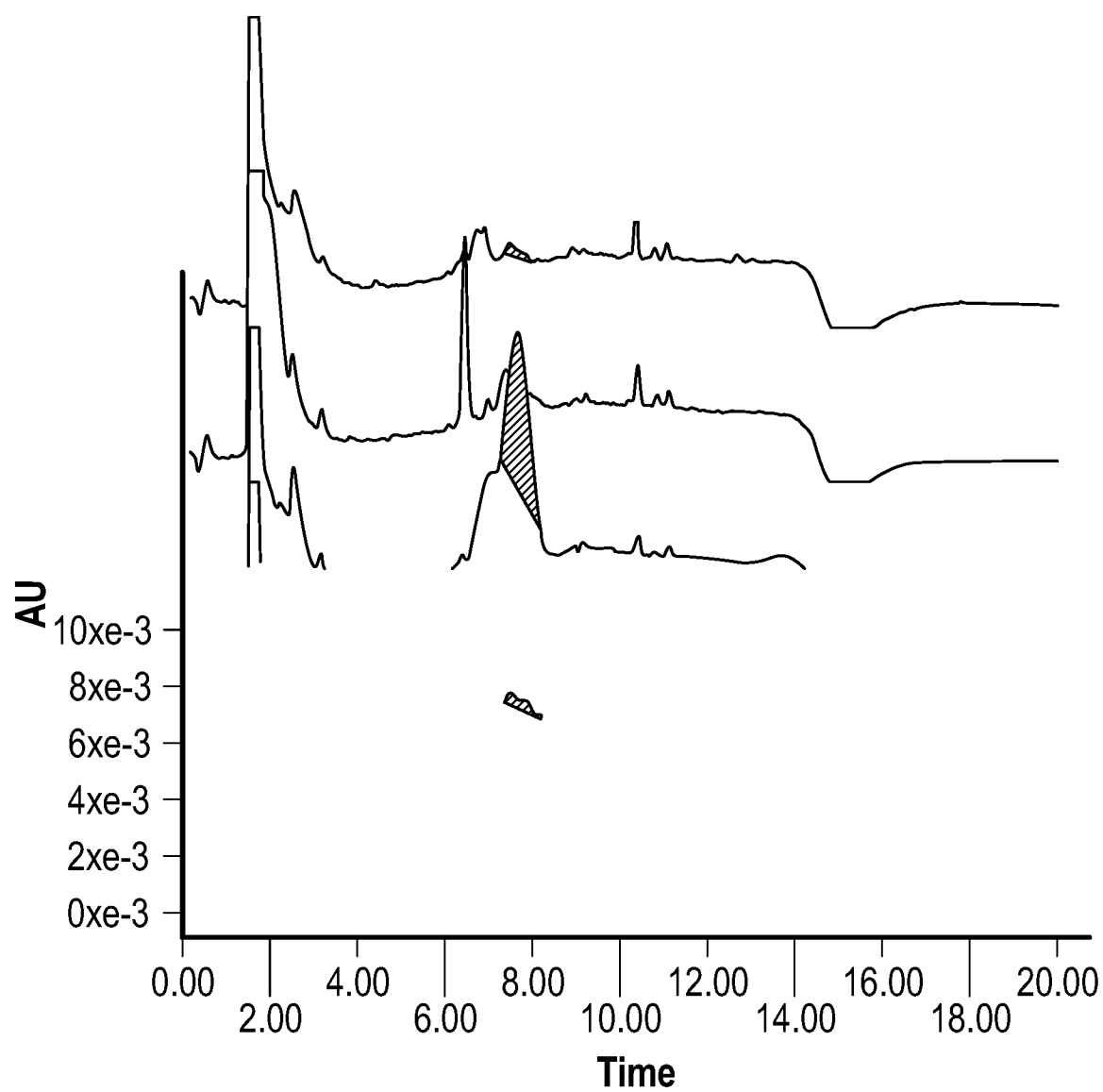


FIG. 28A

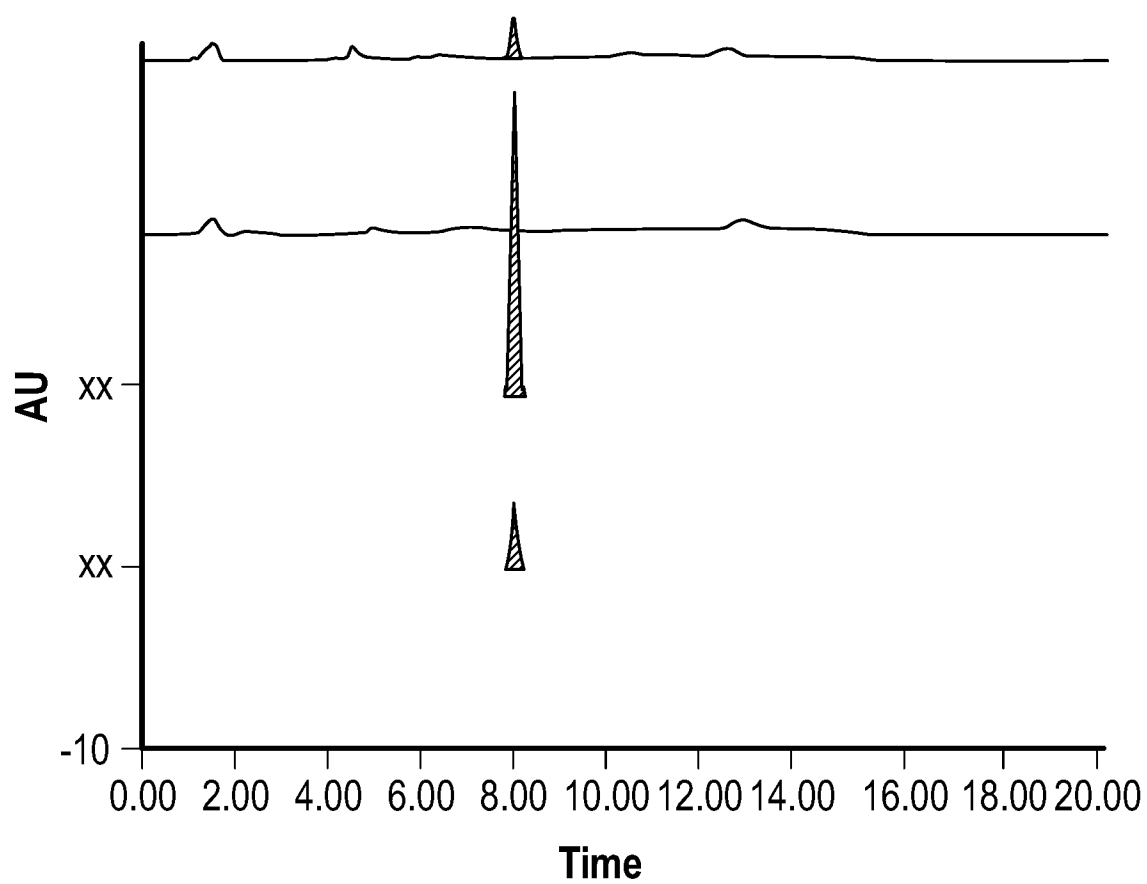


FIG. 28B

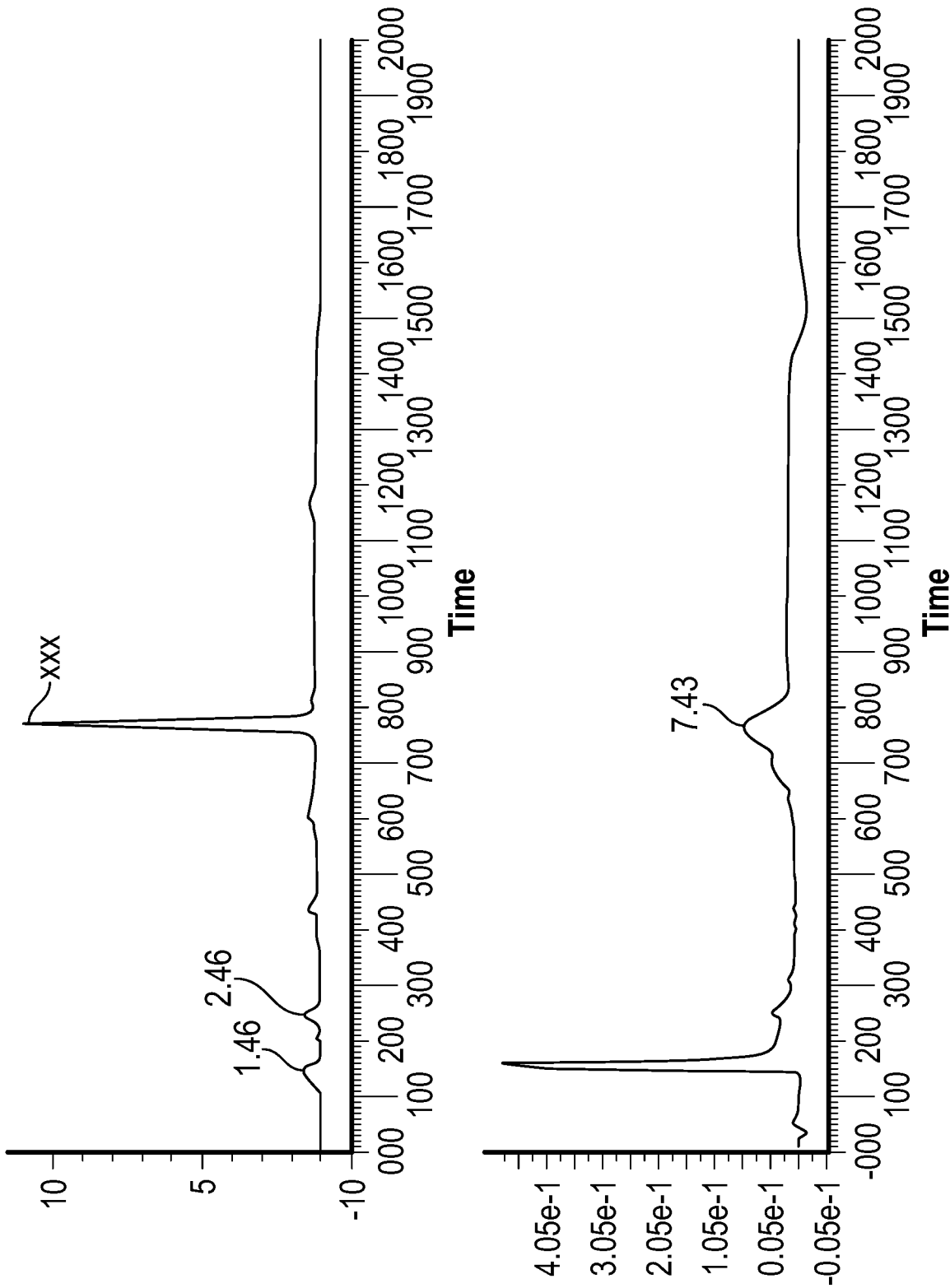


FIG. 29

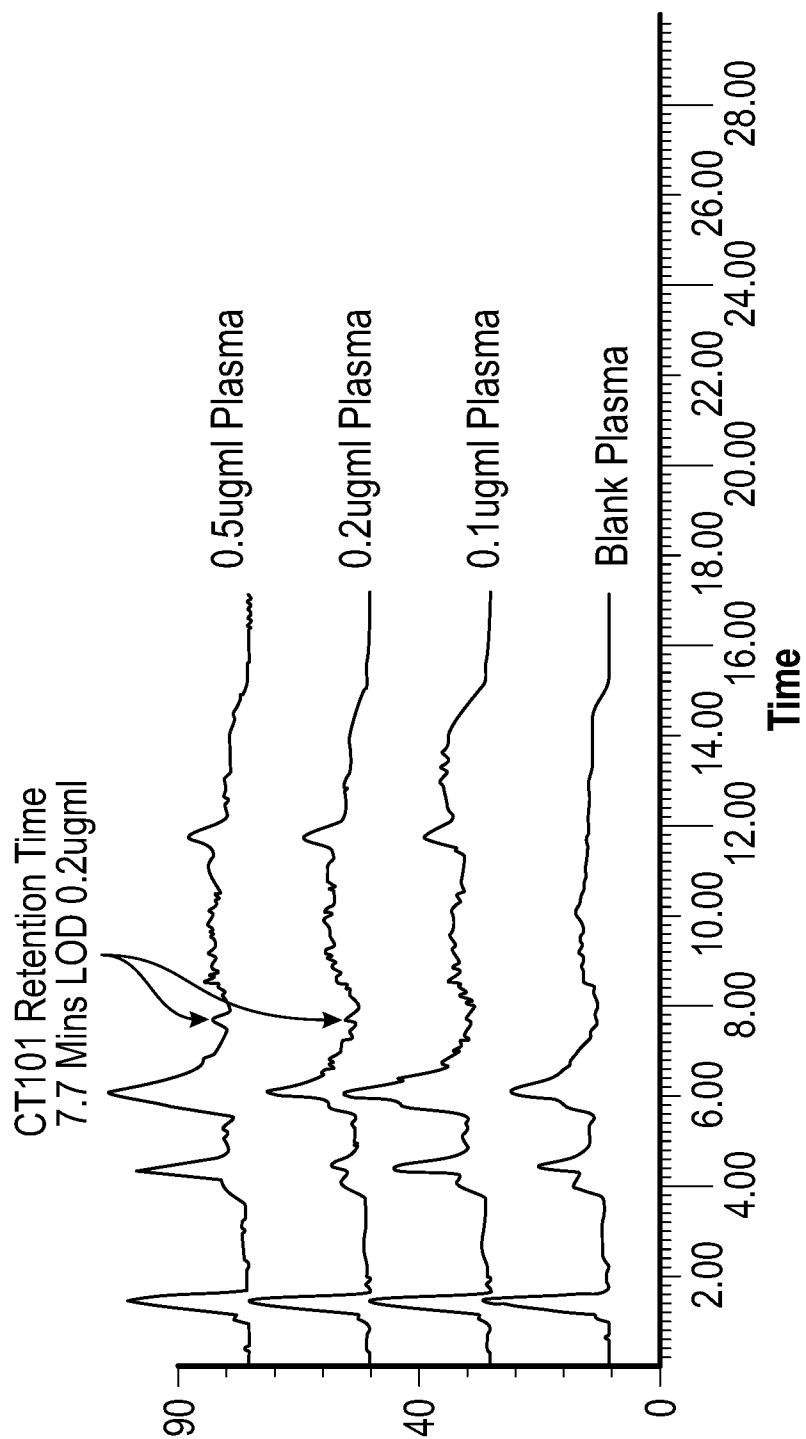
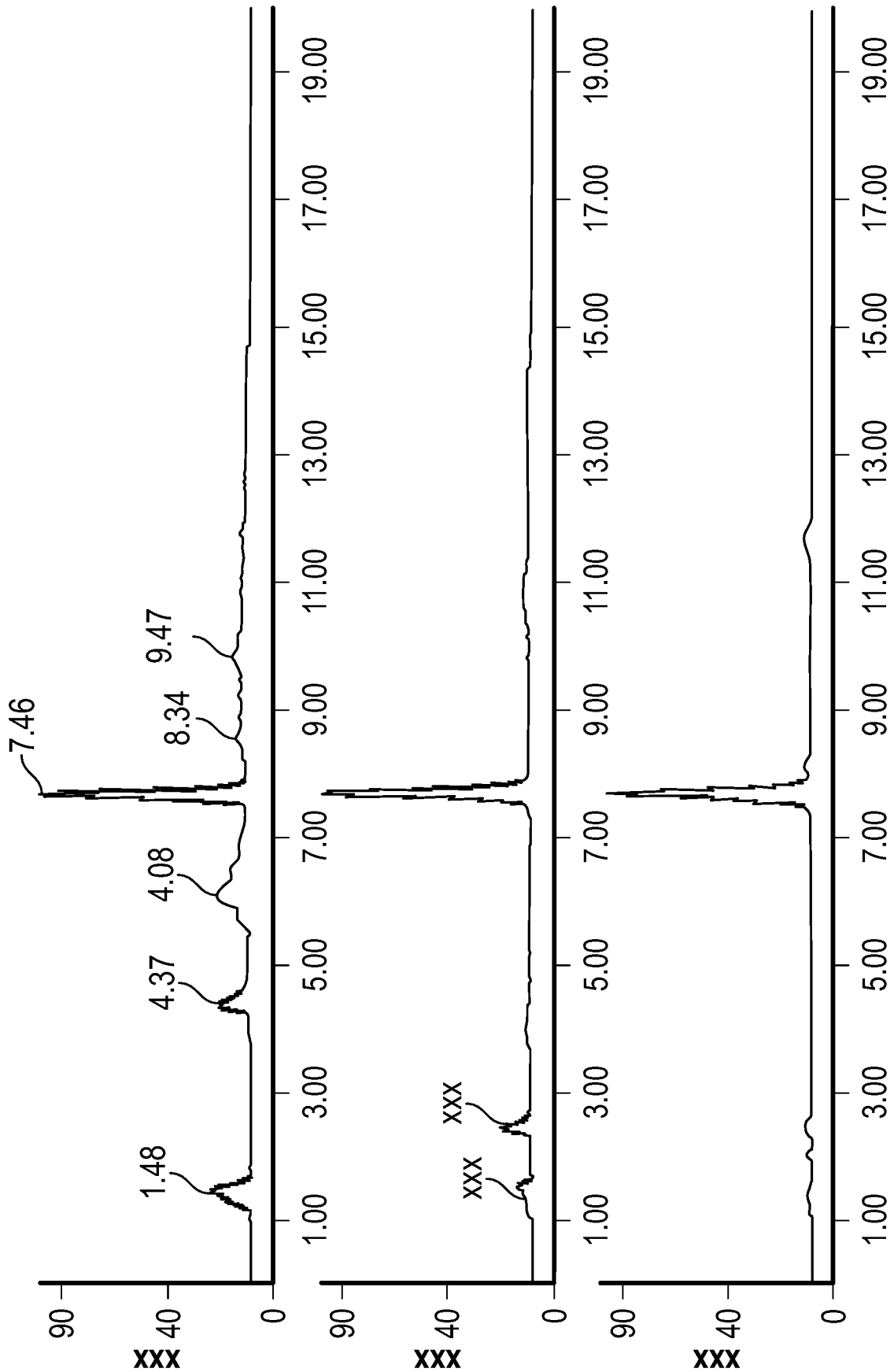
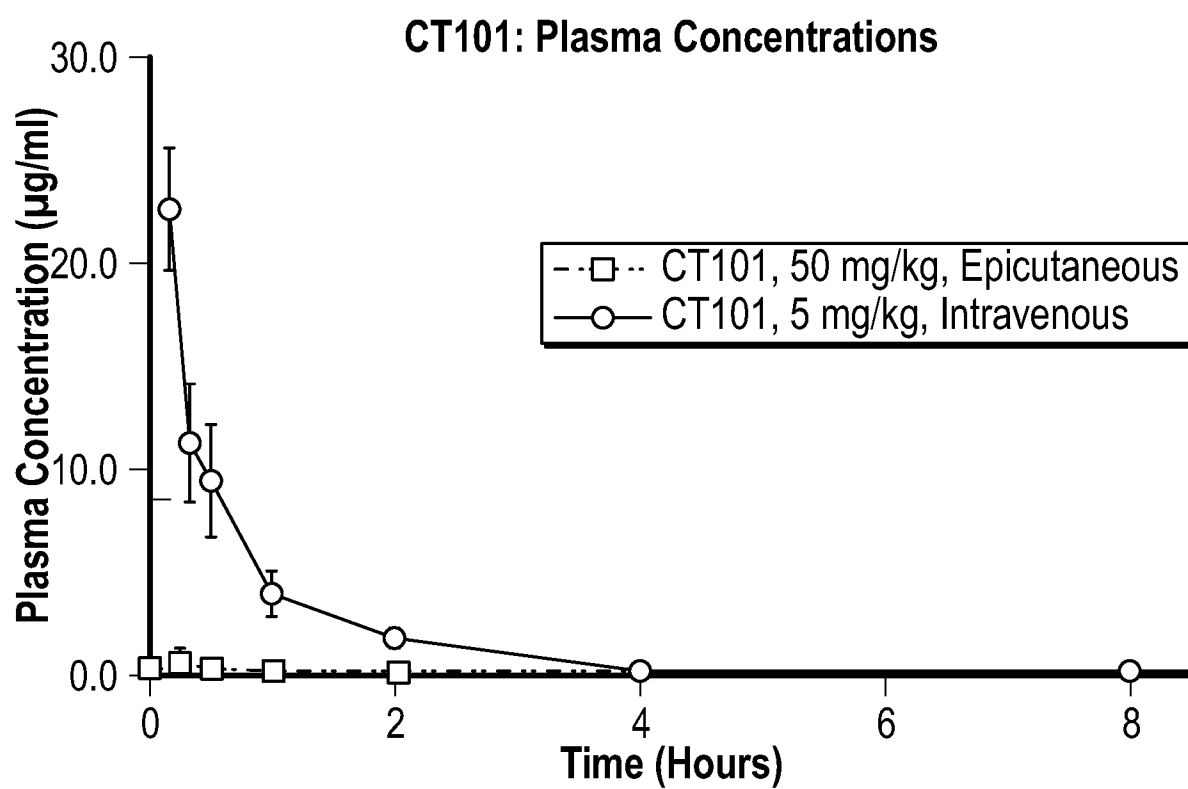
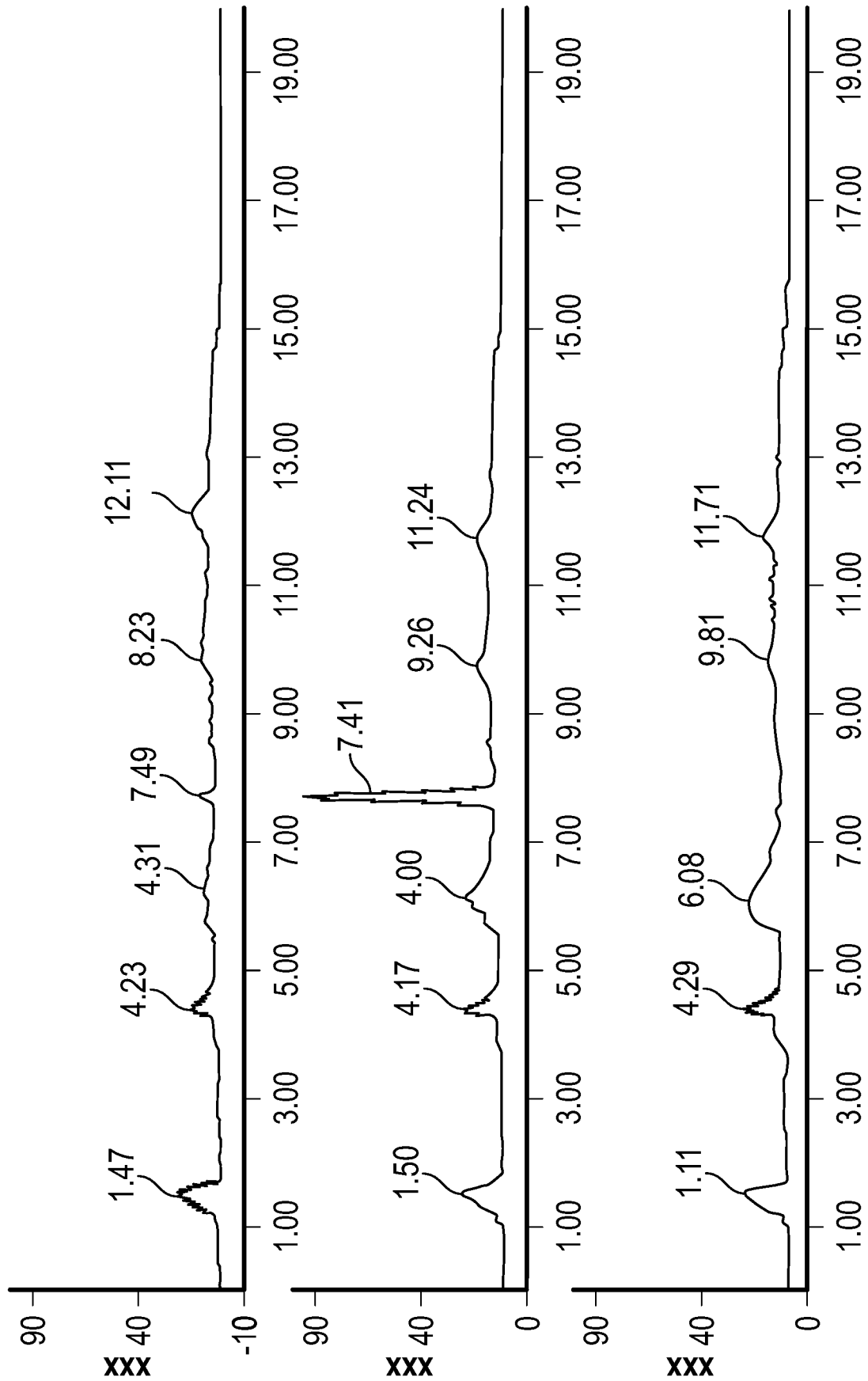


FIG. 30

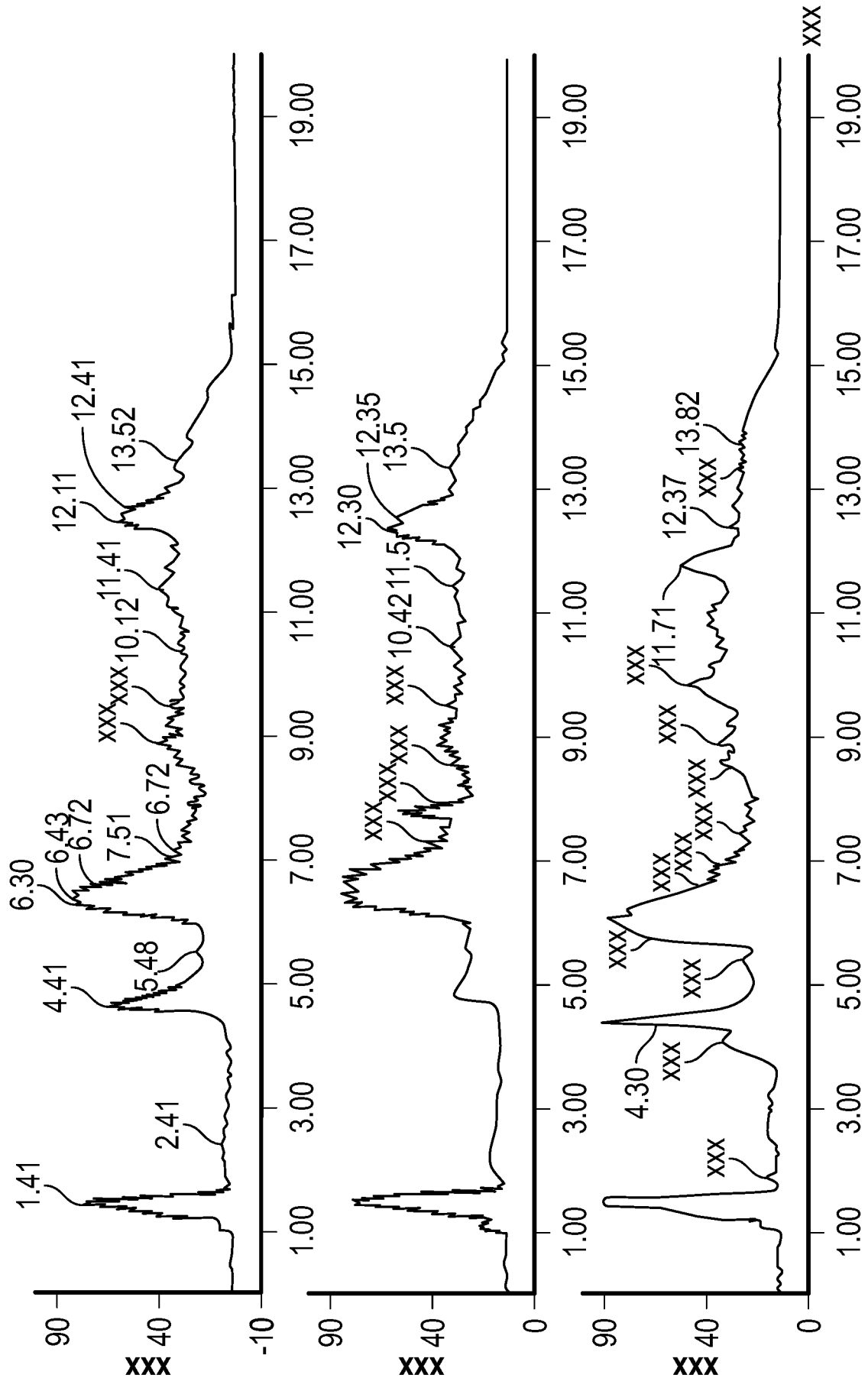


Time
FIG. 31

**FIG. 32**



Time
FIG. 33



Time
FIG. 34

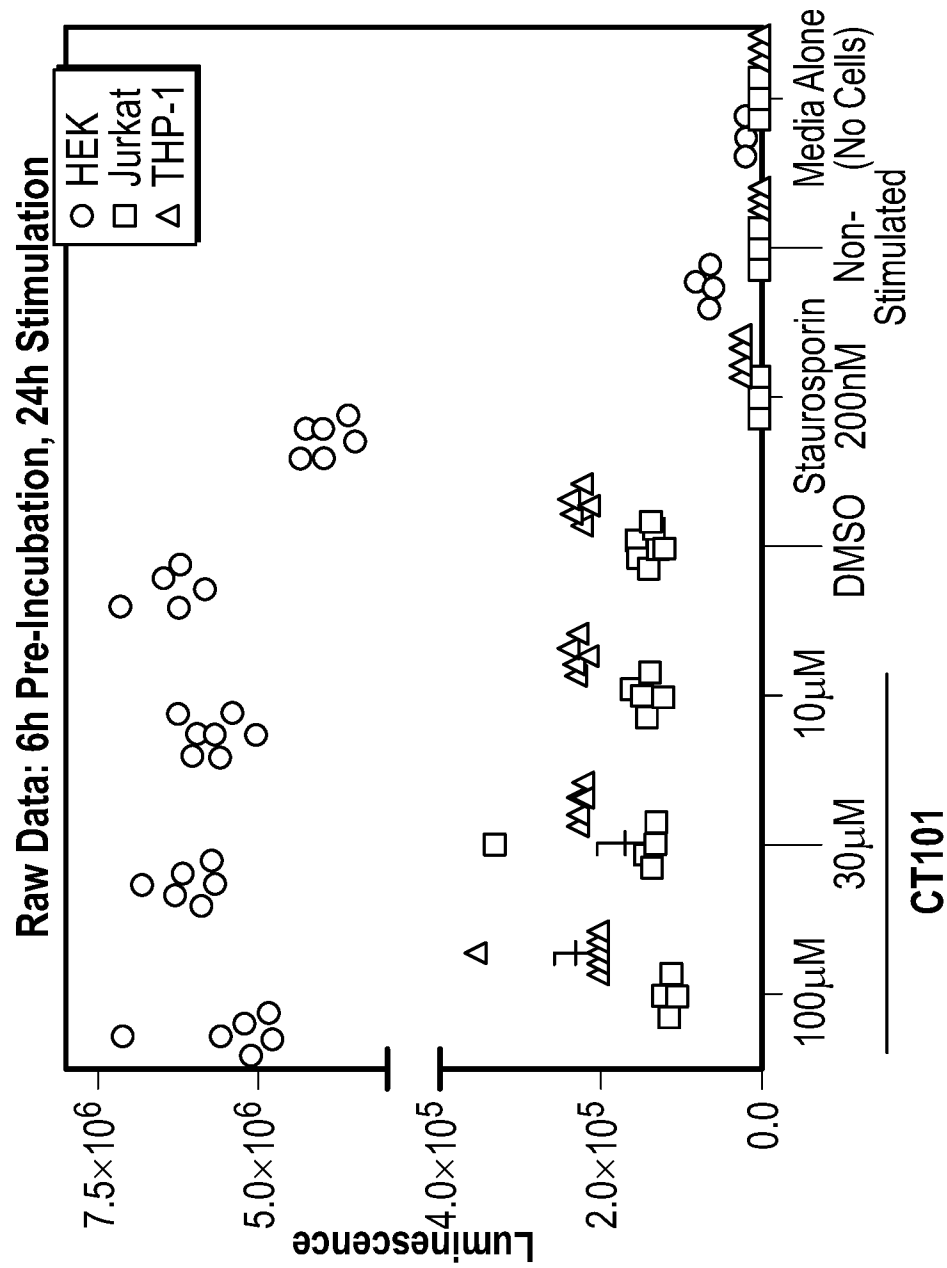
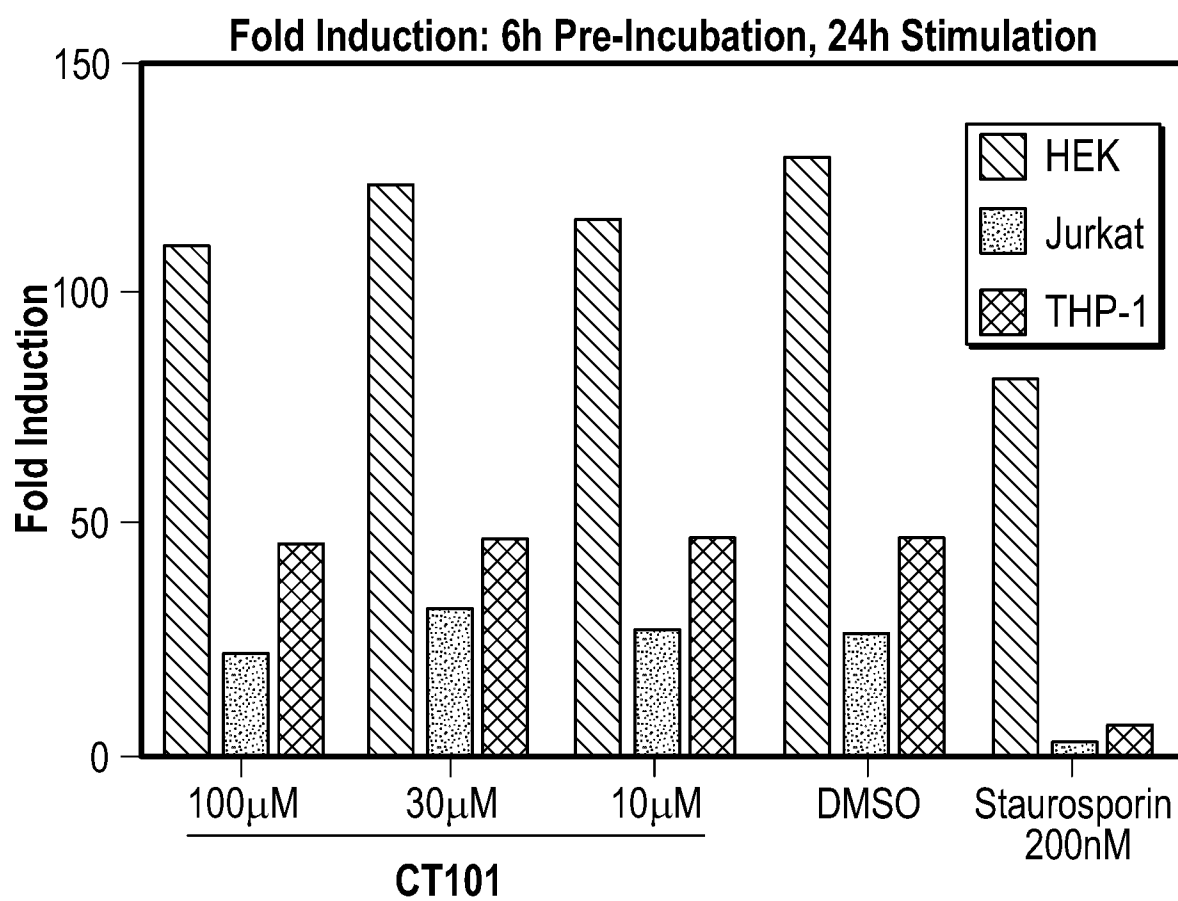


FIG. 35



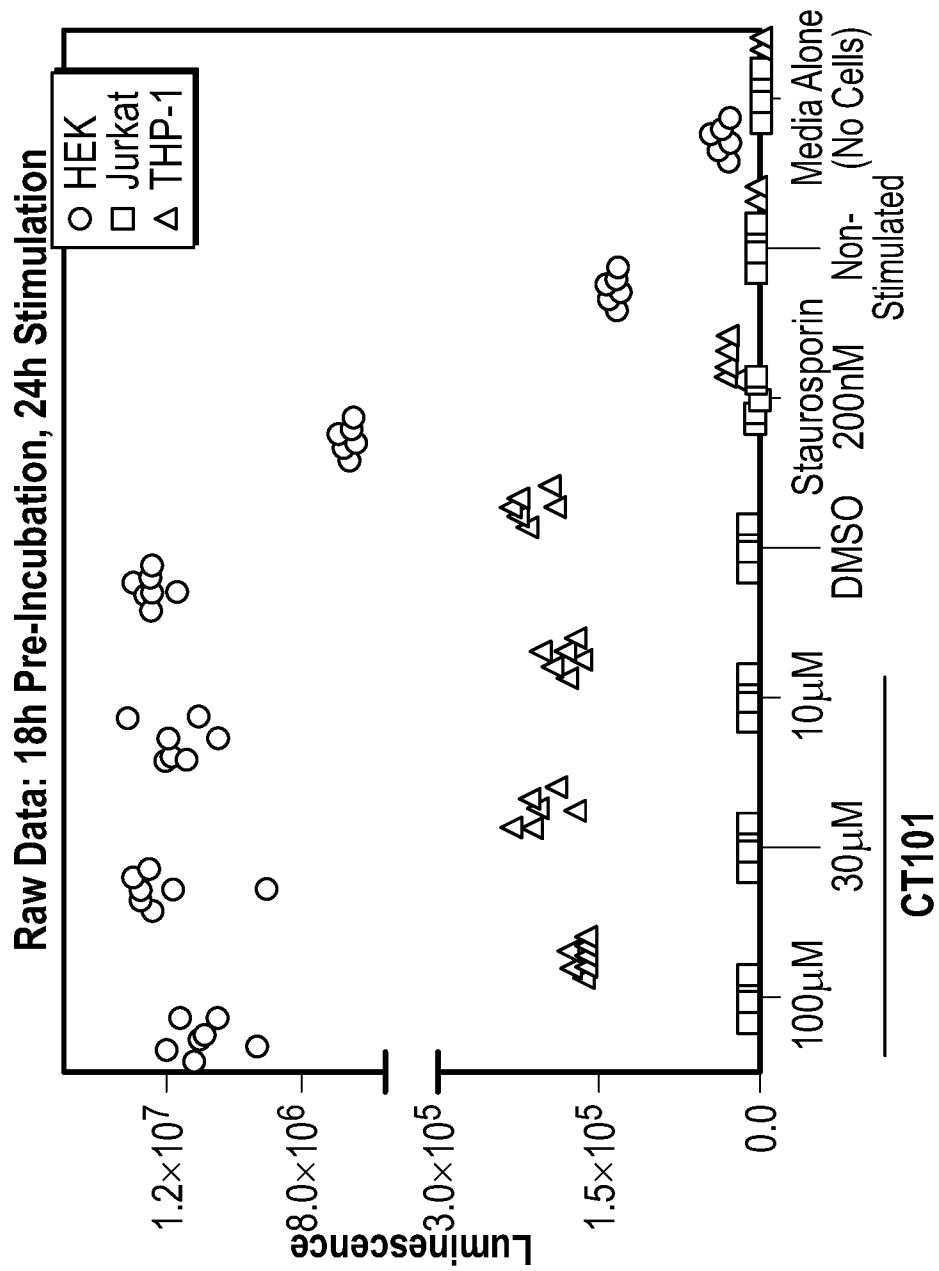
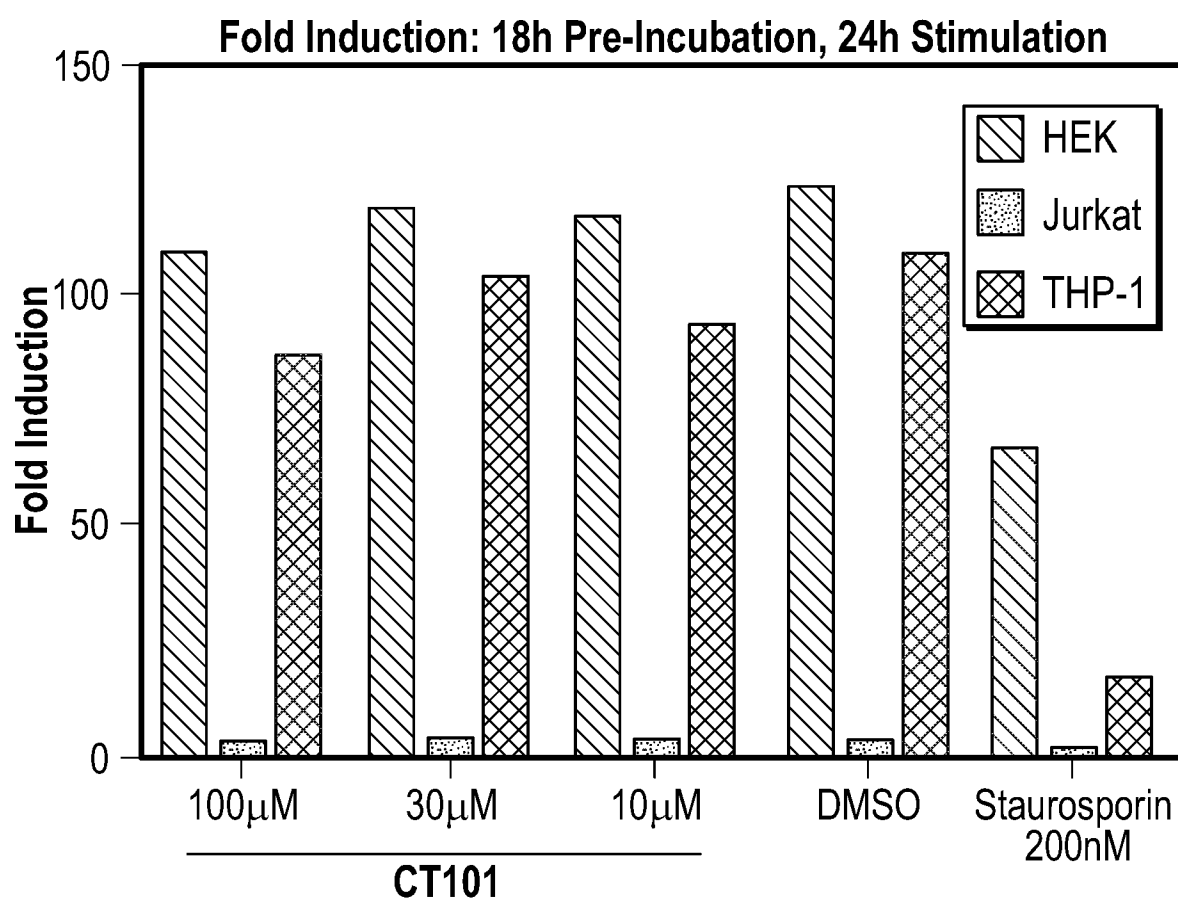


FIG. 37

**FIG. 38**

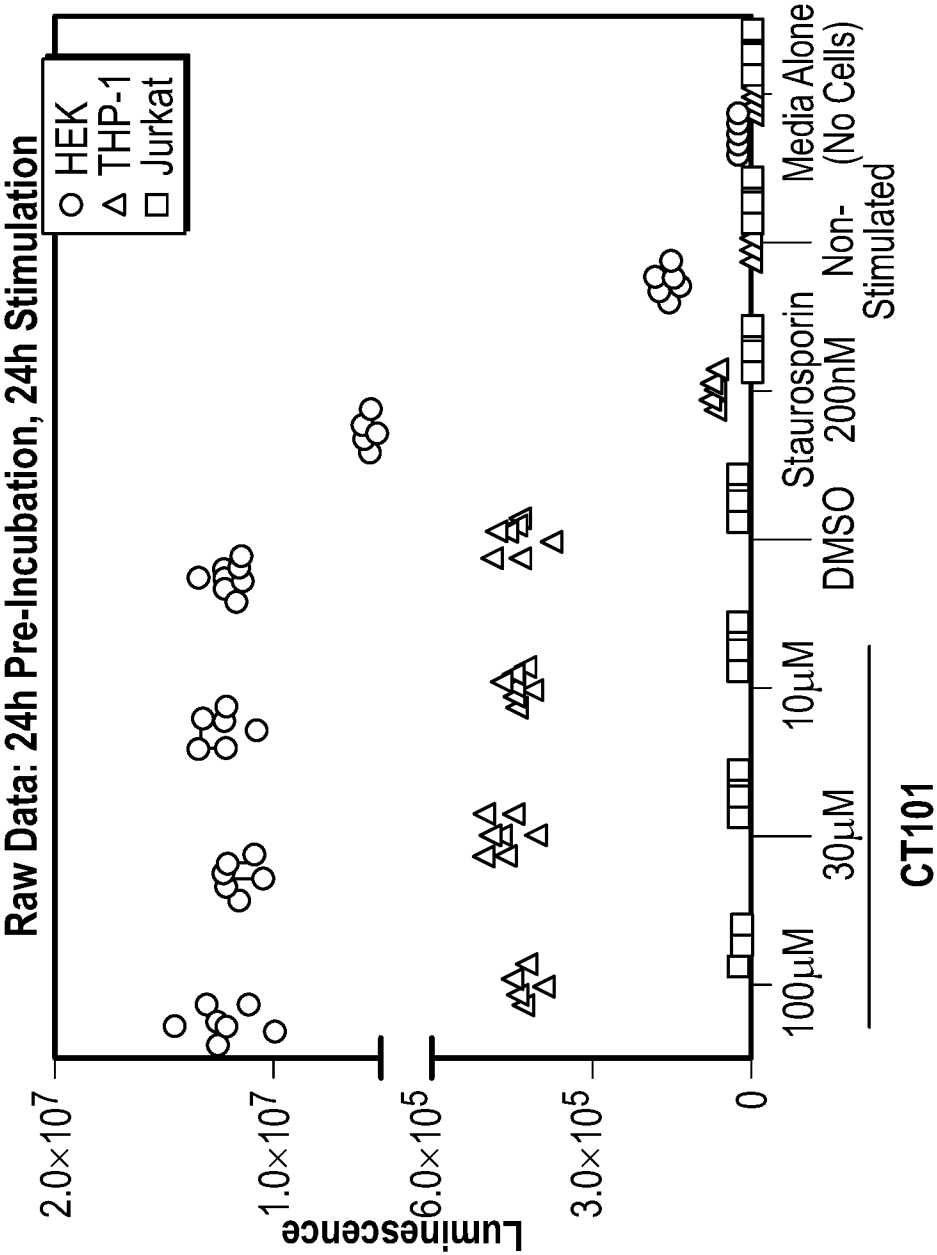
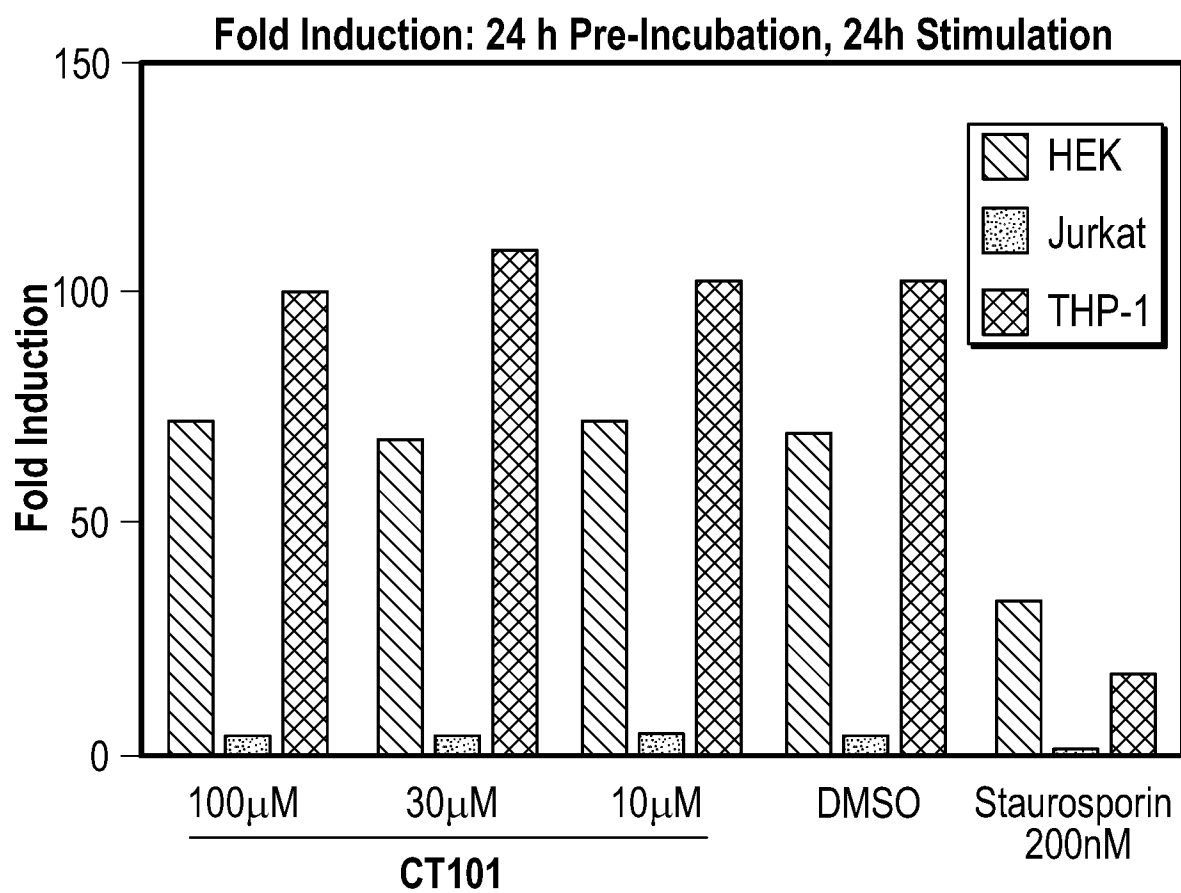


FIG. 39

**FIG. 40**

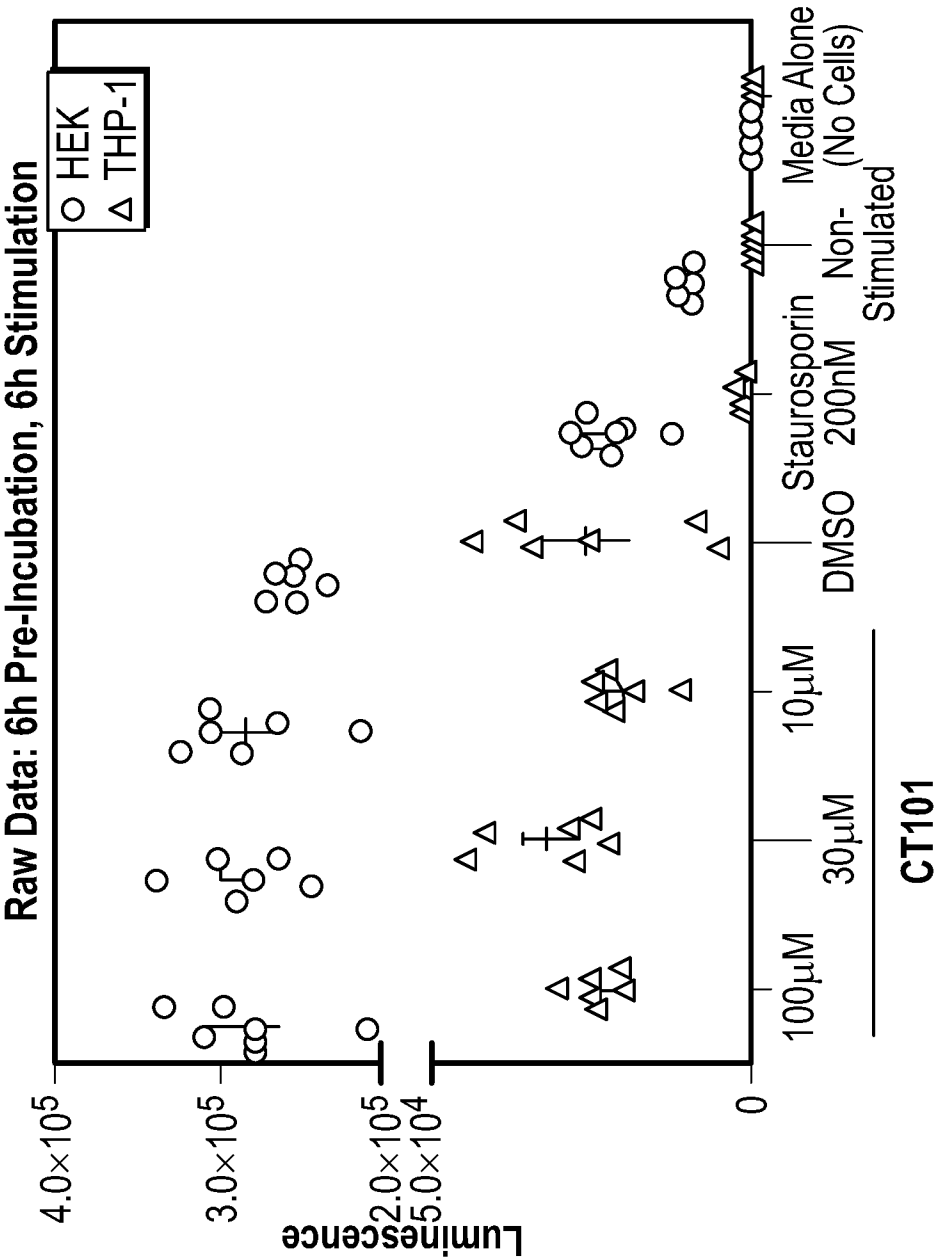


FIG. 41

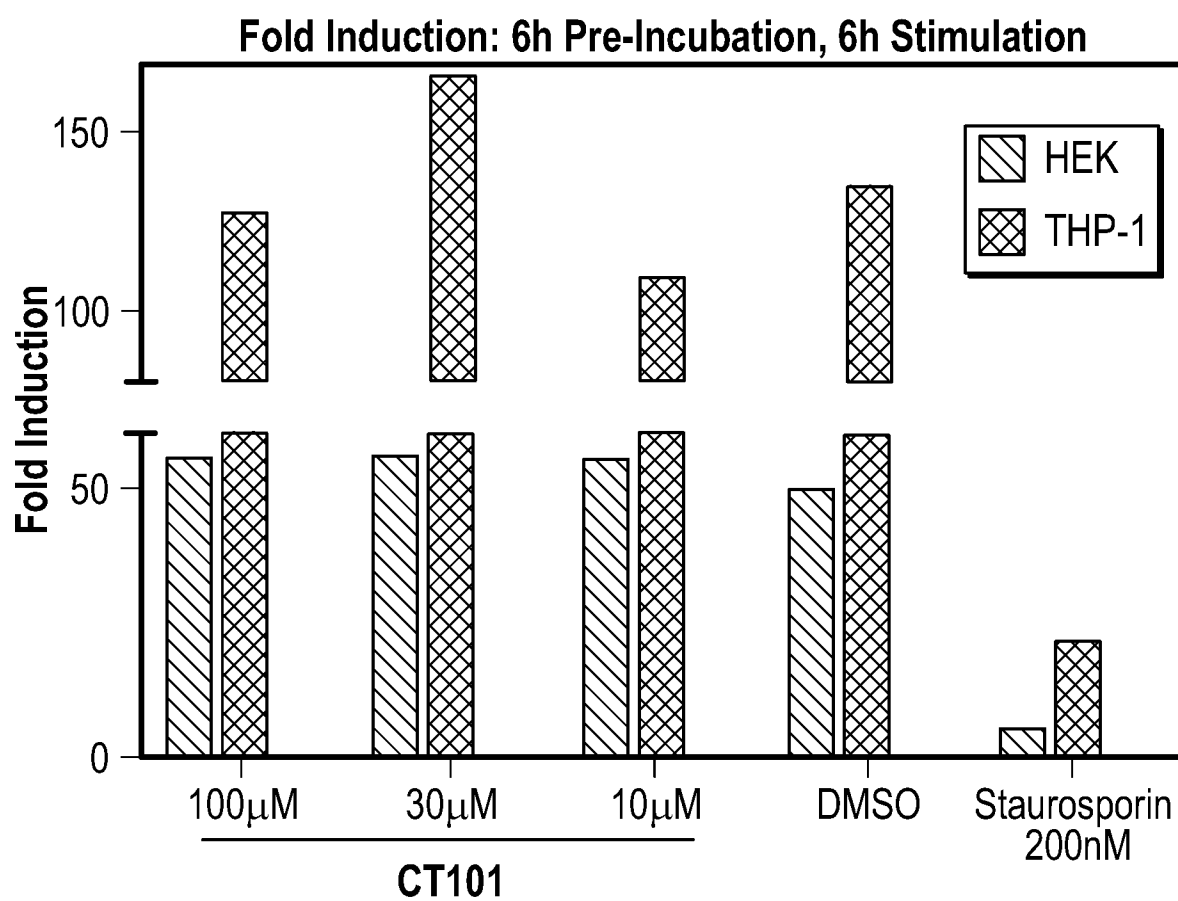


FIG. 42

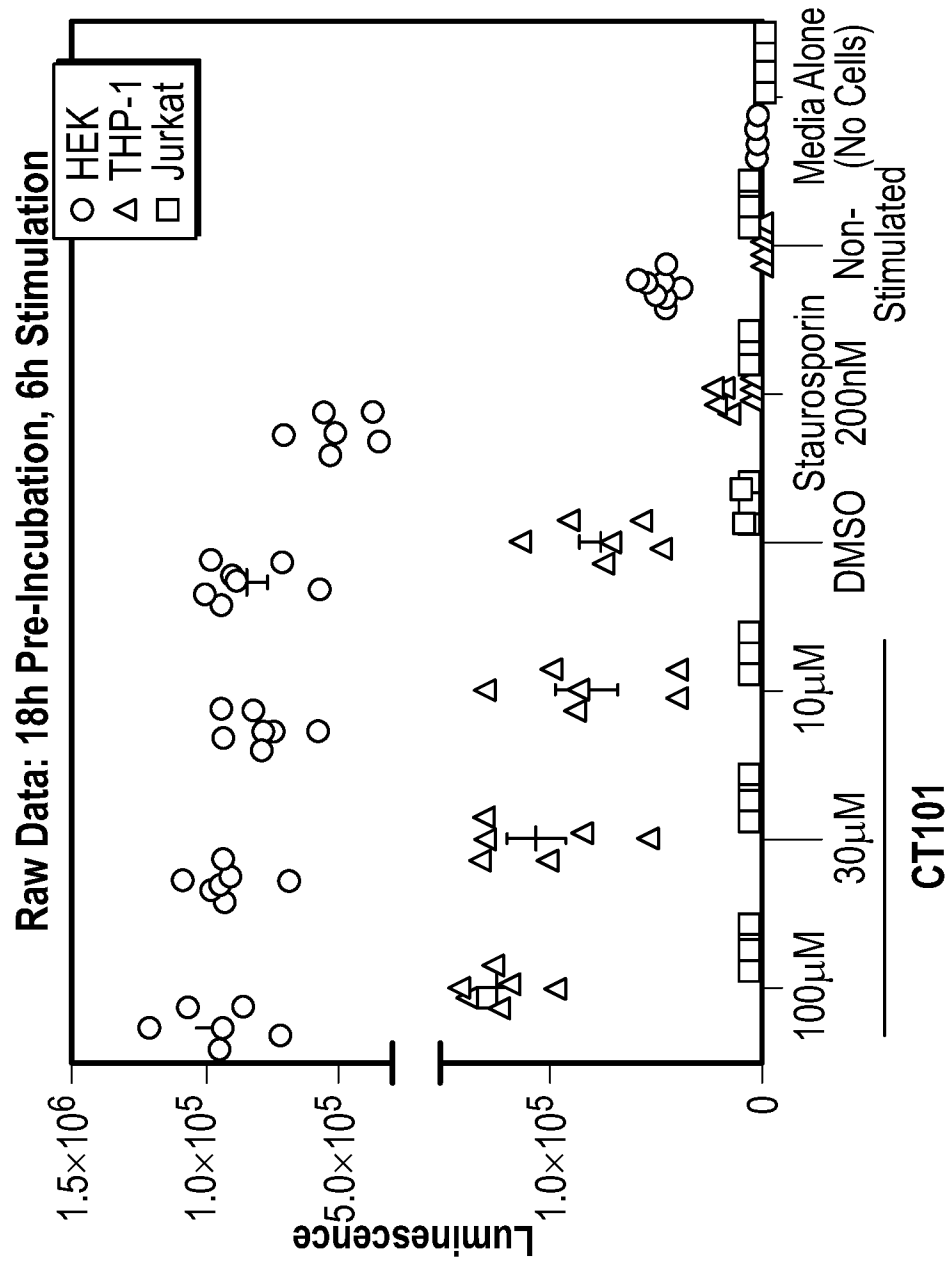


FIG. 43

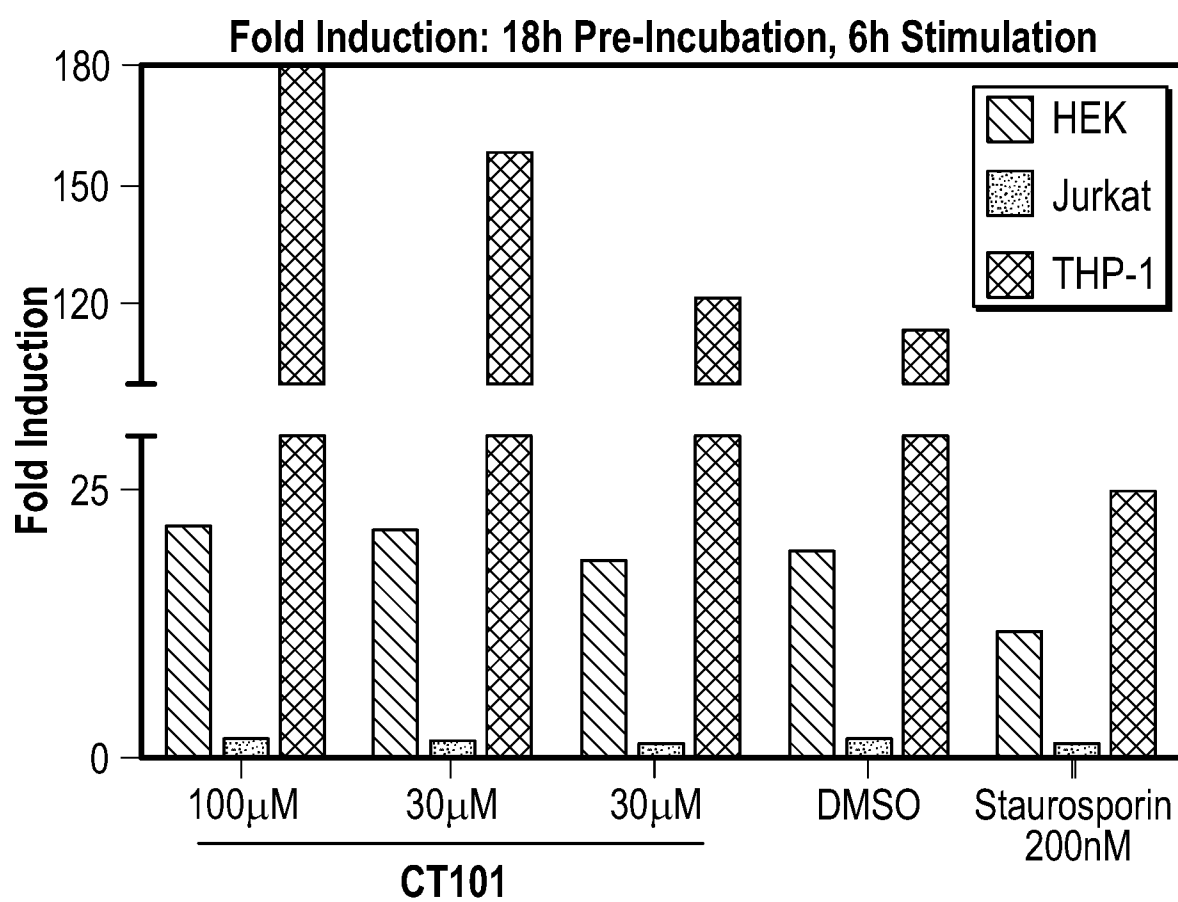


FIG. 44

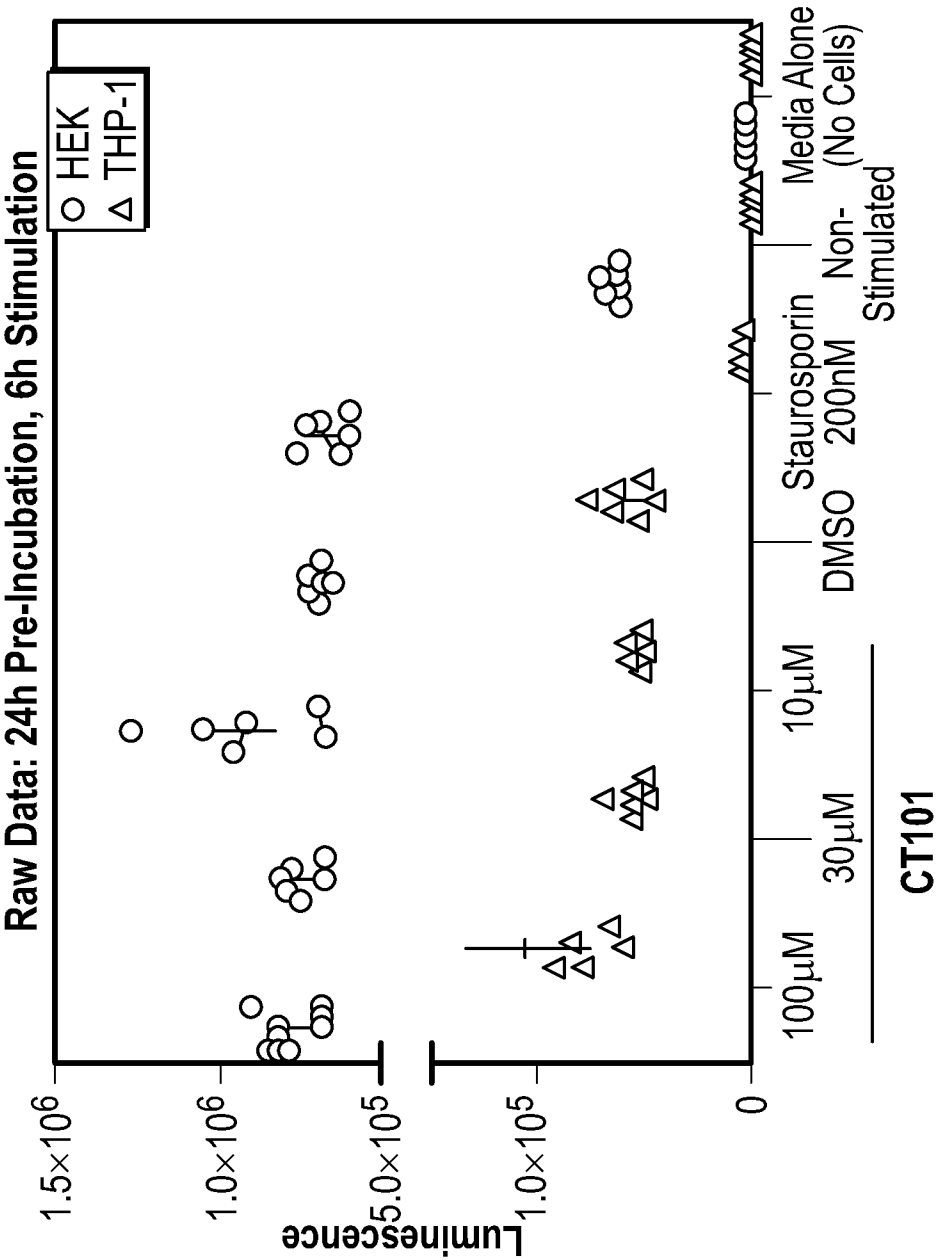
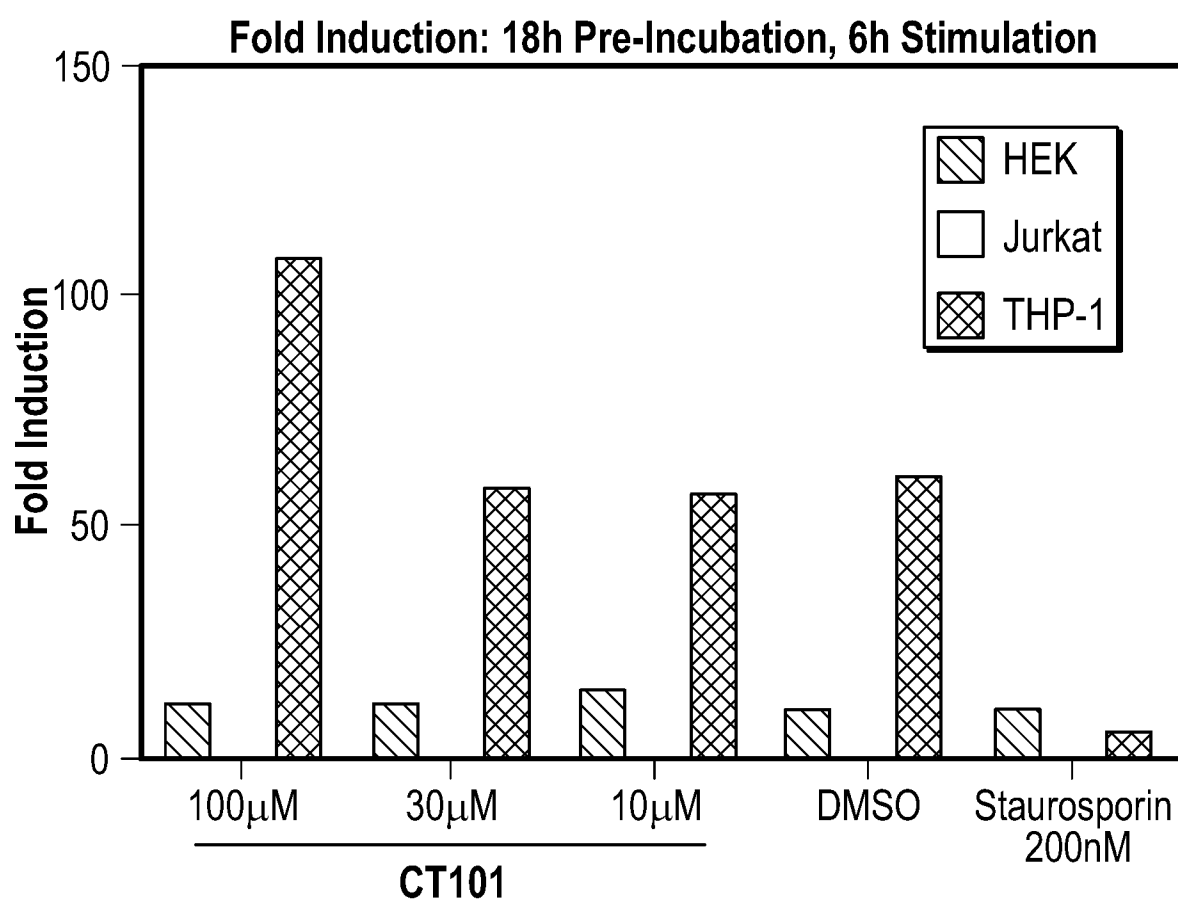


FIG. 45

**FIG. 46**

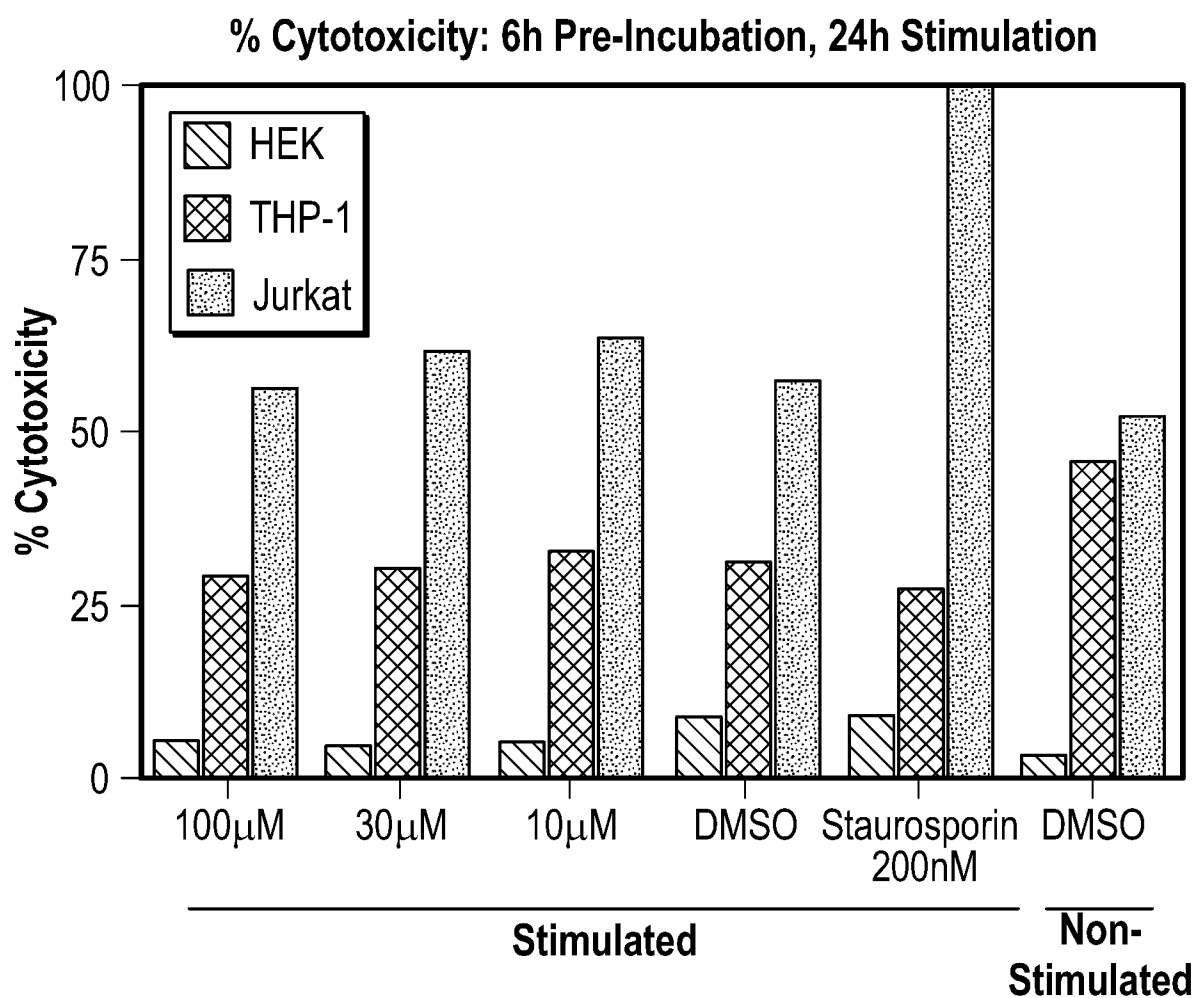
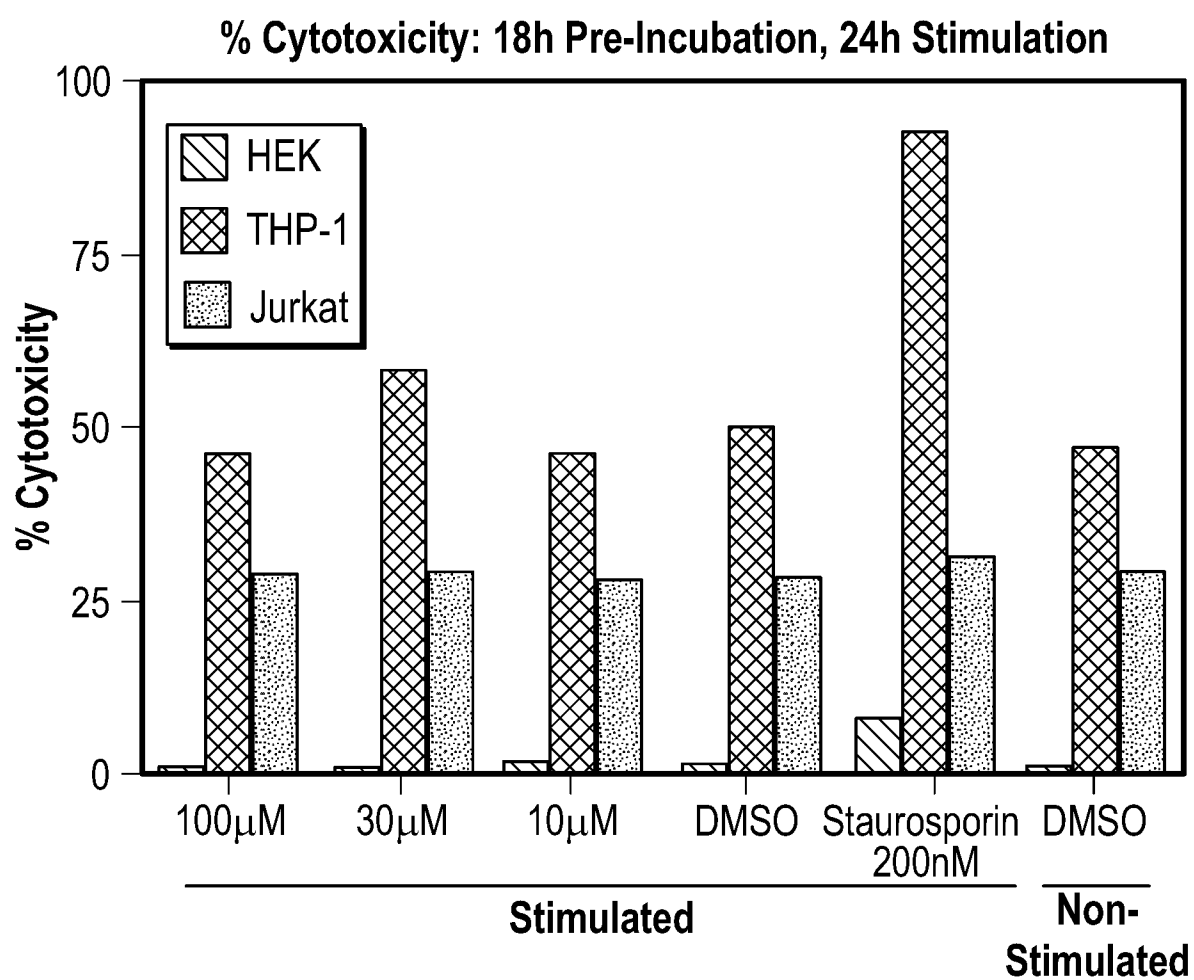


FIG. 47

**FIG. 48**

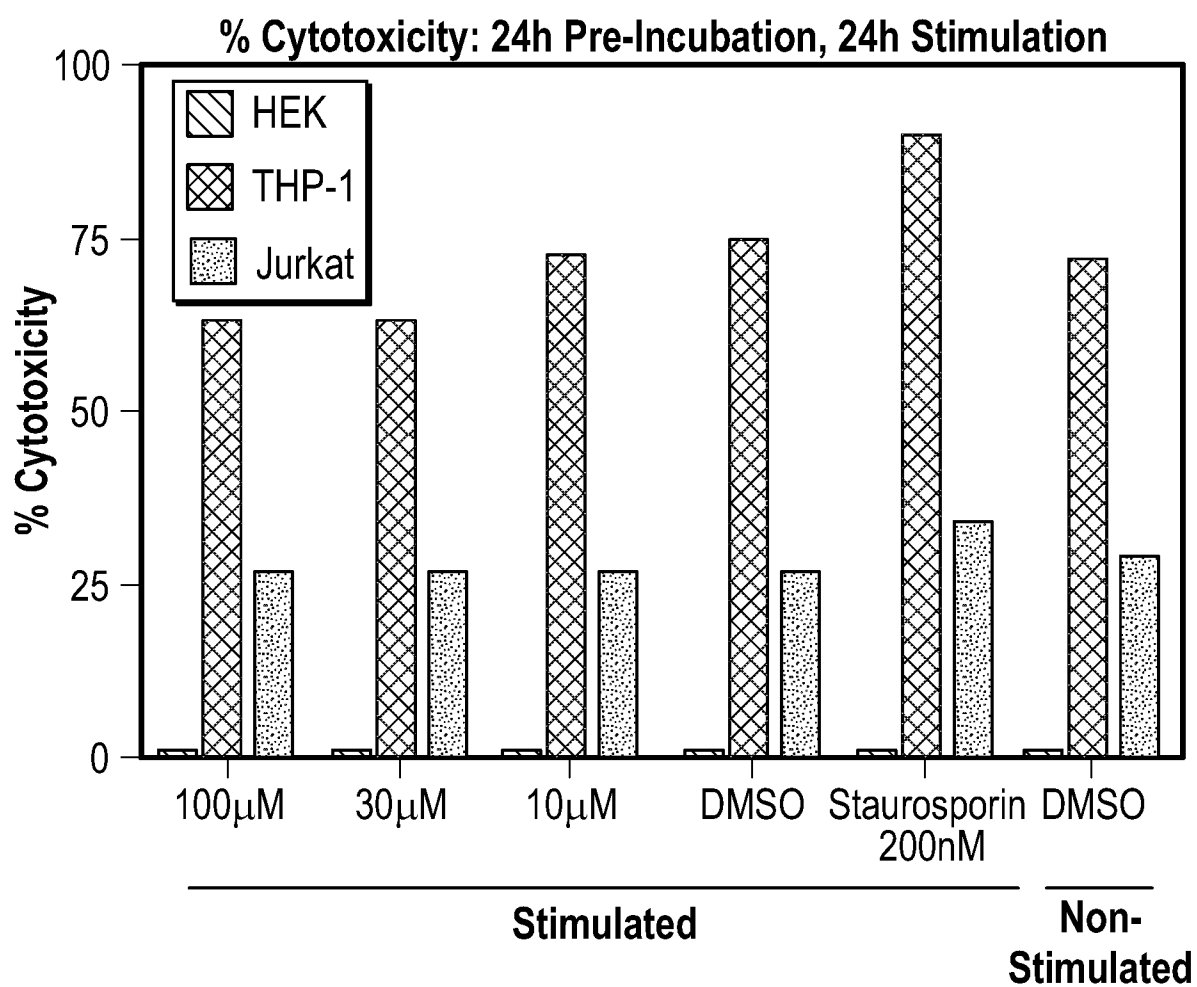


FIG. 49

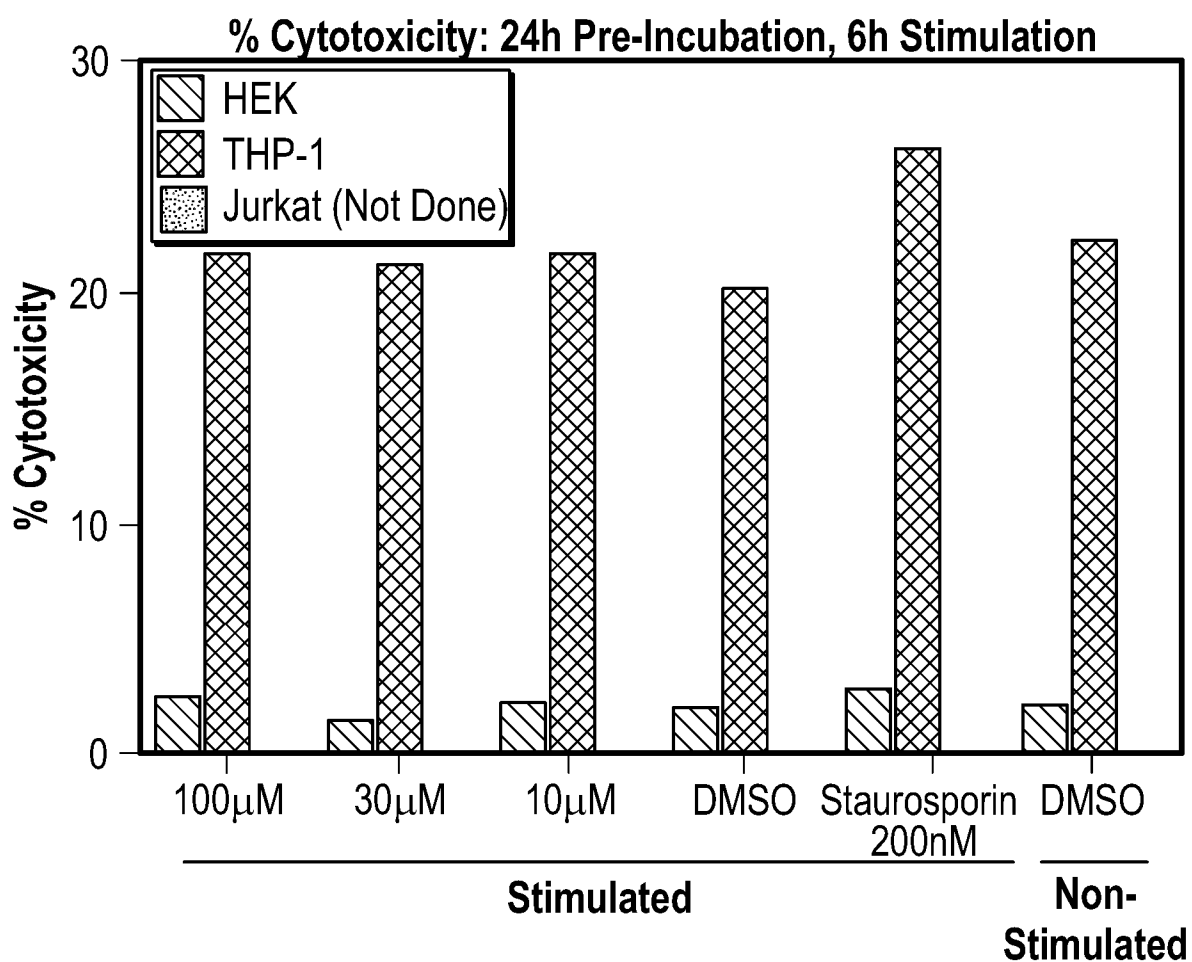


FIG. 50

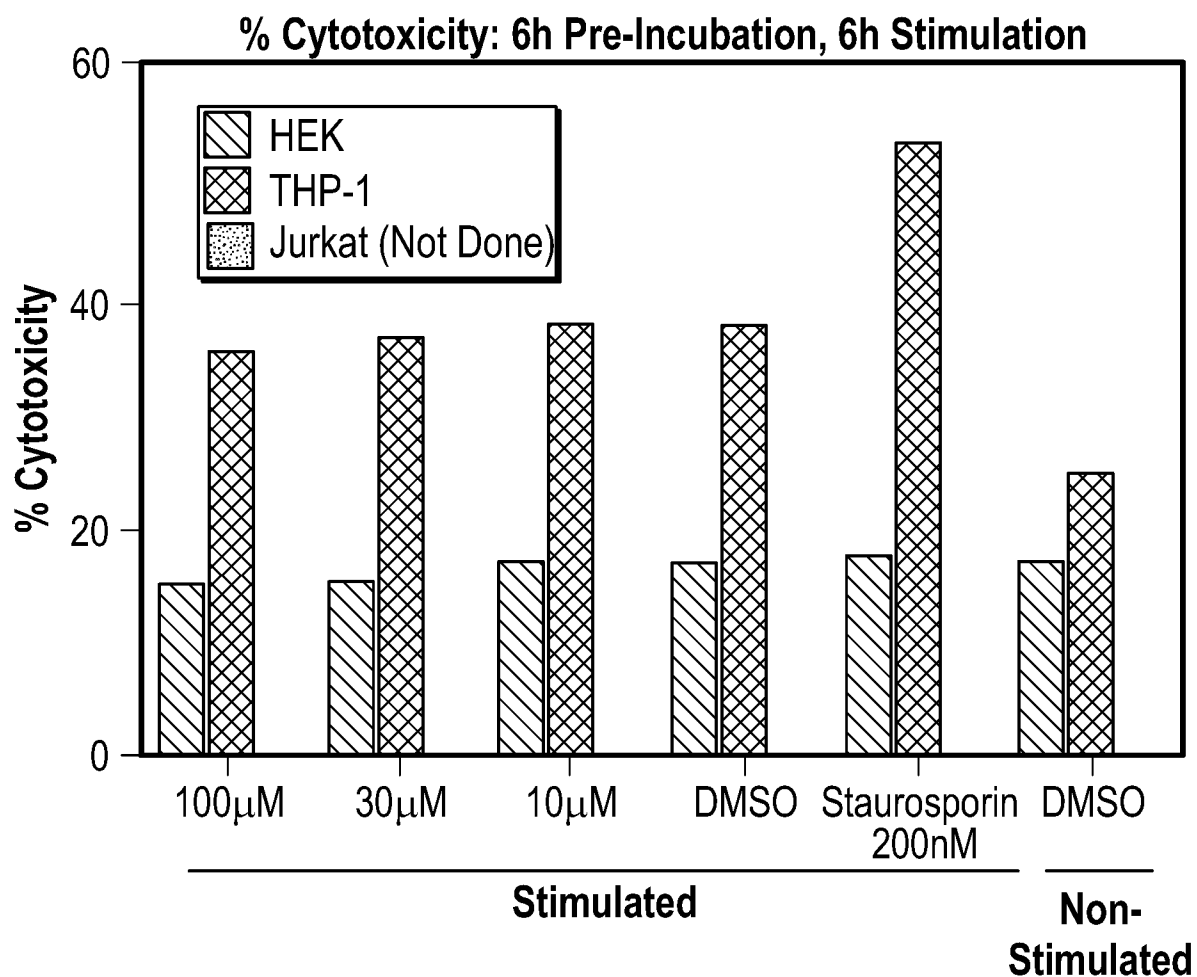
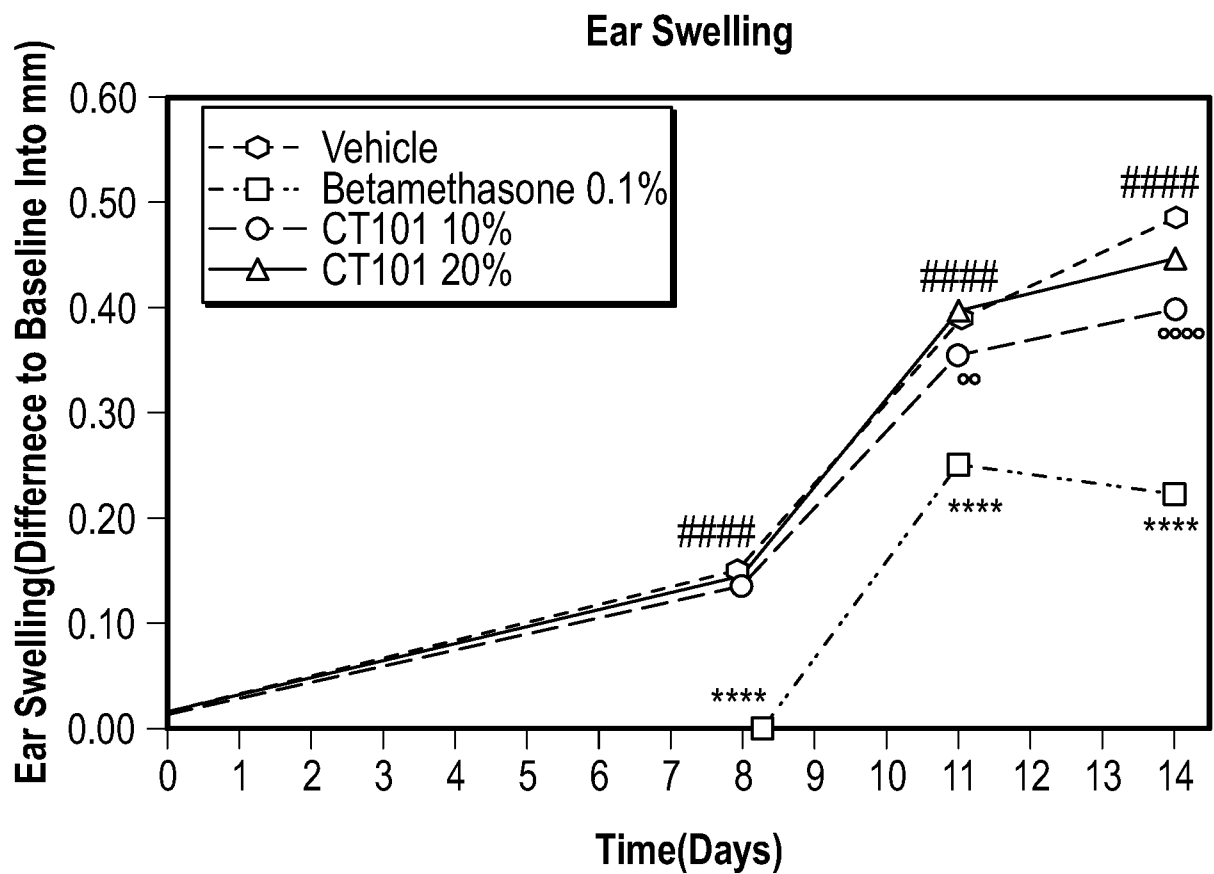
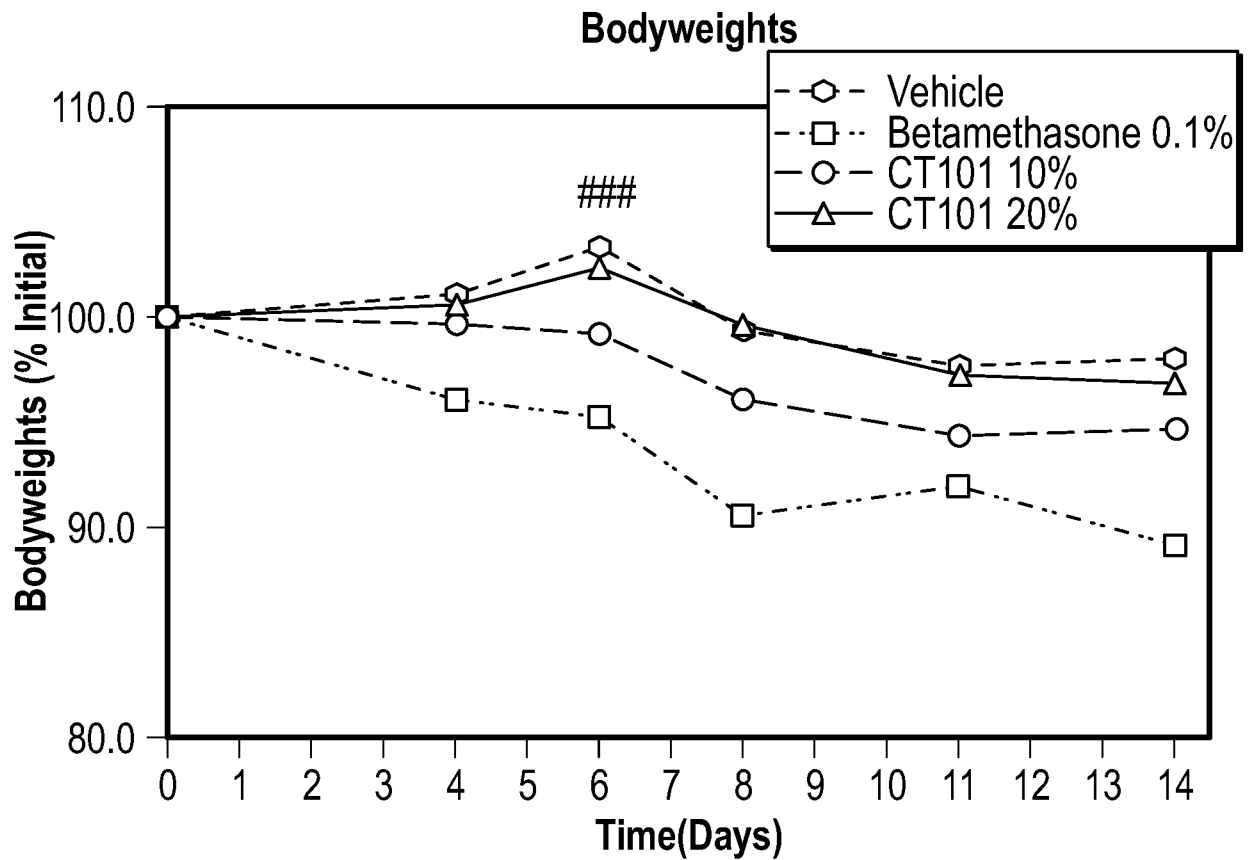
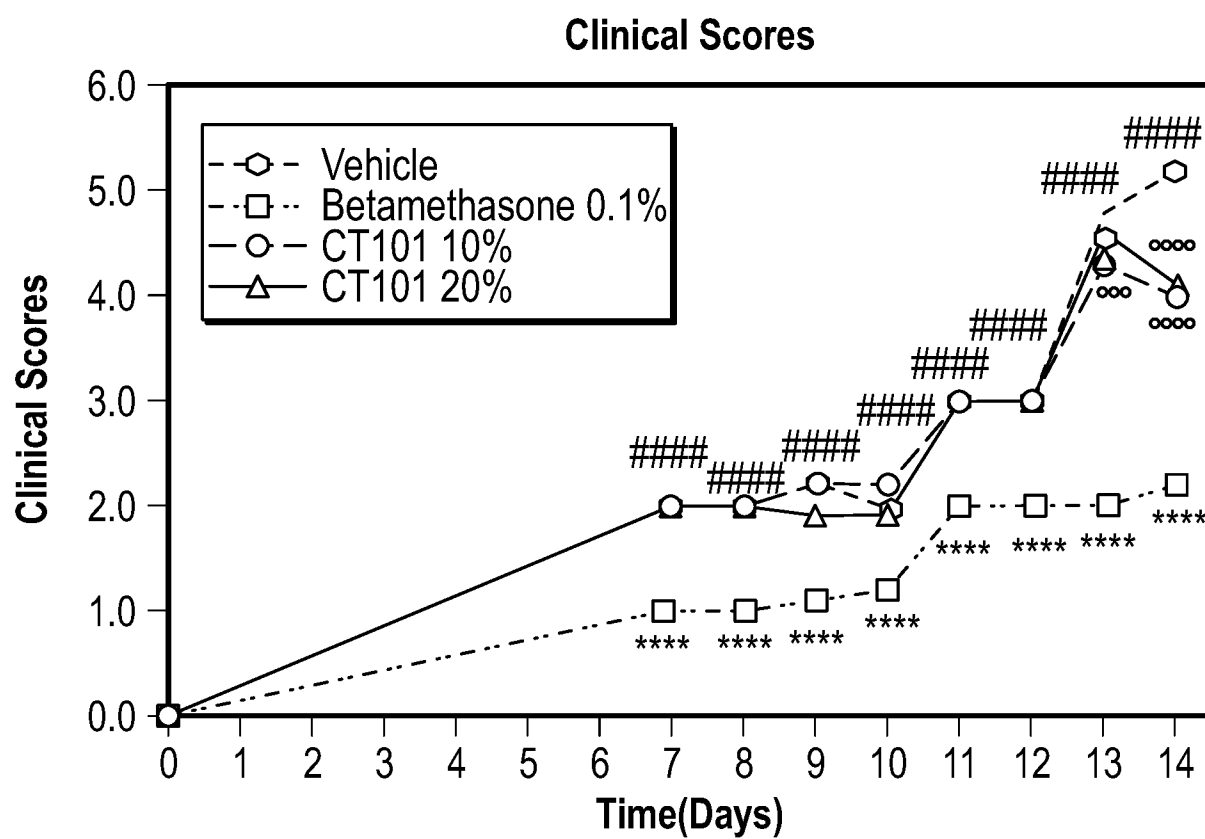


FIG. 51



**FIG. 54**

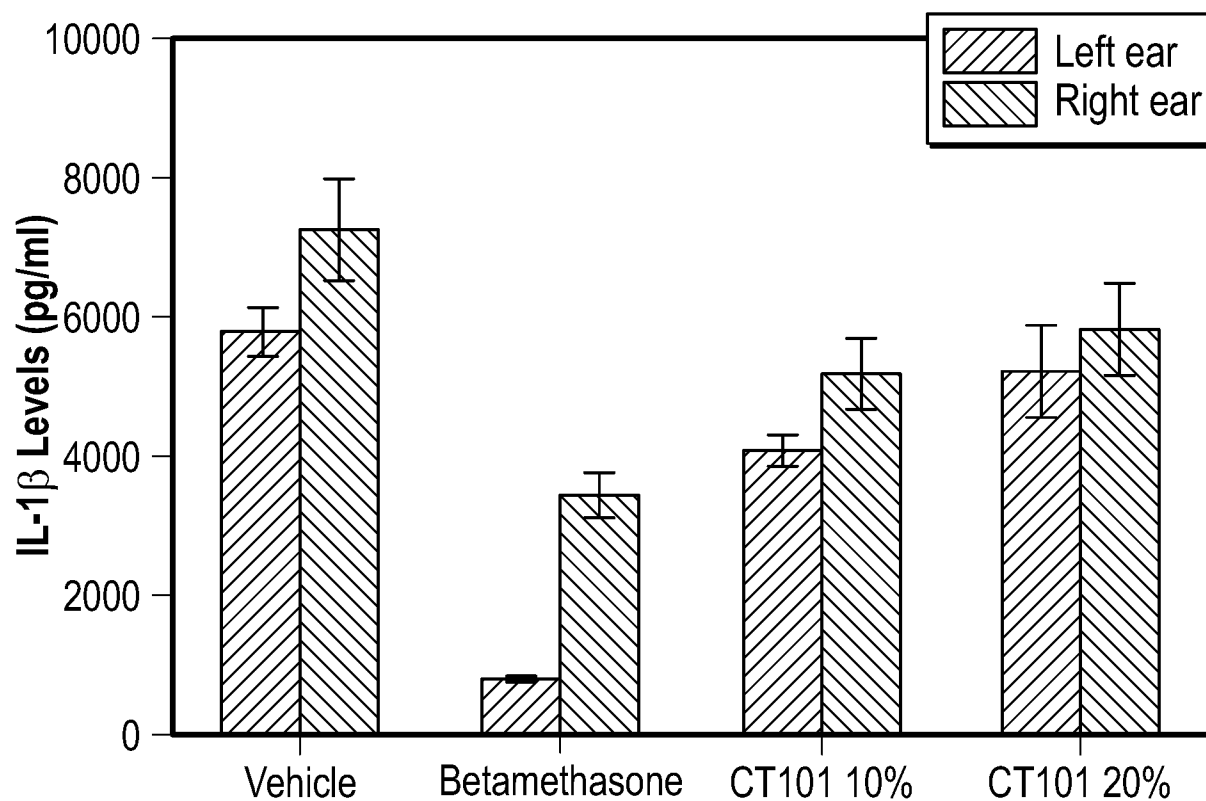


FIG. 55A

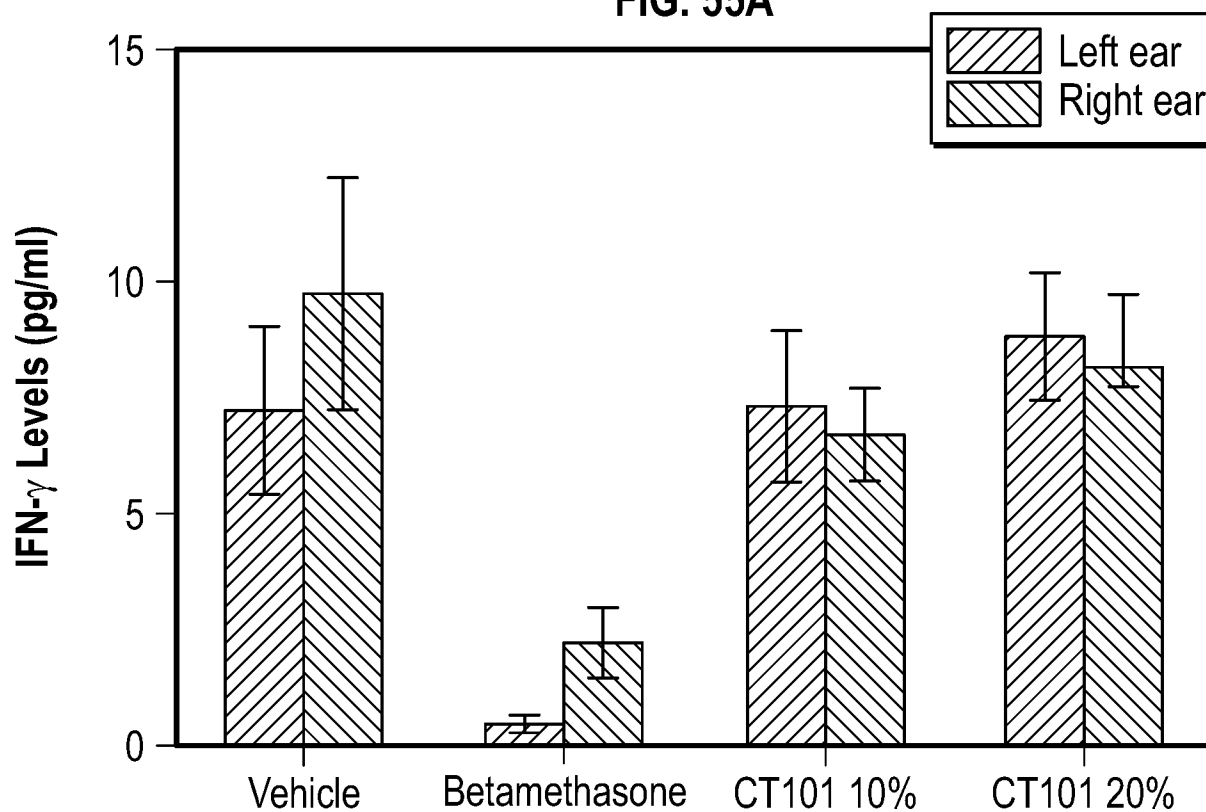


FIG. 55B

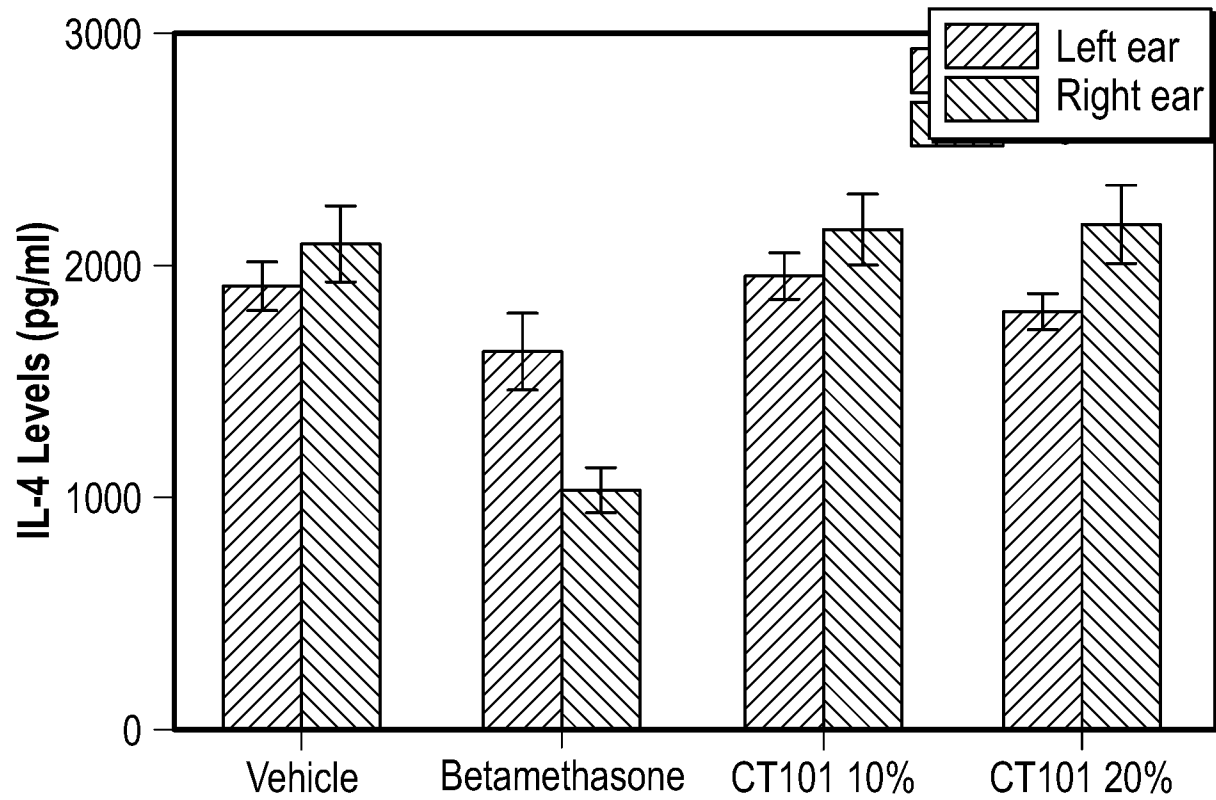


FIG. 55C

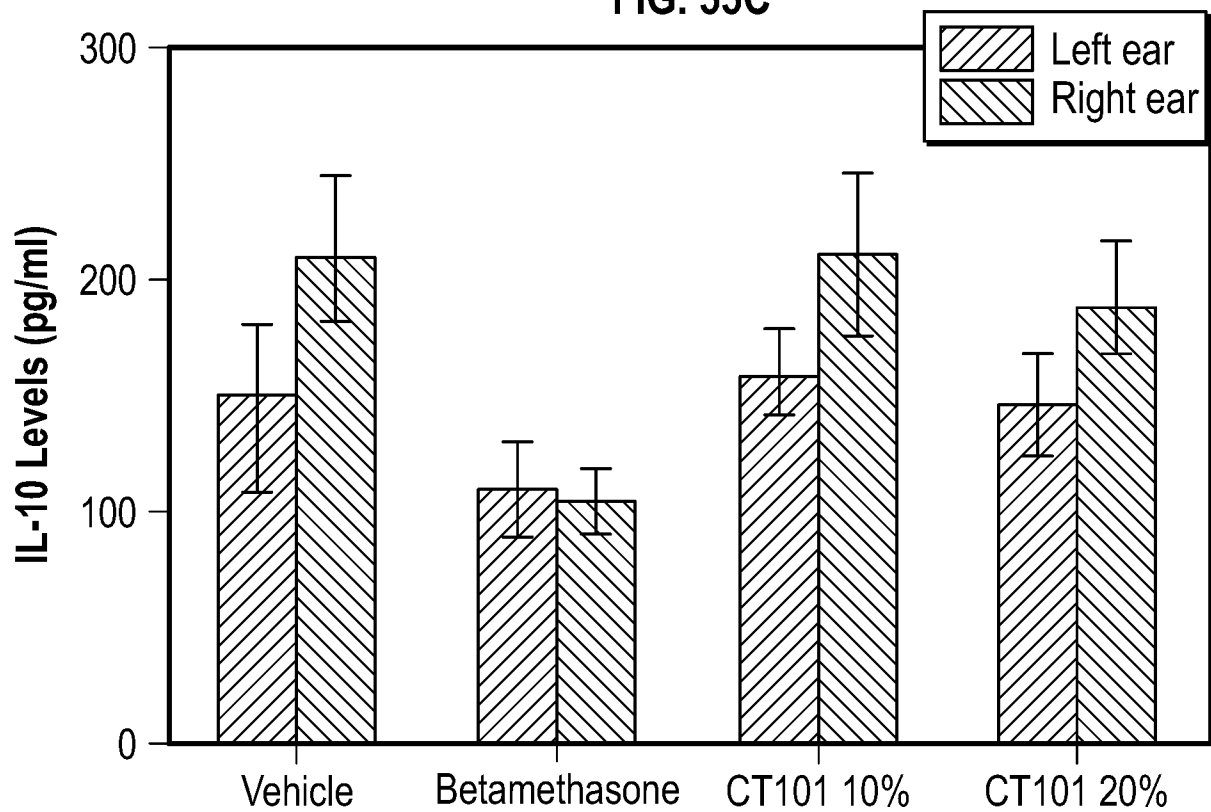
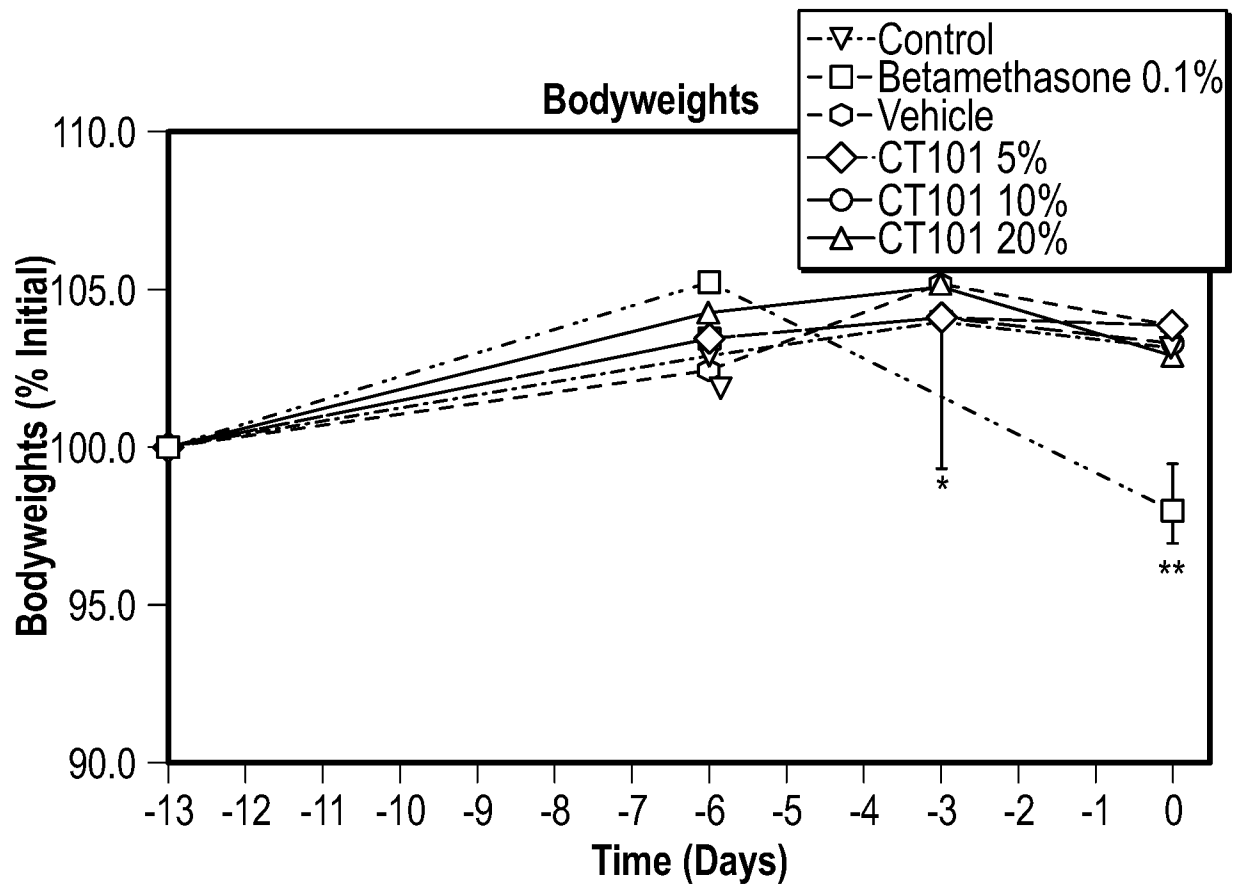
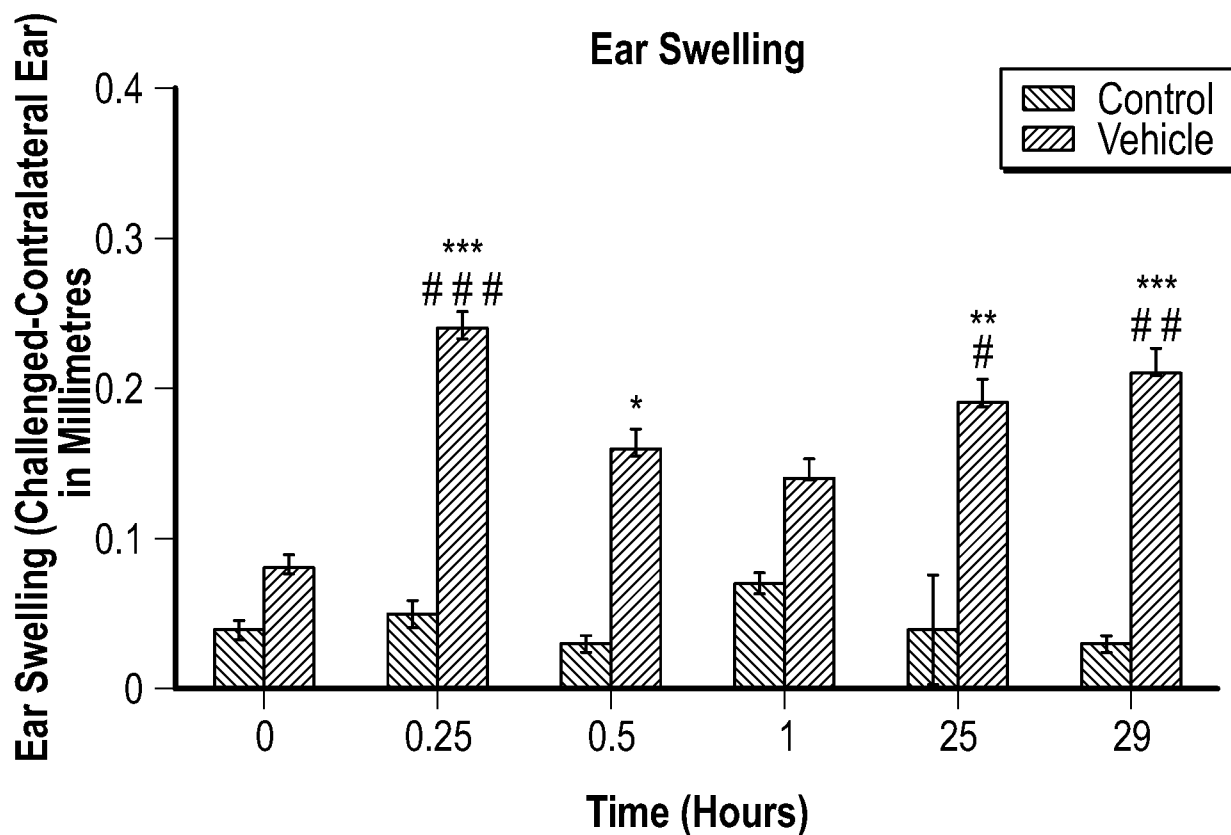
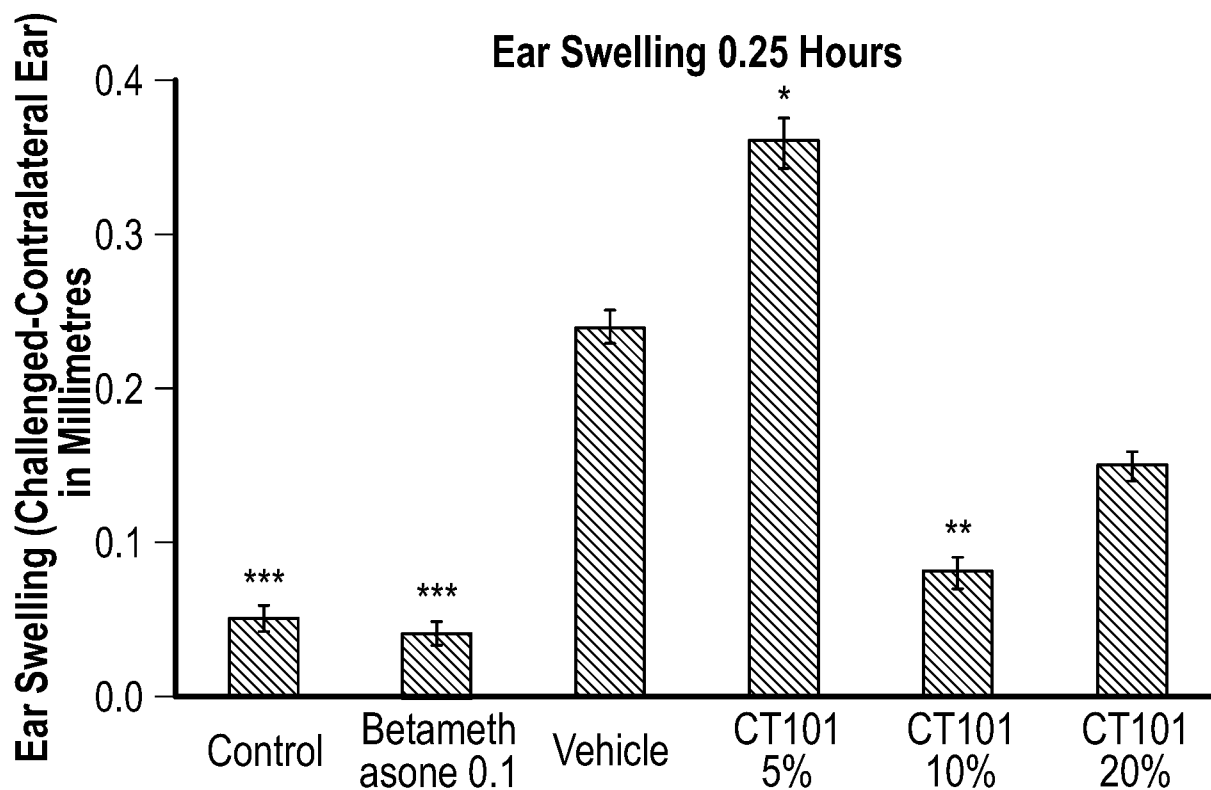
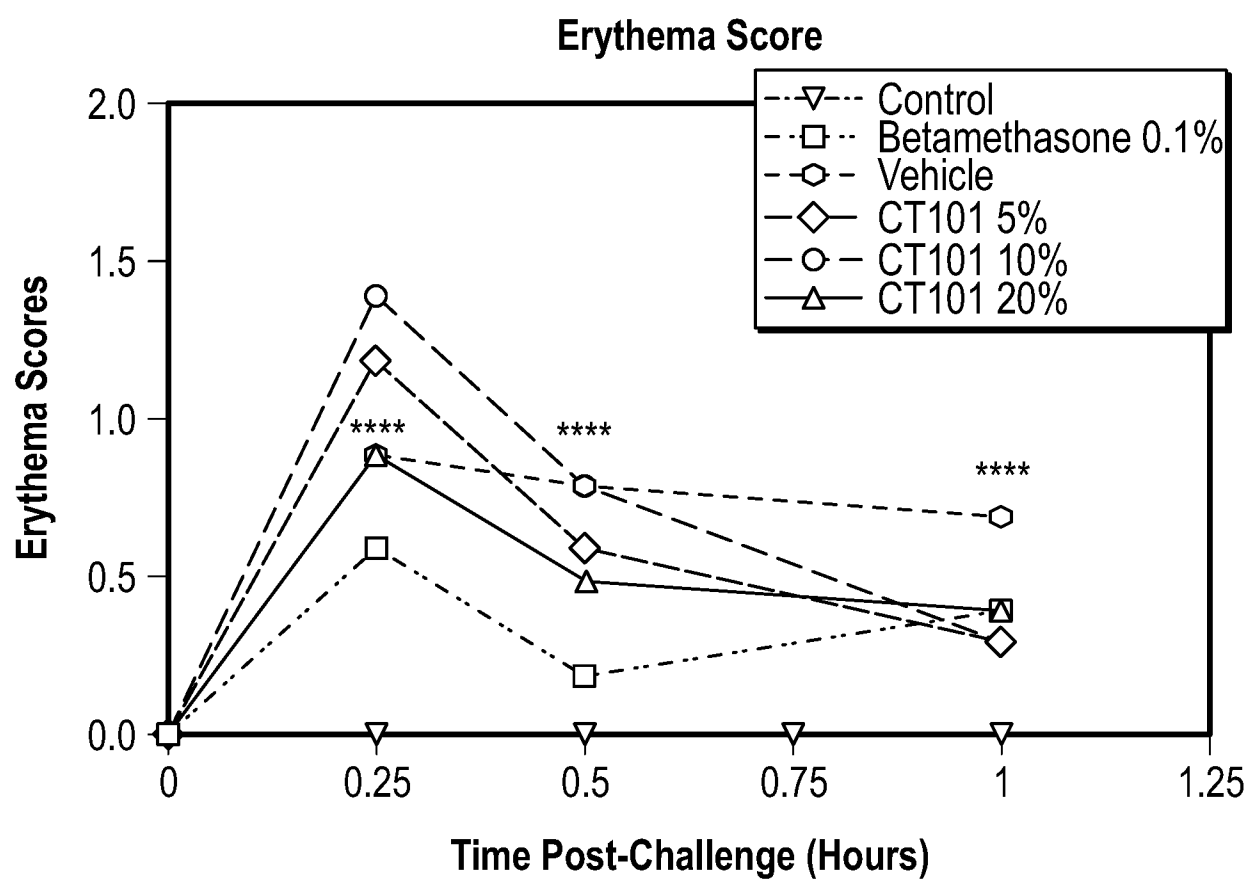


FIG. 55D

**FIG. 56**

**FIG. 57****FIG. 58**

**FIG.59**

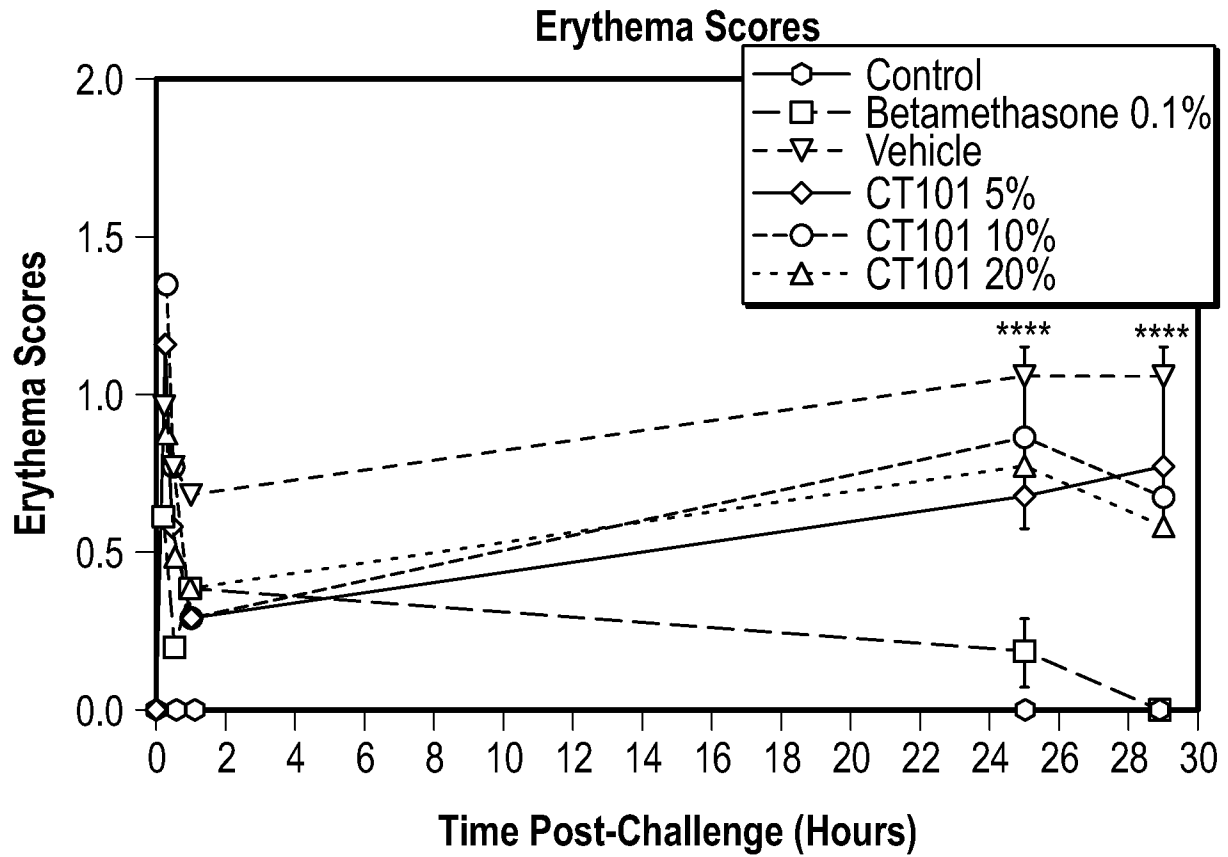


FIG. 60

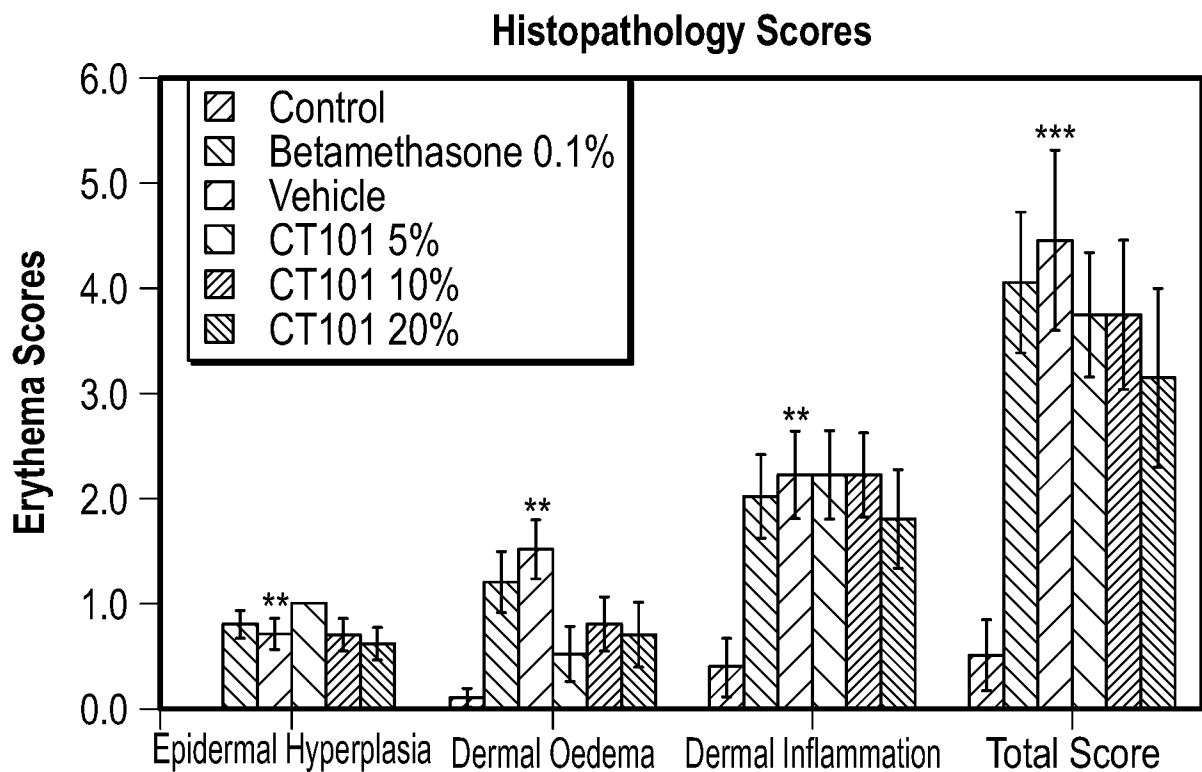


FIG. 61

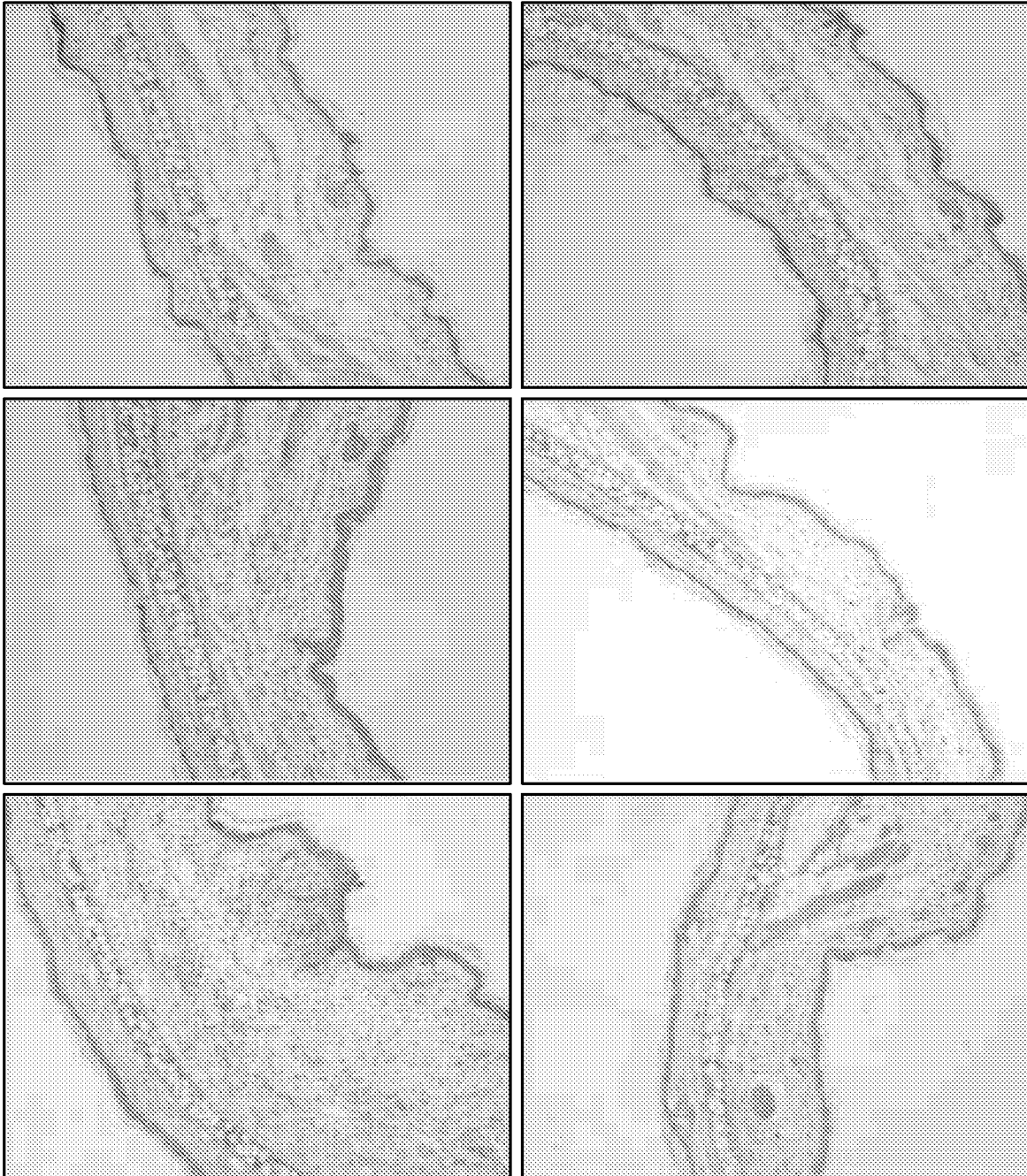


FIG. 62

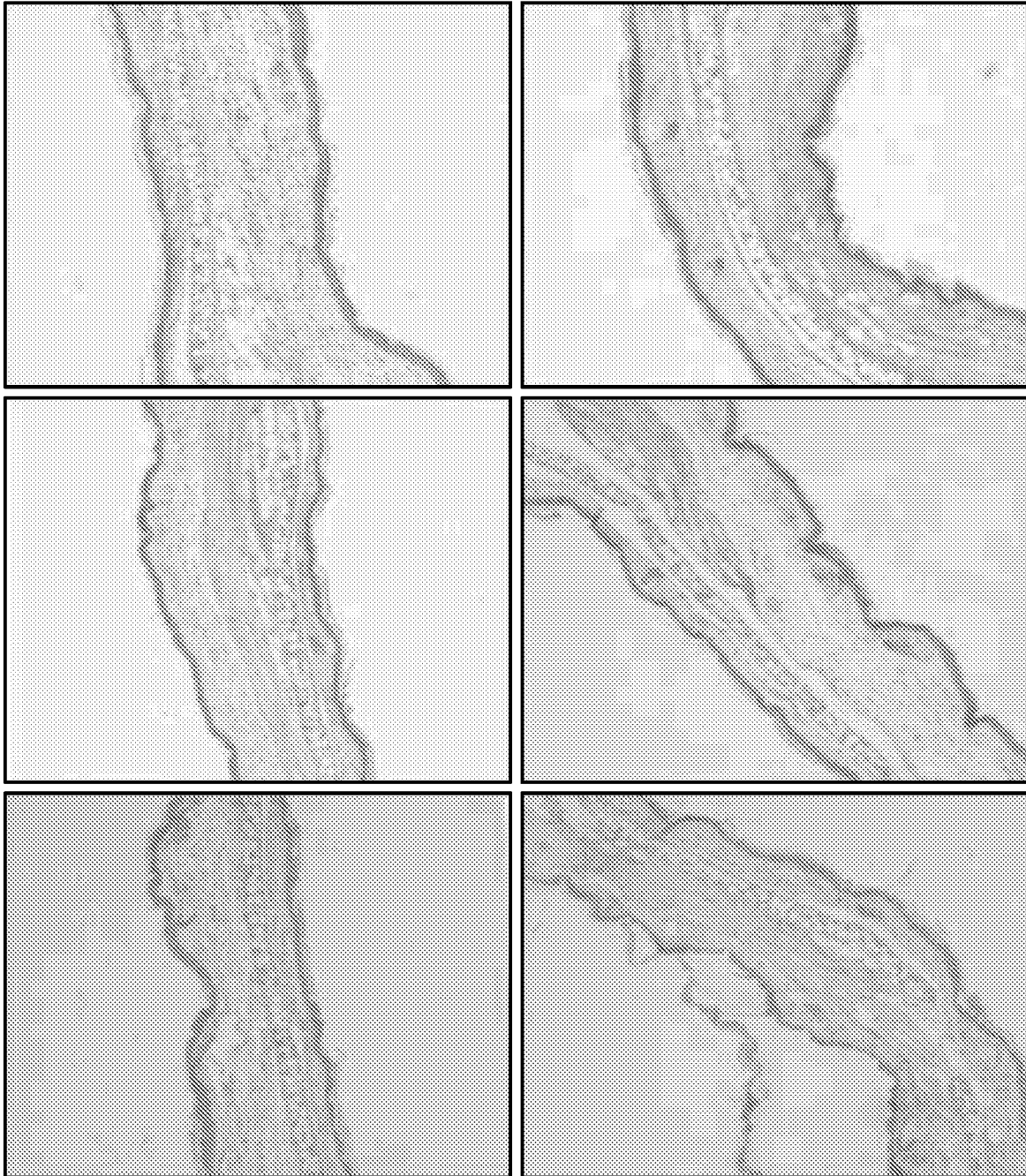


FIG. 63

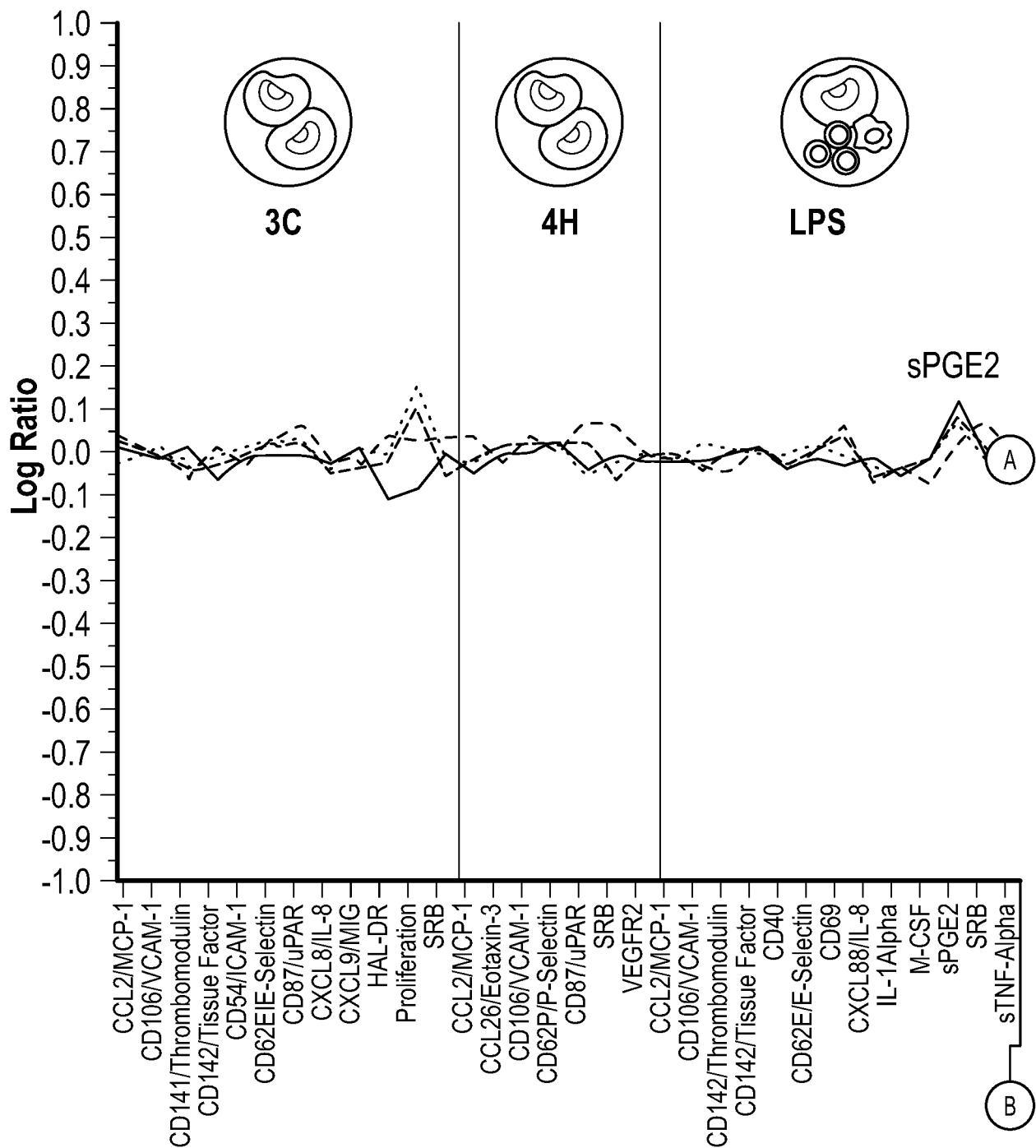


FIG. 64

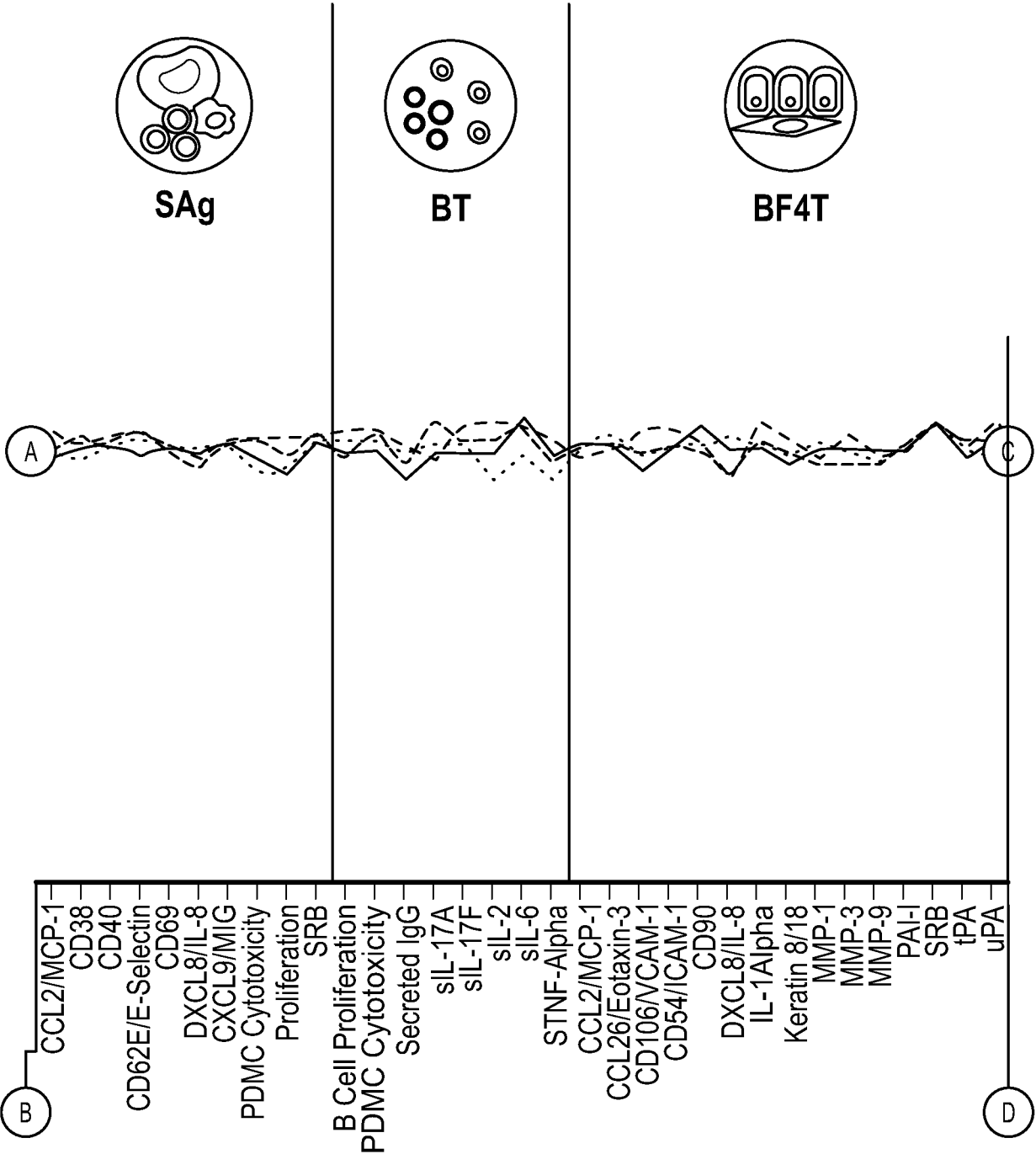


FIG. 64
(Continued)

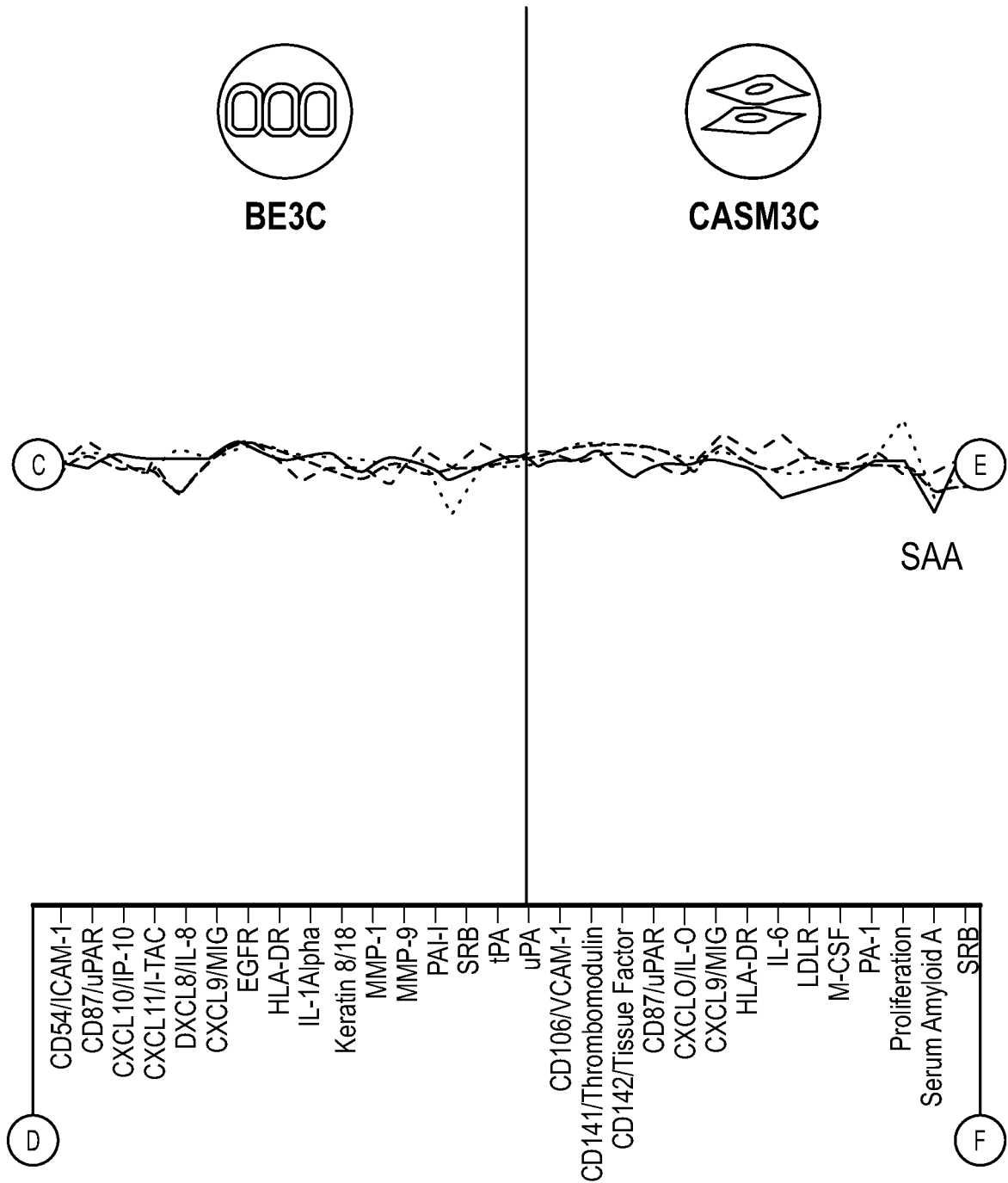


FIG. 64
(Continued)

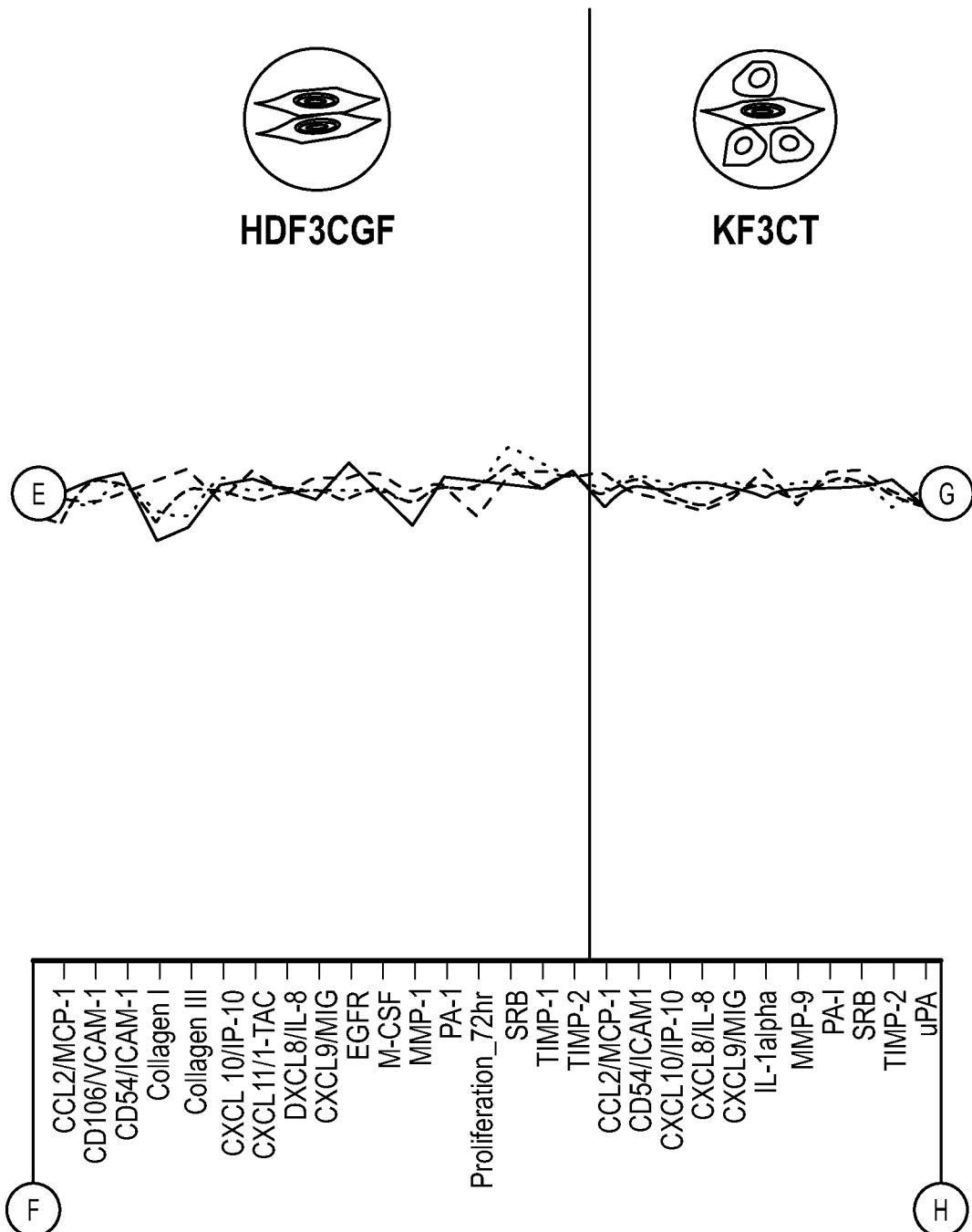


FIG. 64
(Continued)

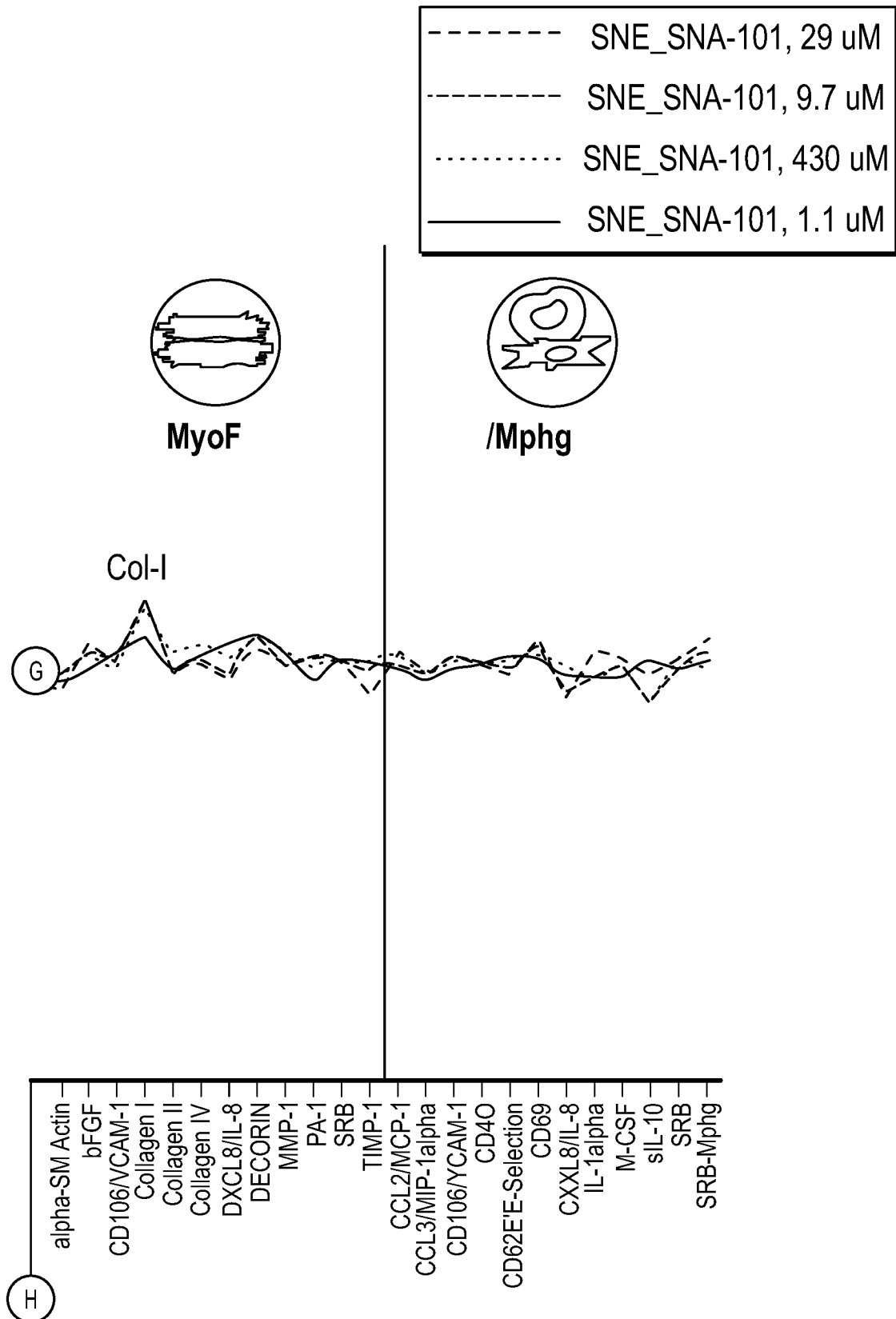


FIG. 64
(Continued)

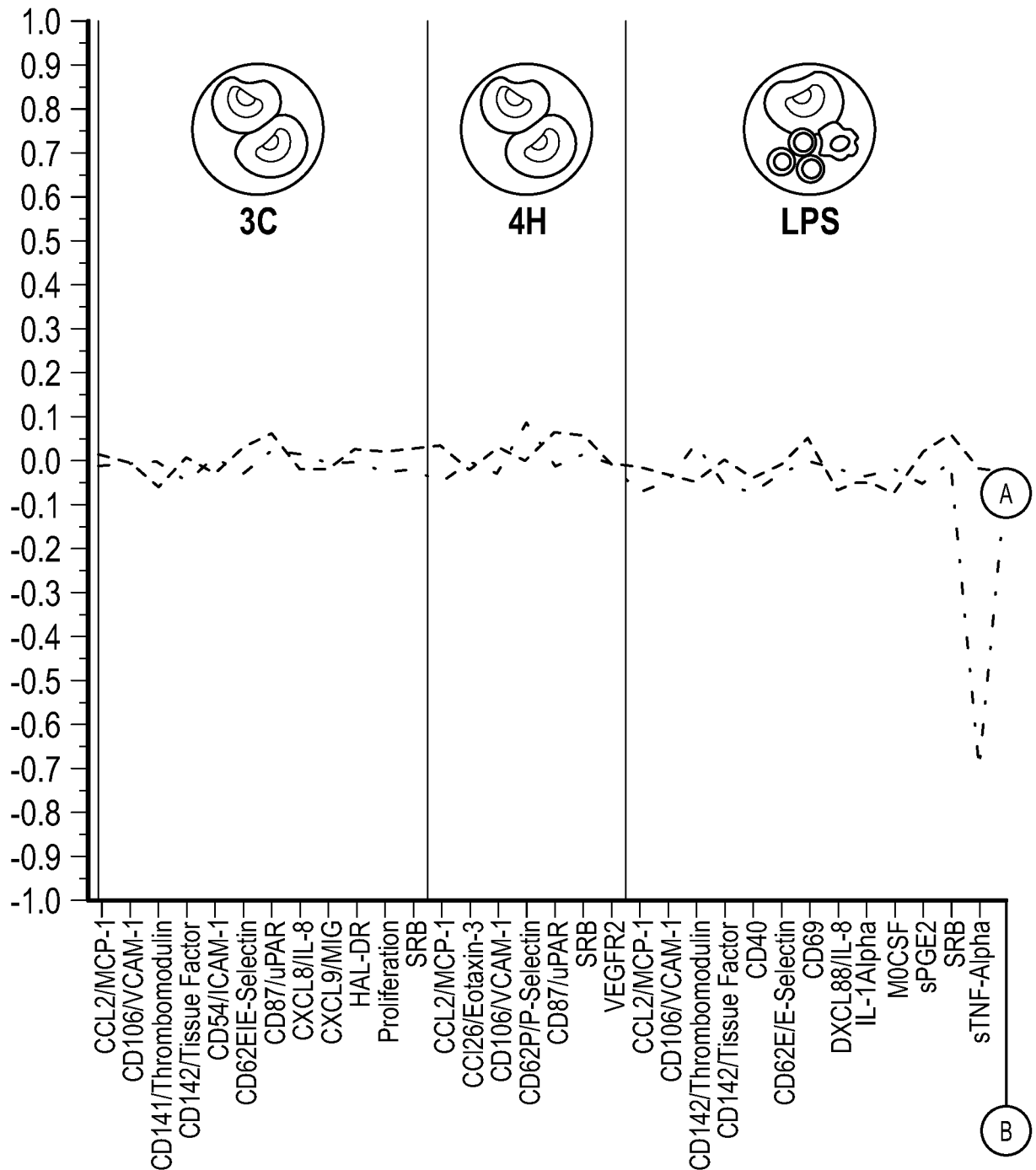


FIG. 65

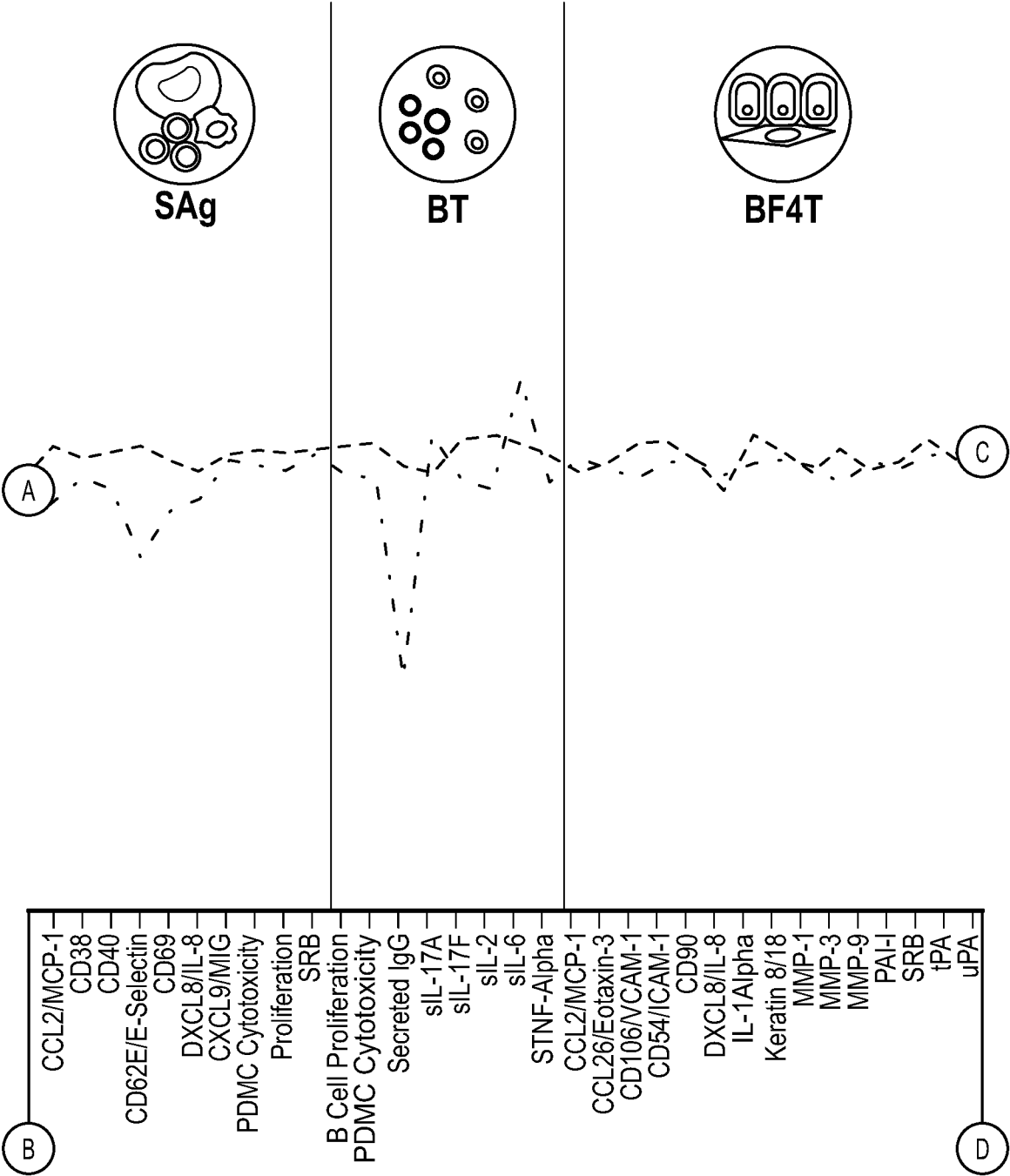


FIG. 65

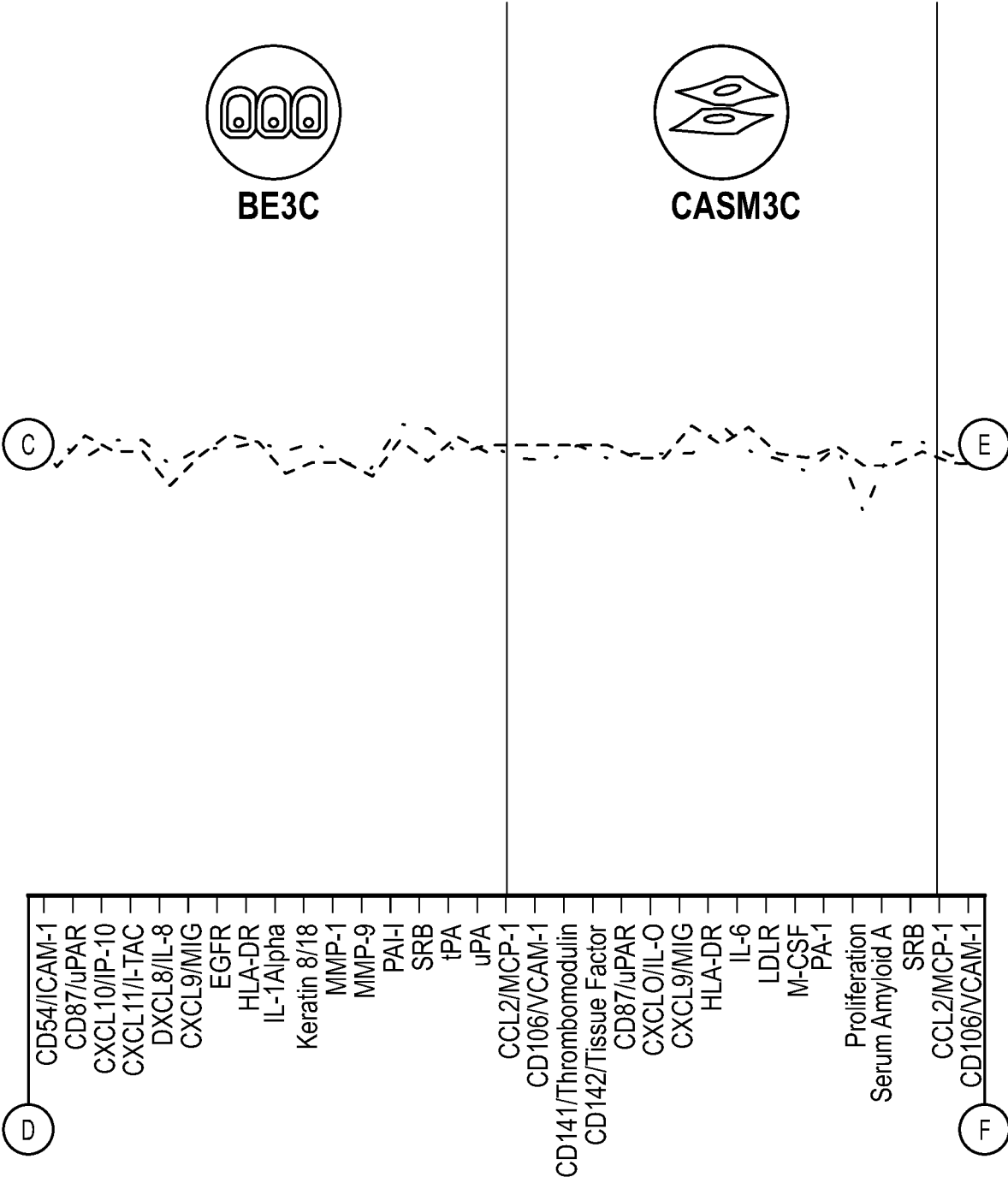


FIG. 65
(Continued)

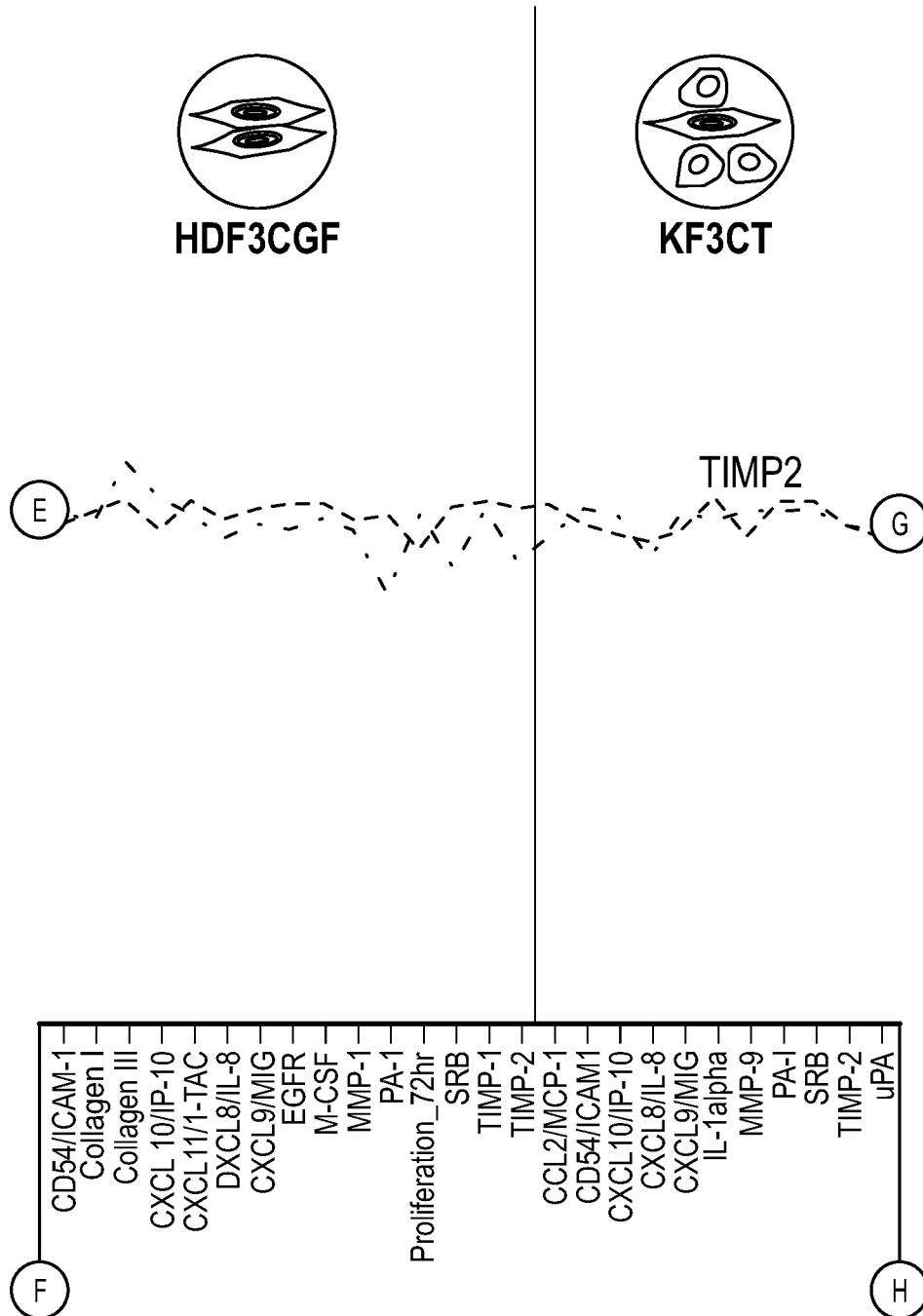


FIG. 65
(Continued)

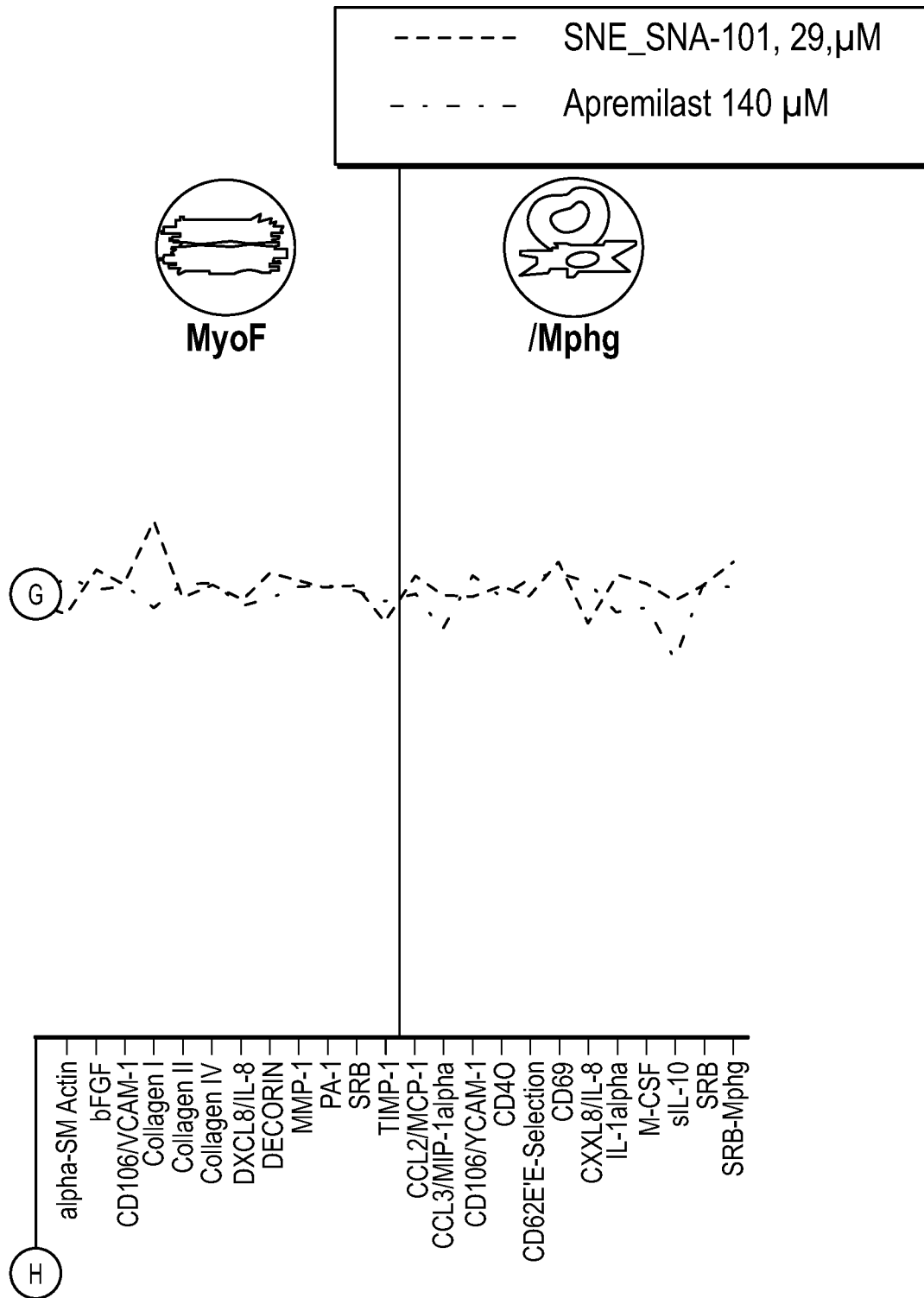


FIG. 65
(Continued)

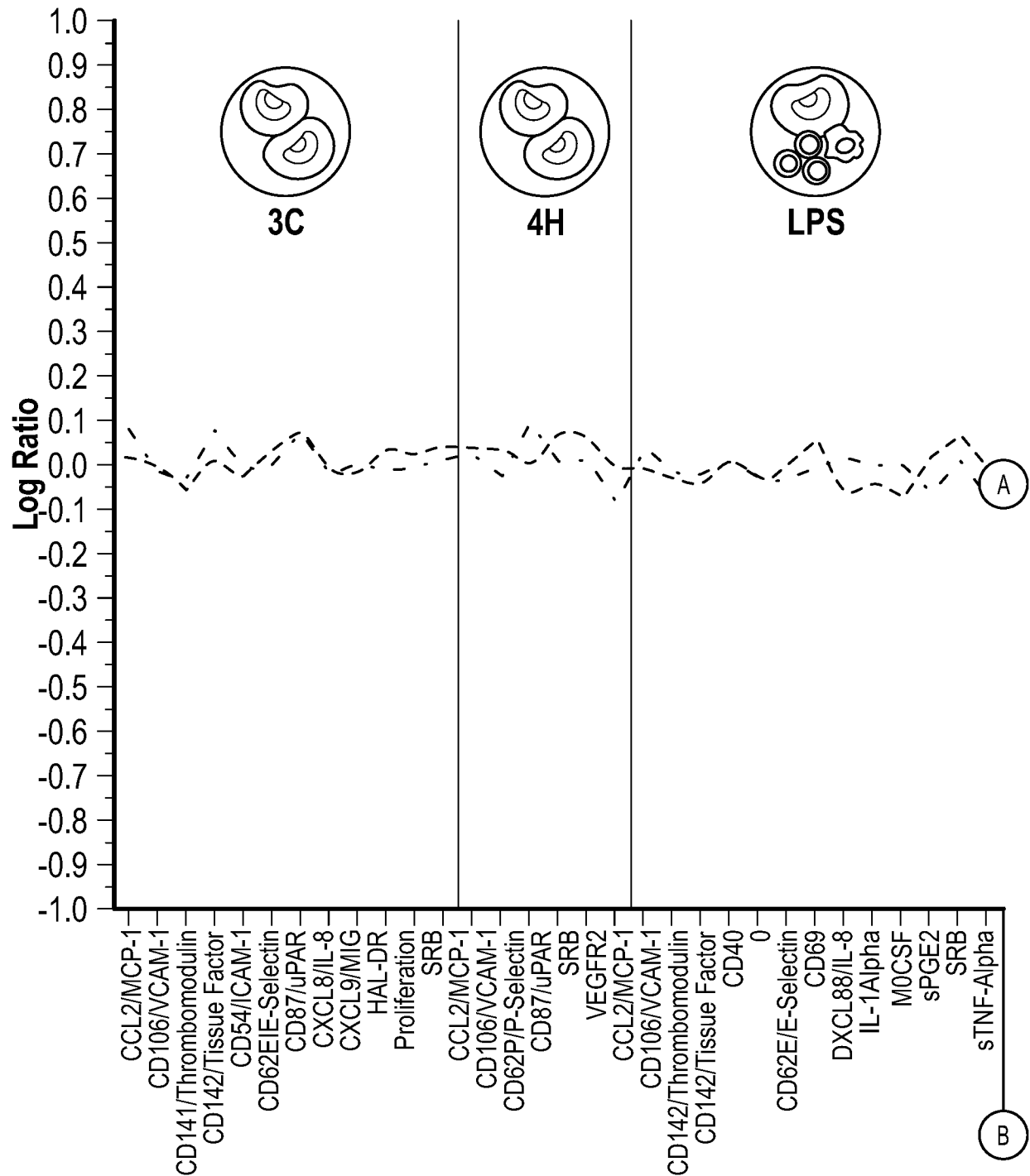


FIG. 66

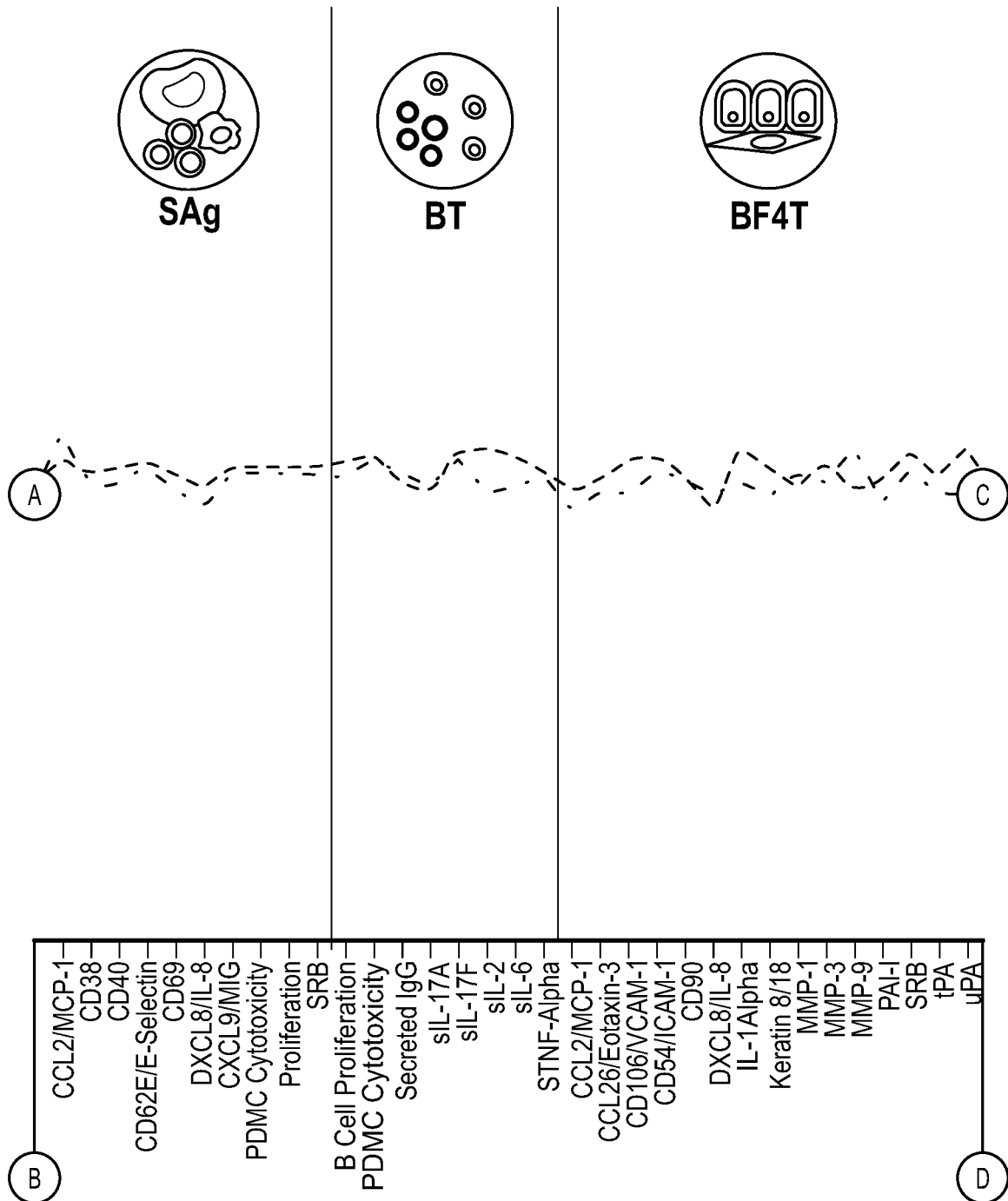


FIG. 66
(Continued)

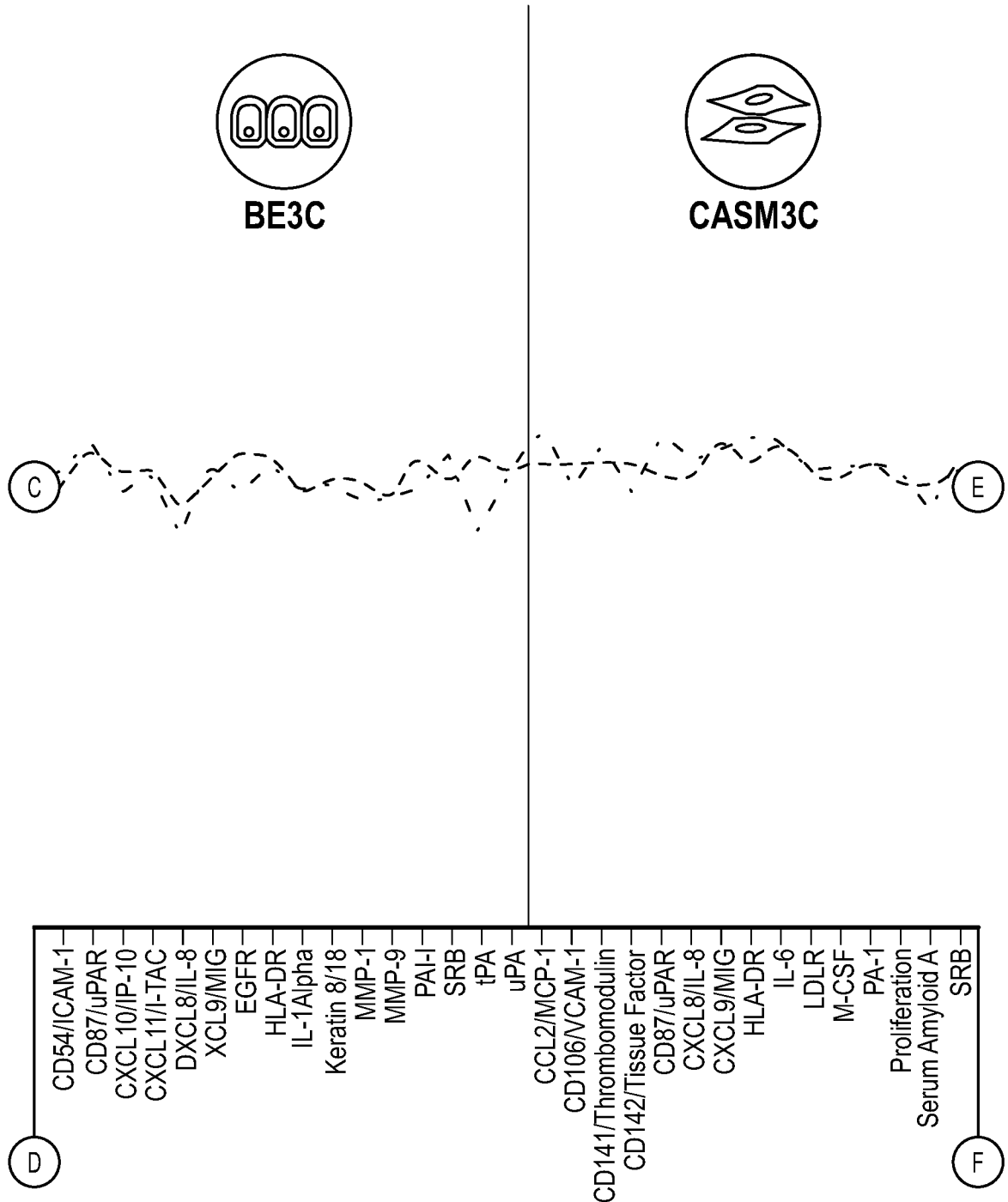


FIG. 66
(Continued)

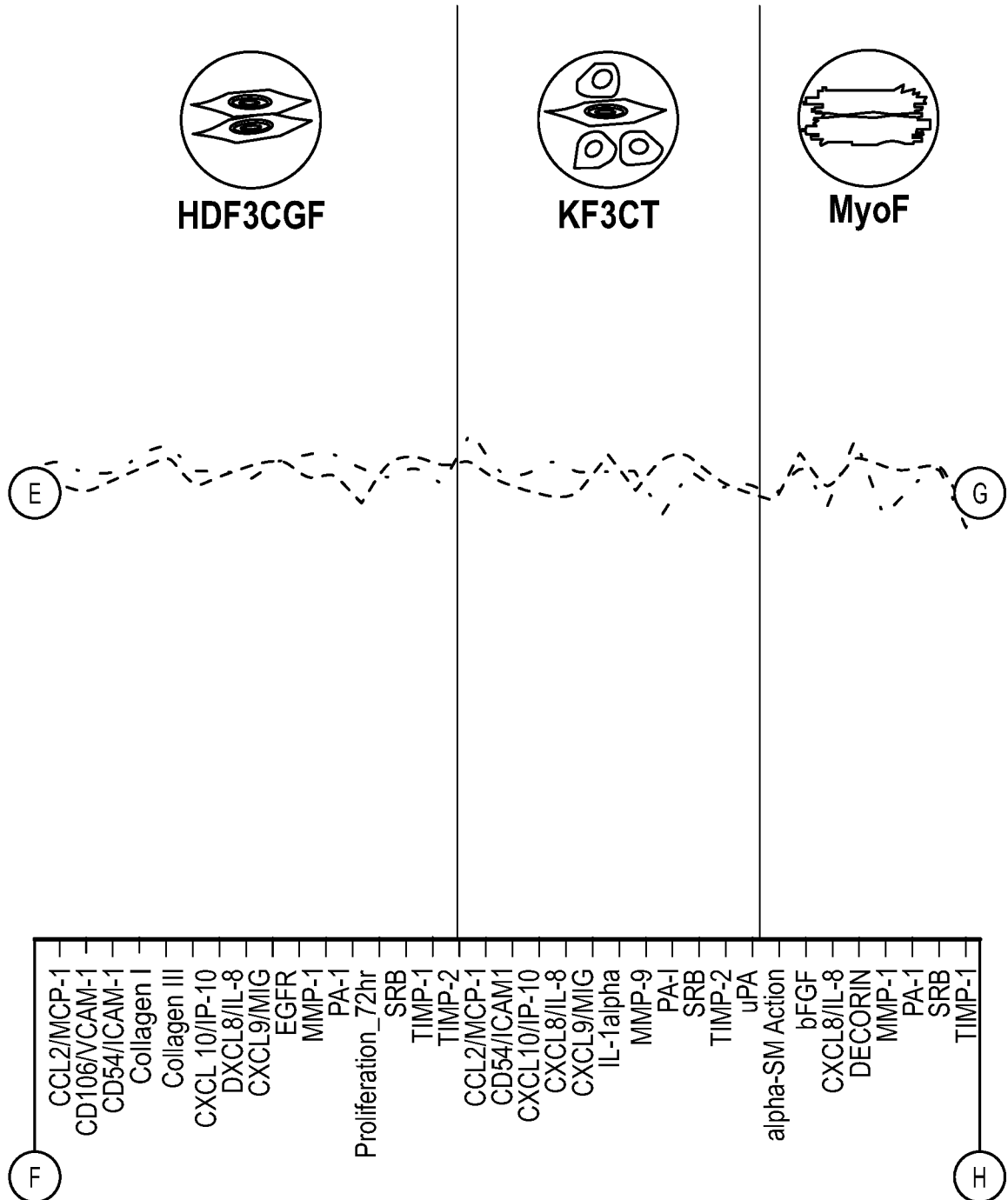


FIG. 66
(Continued)

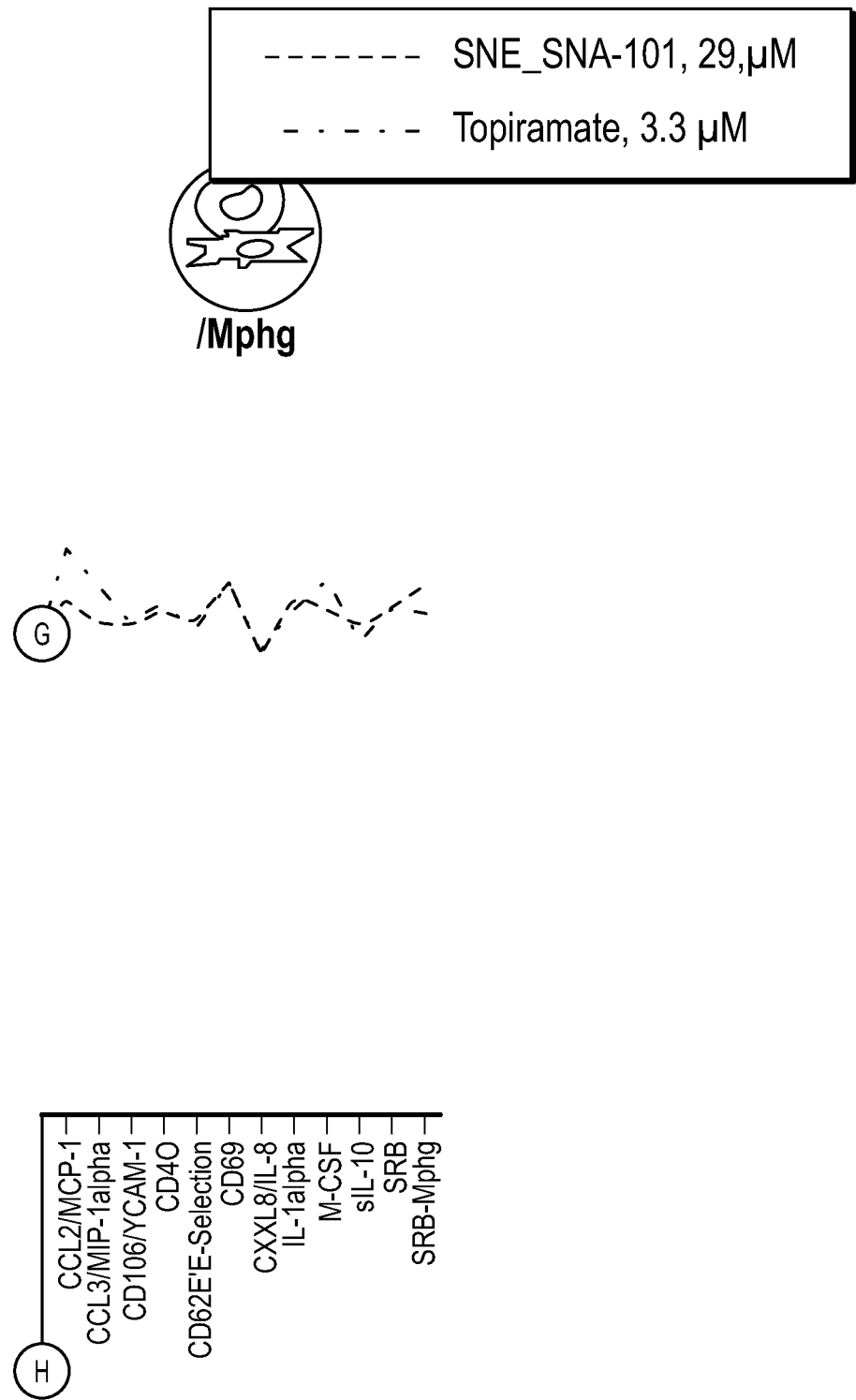


FIG. 66
(Continued)

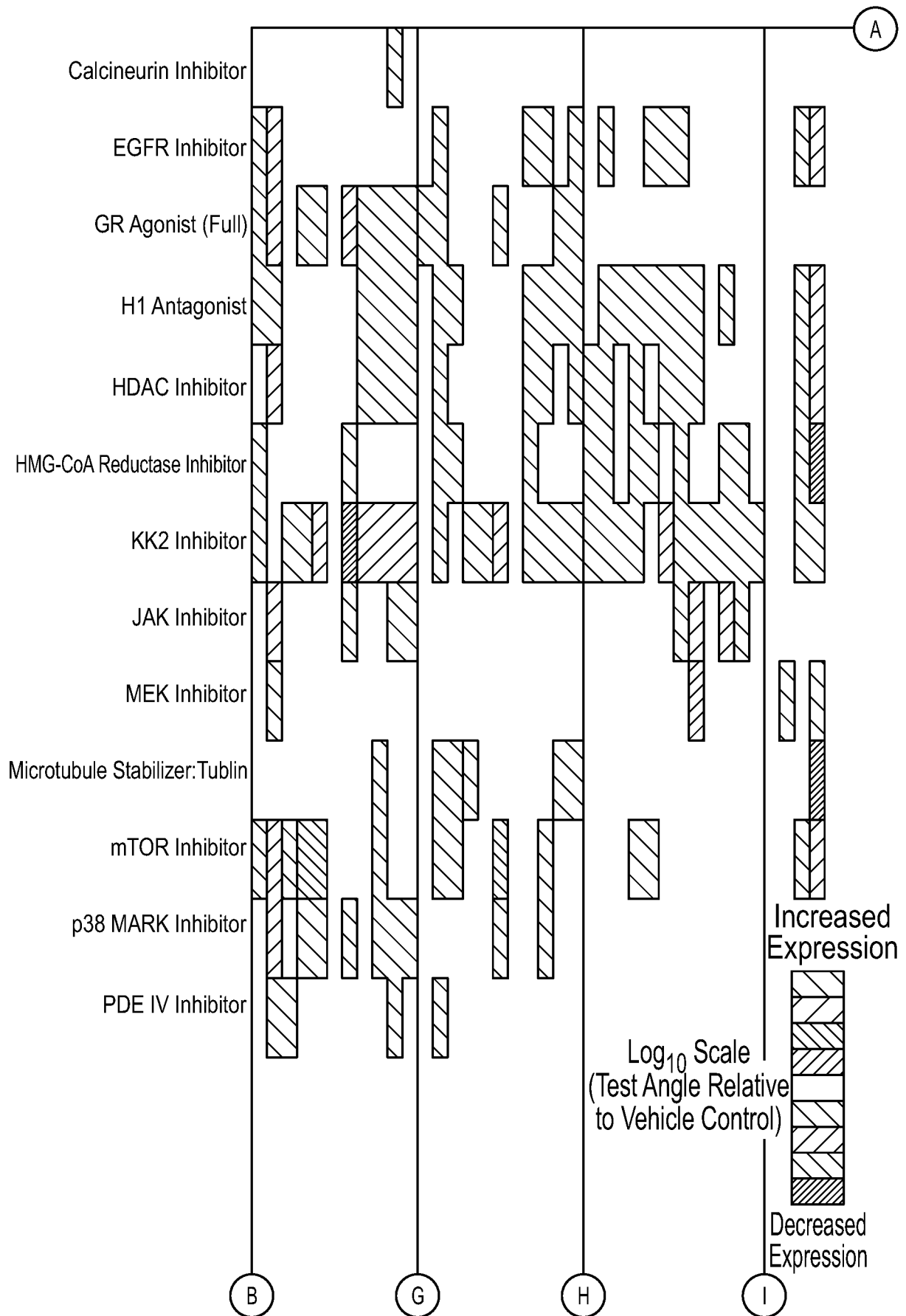


FIG. 67

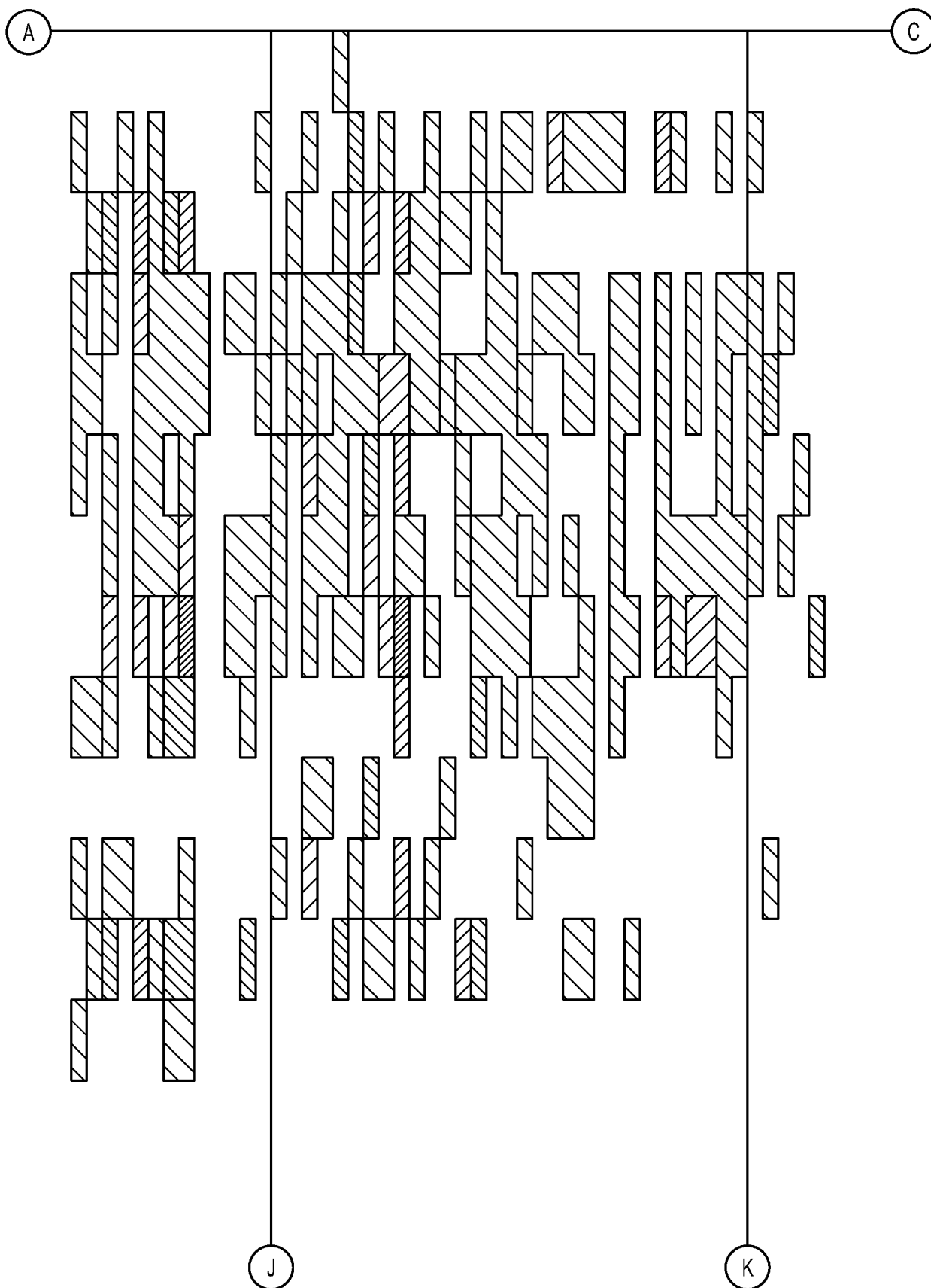


FIG. 67
(Continued)

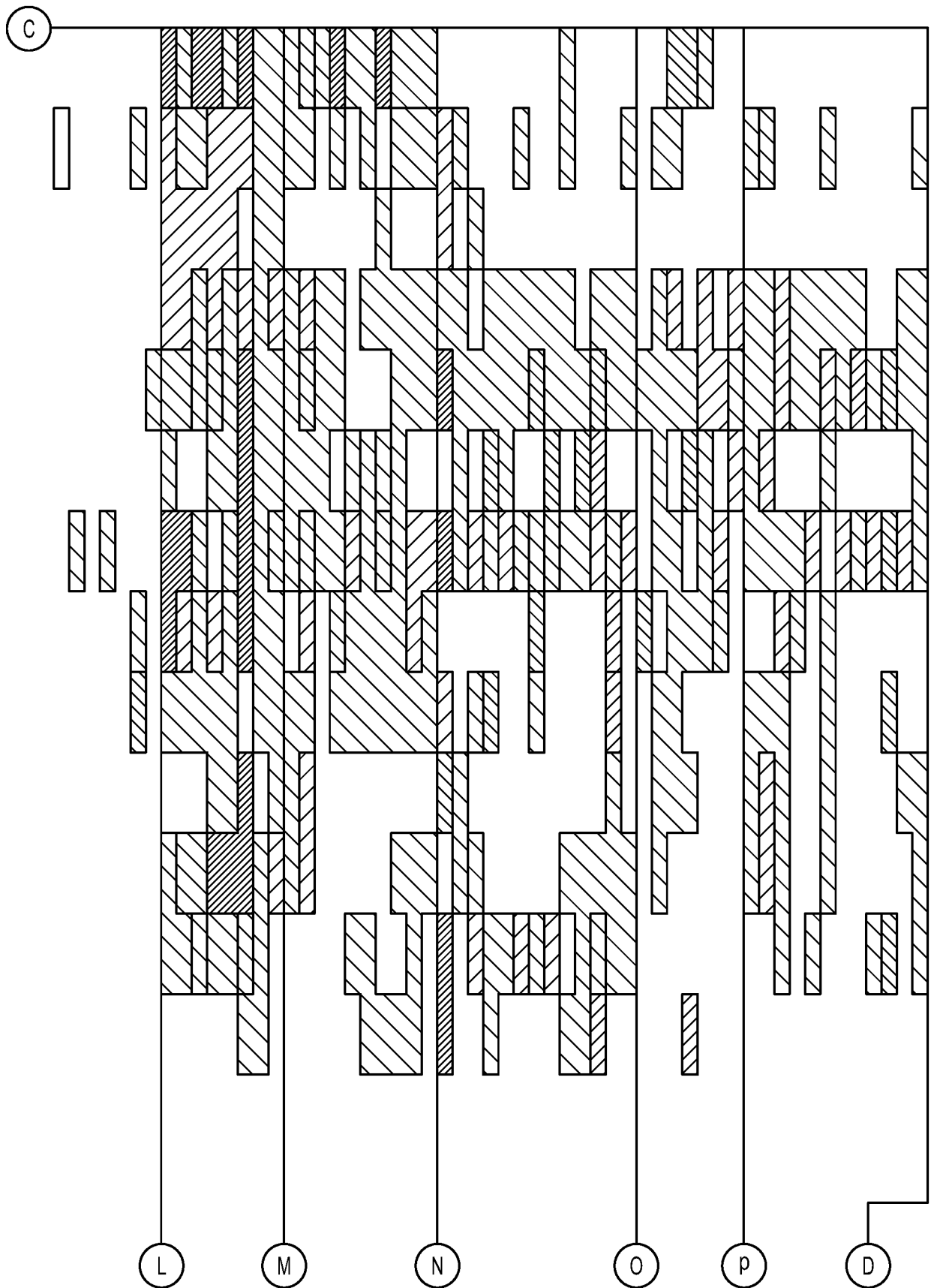


FIG. 67
(Continued)

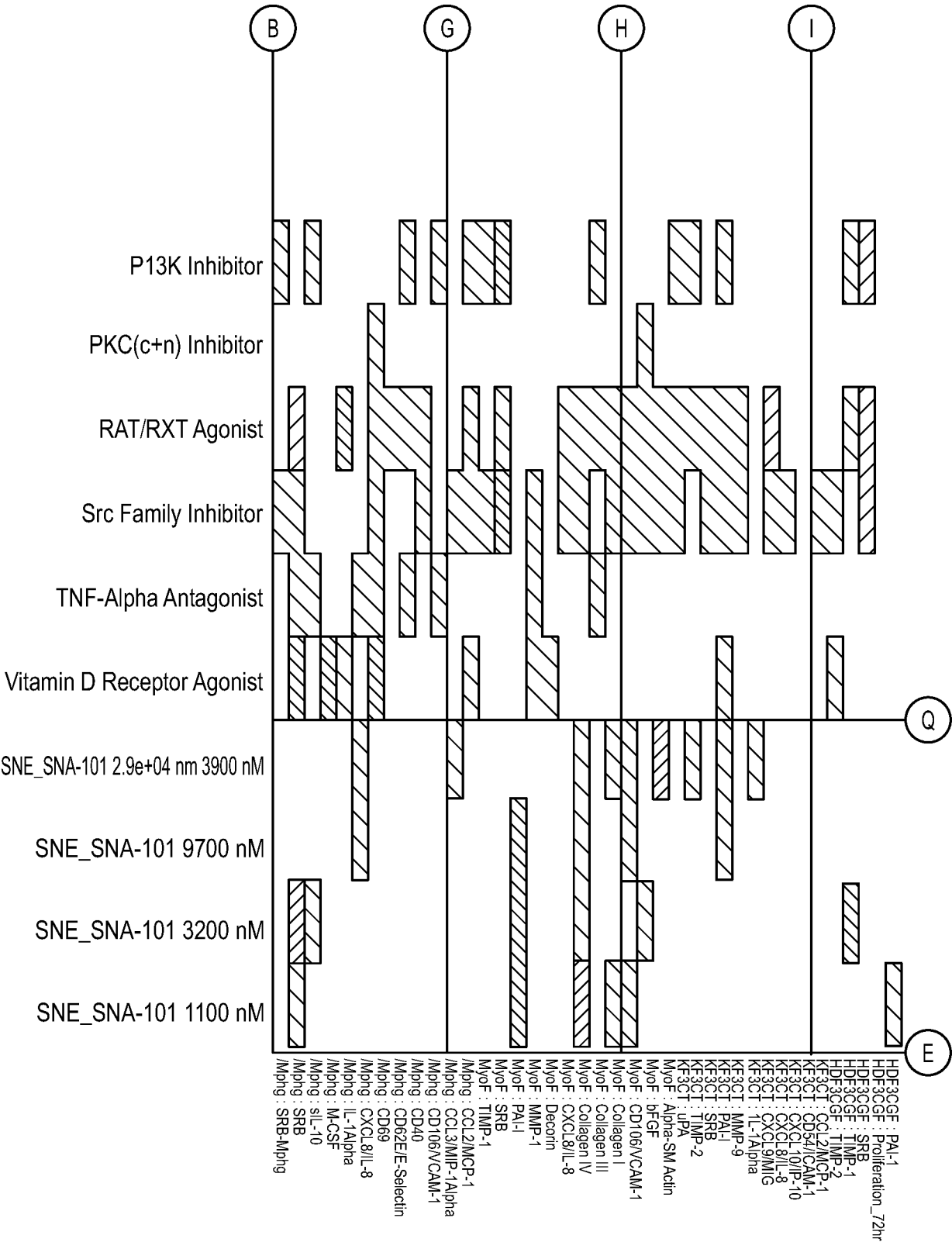


FIG. 67
(Continued)

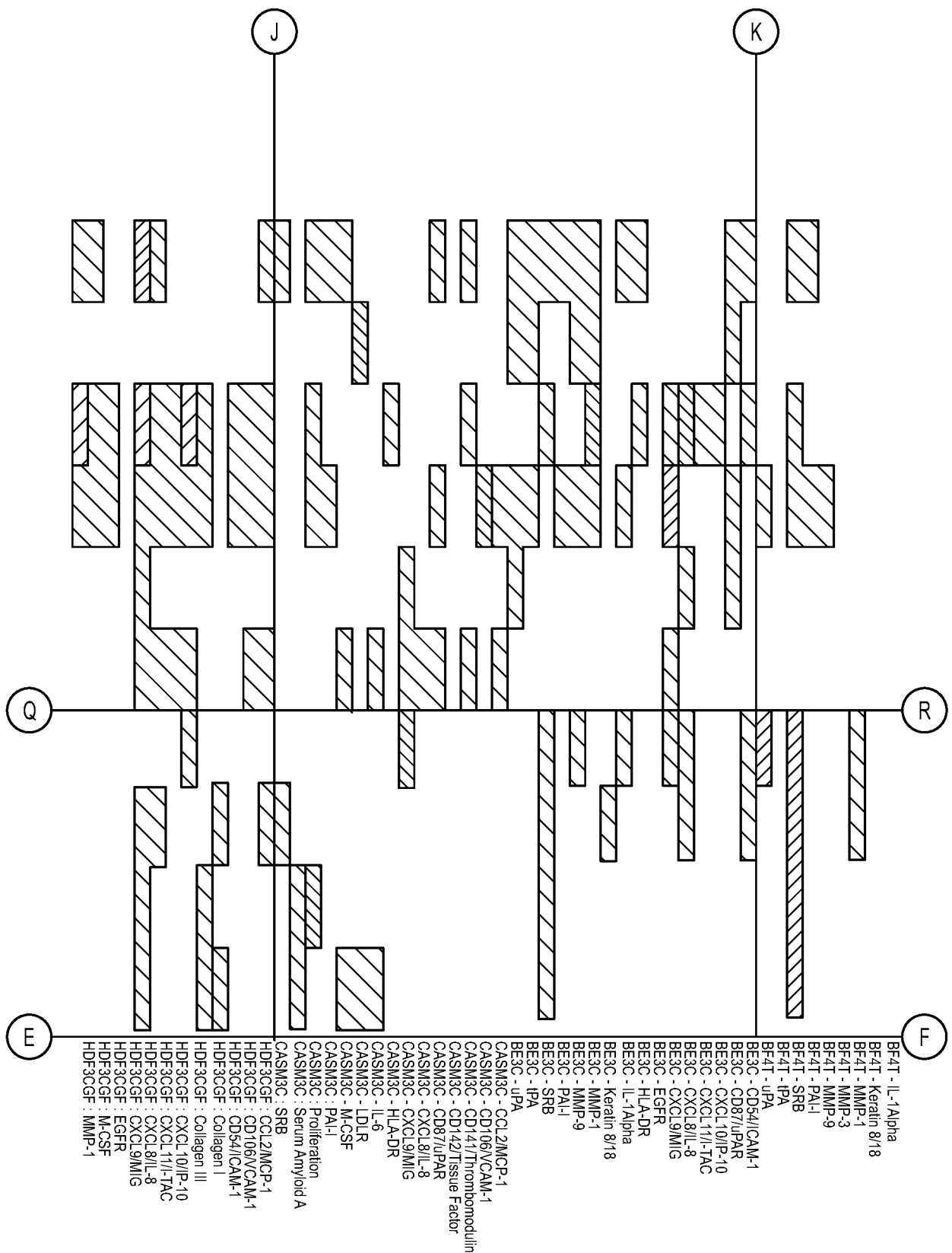


FIG. 67
(Continued)

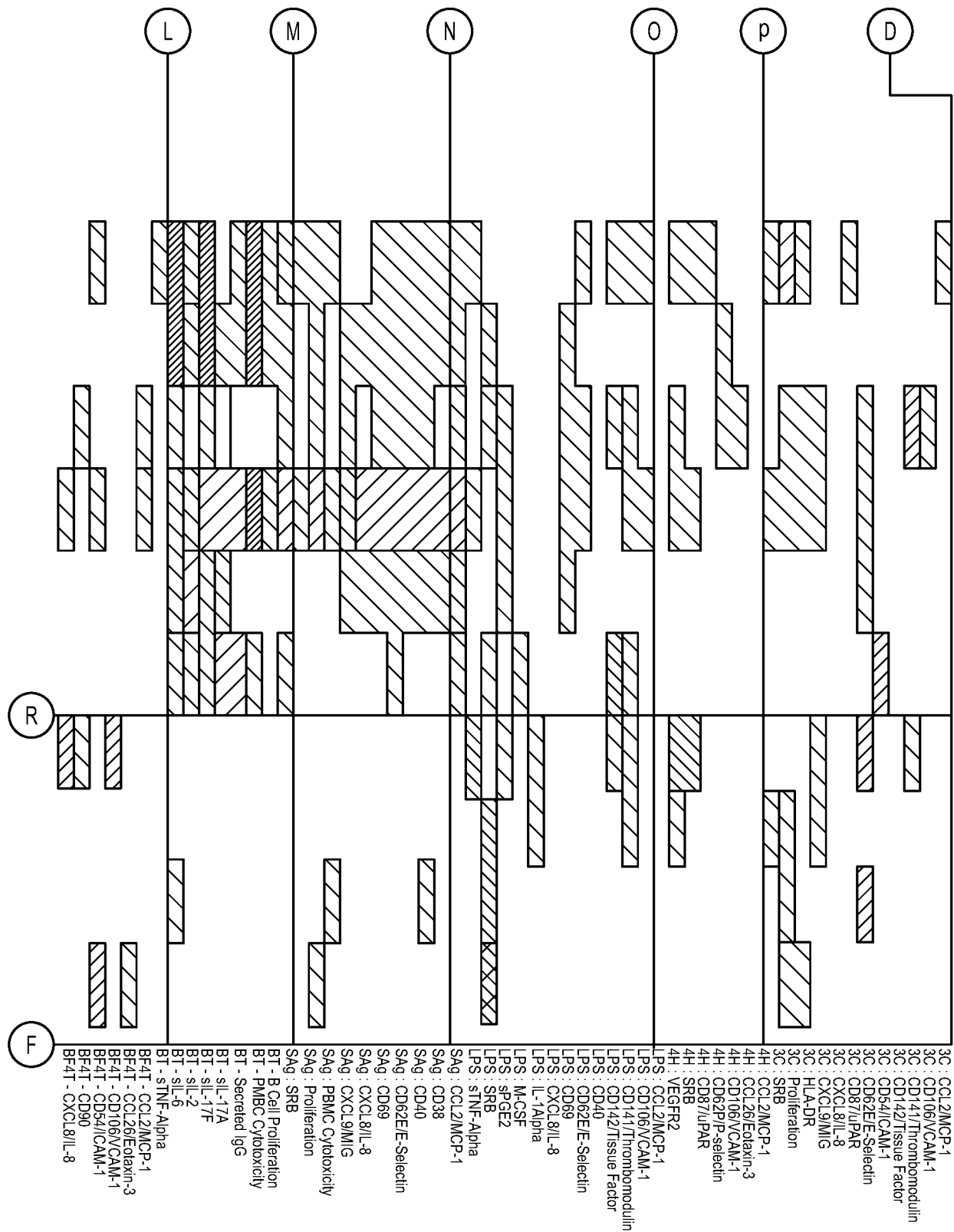


FIG. 67
(Continued)

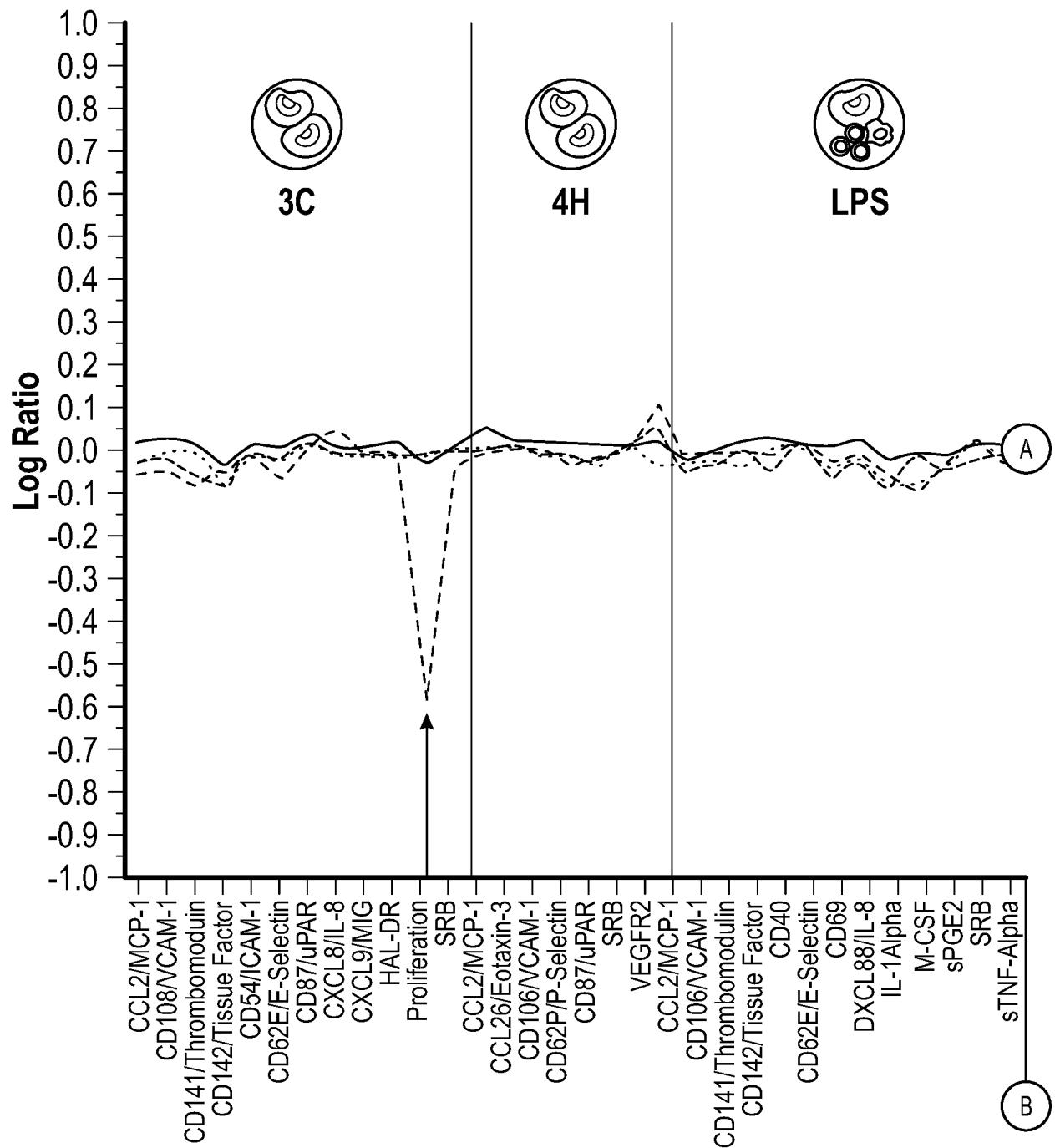


FIG. 68

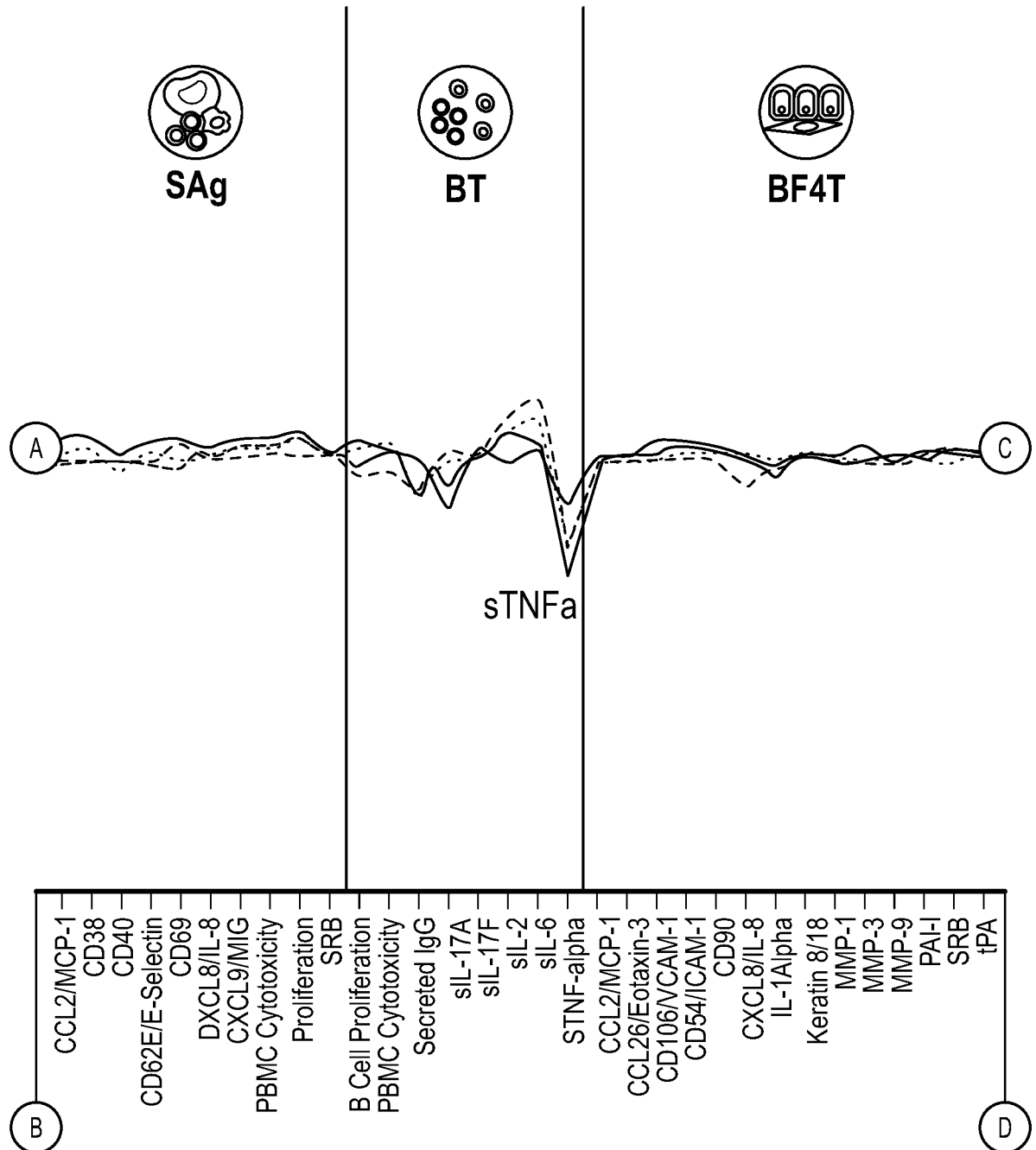


FIG. 68
(Continued)

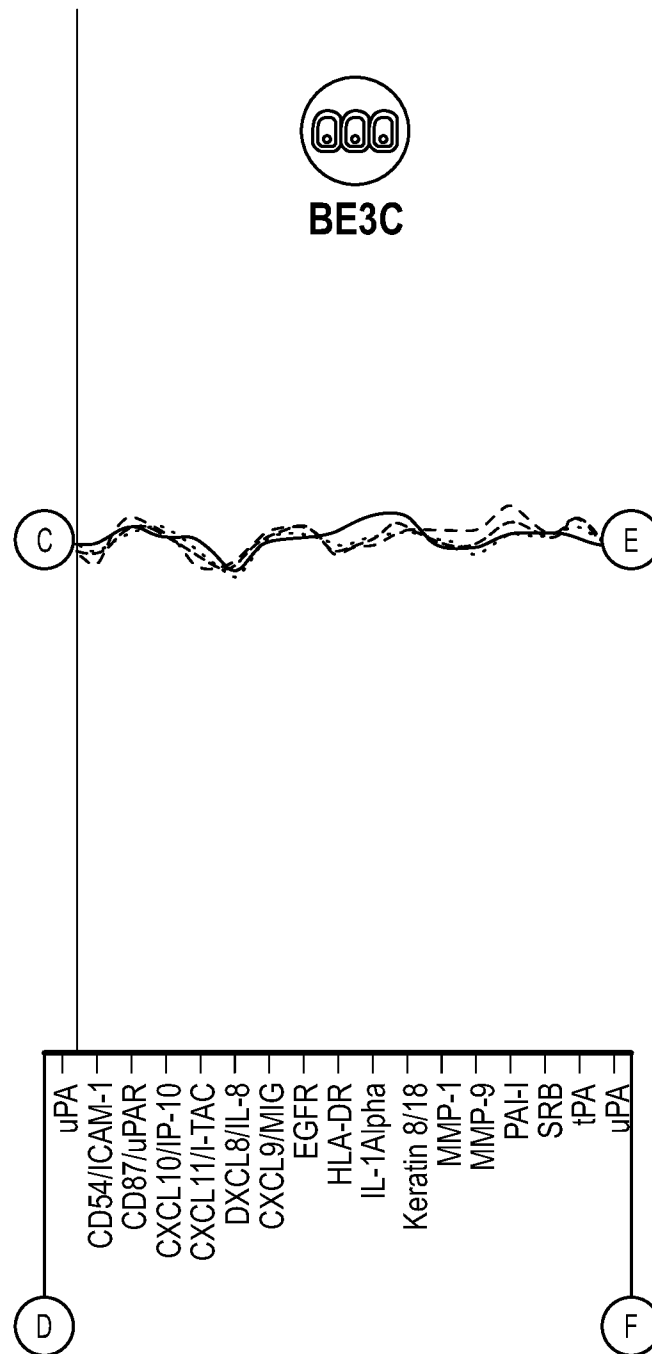


FIG. 68
(Continued)

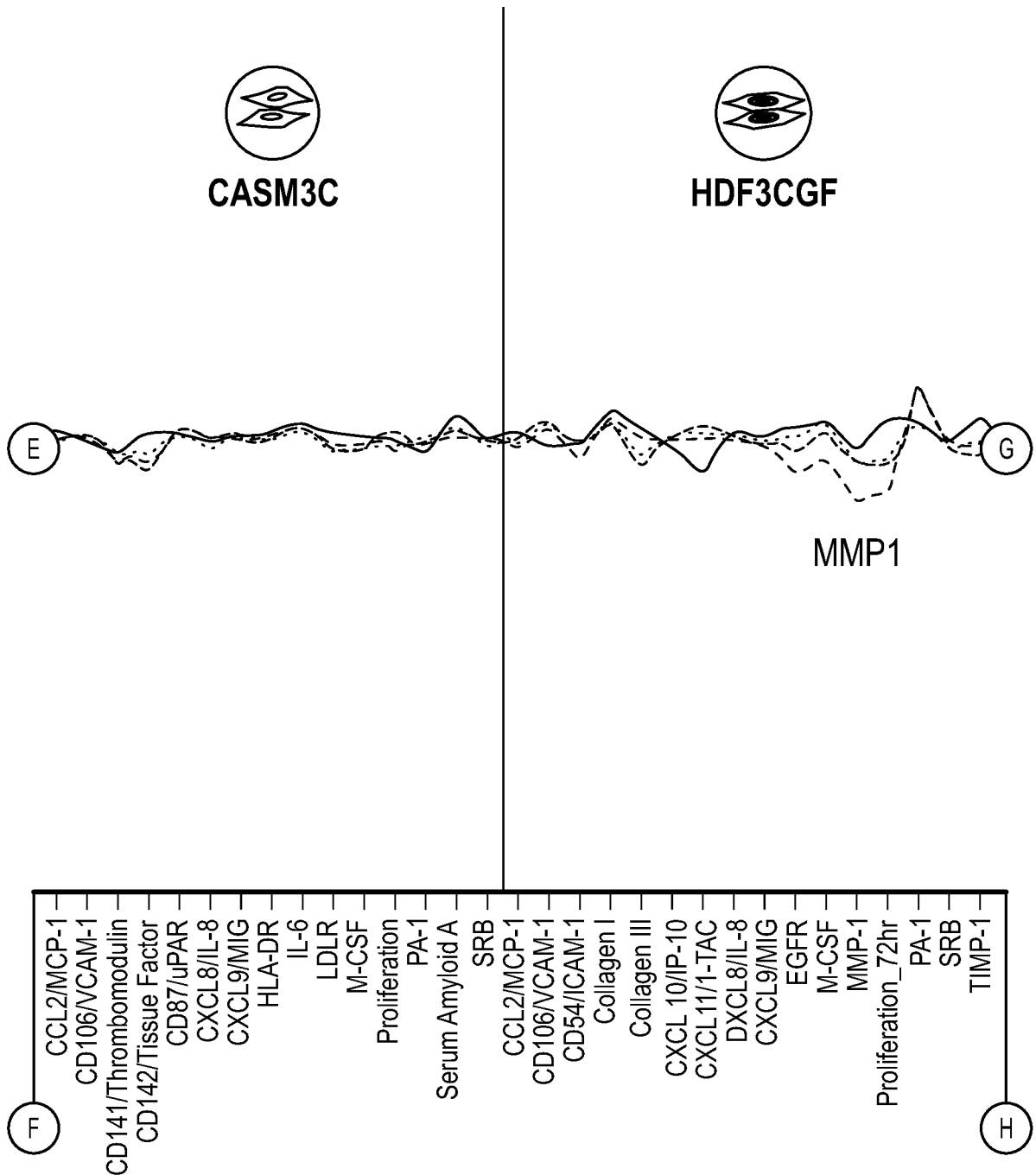


FIG. 68
(Continued)

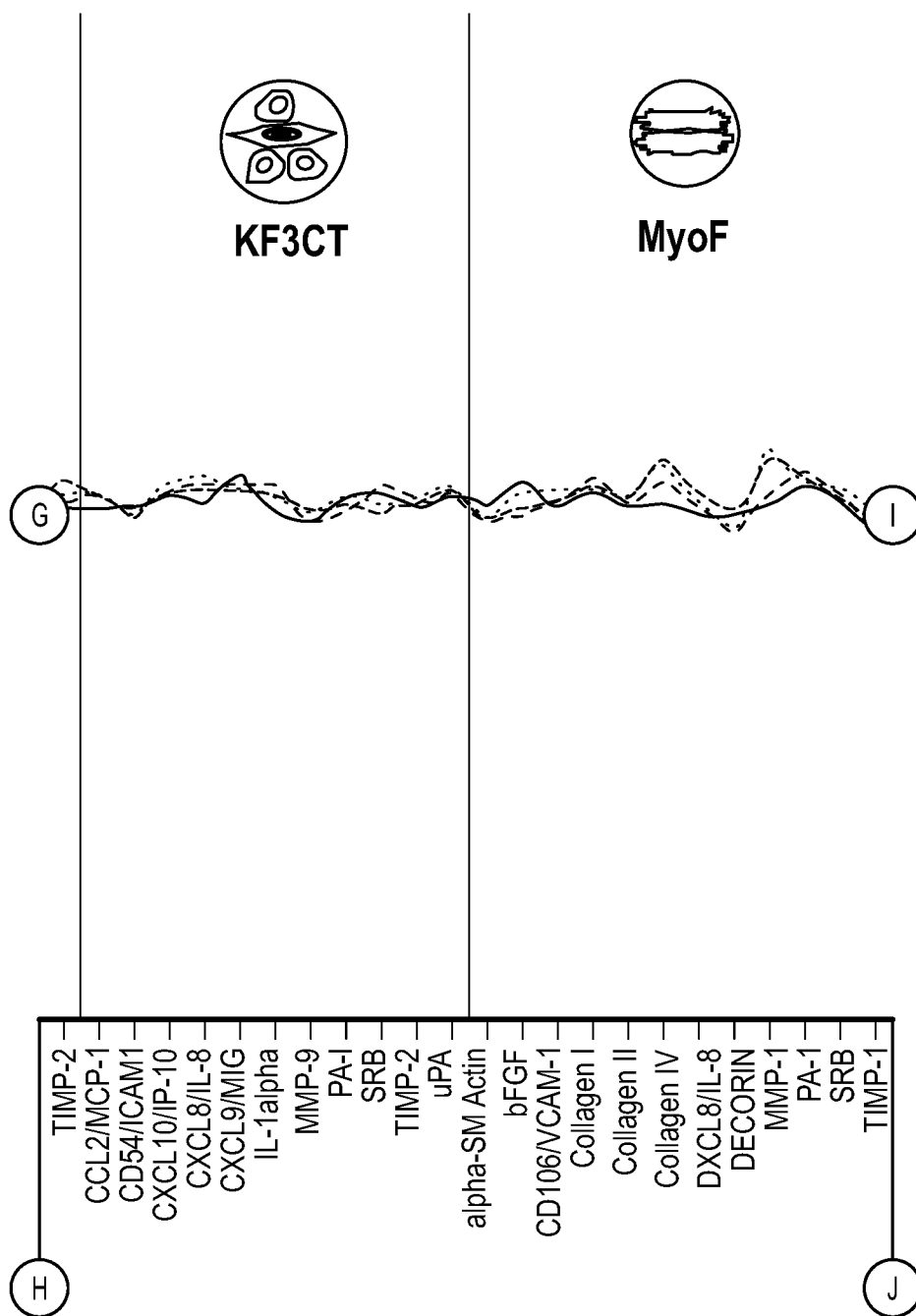


FIG. 68
(Continued)

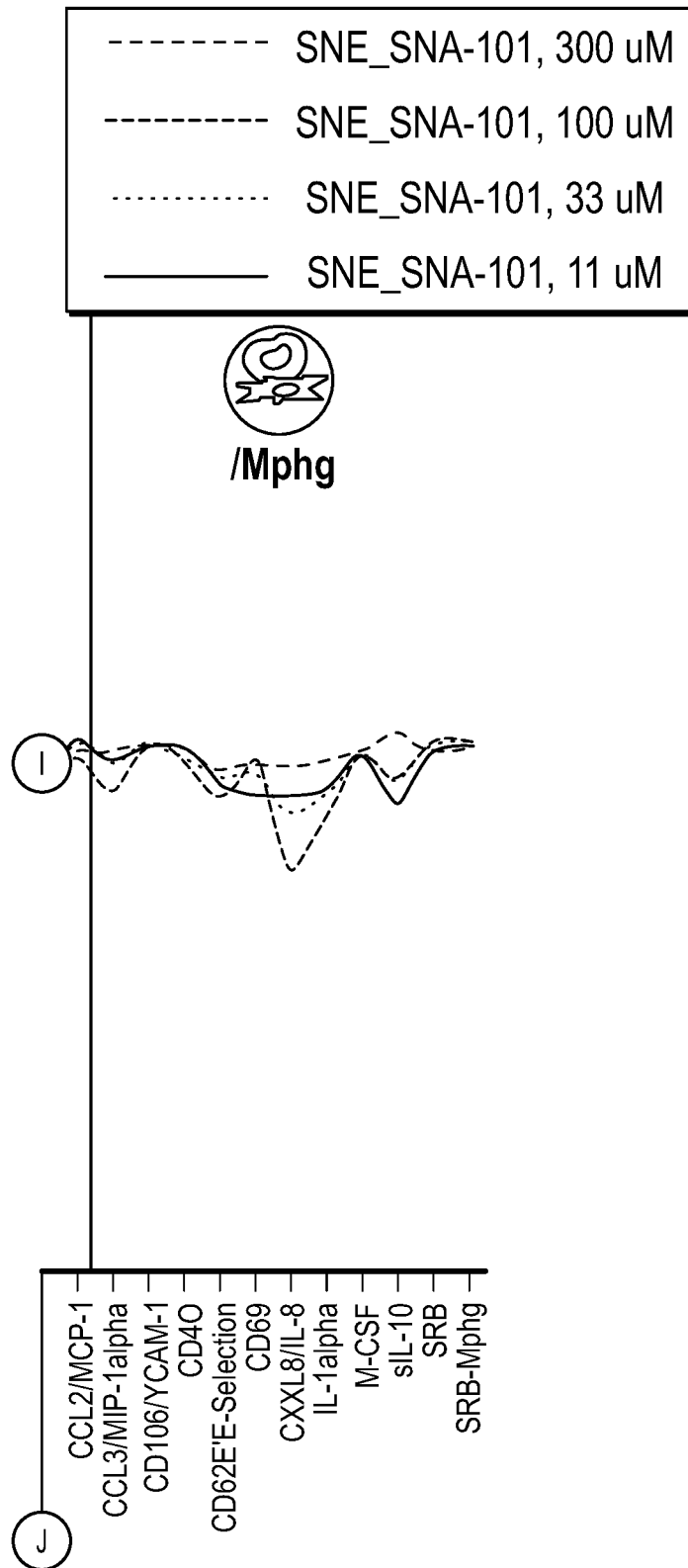


FIG. 68
(Continued)

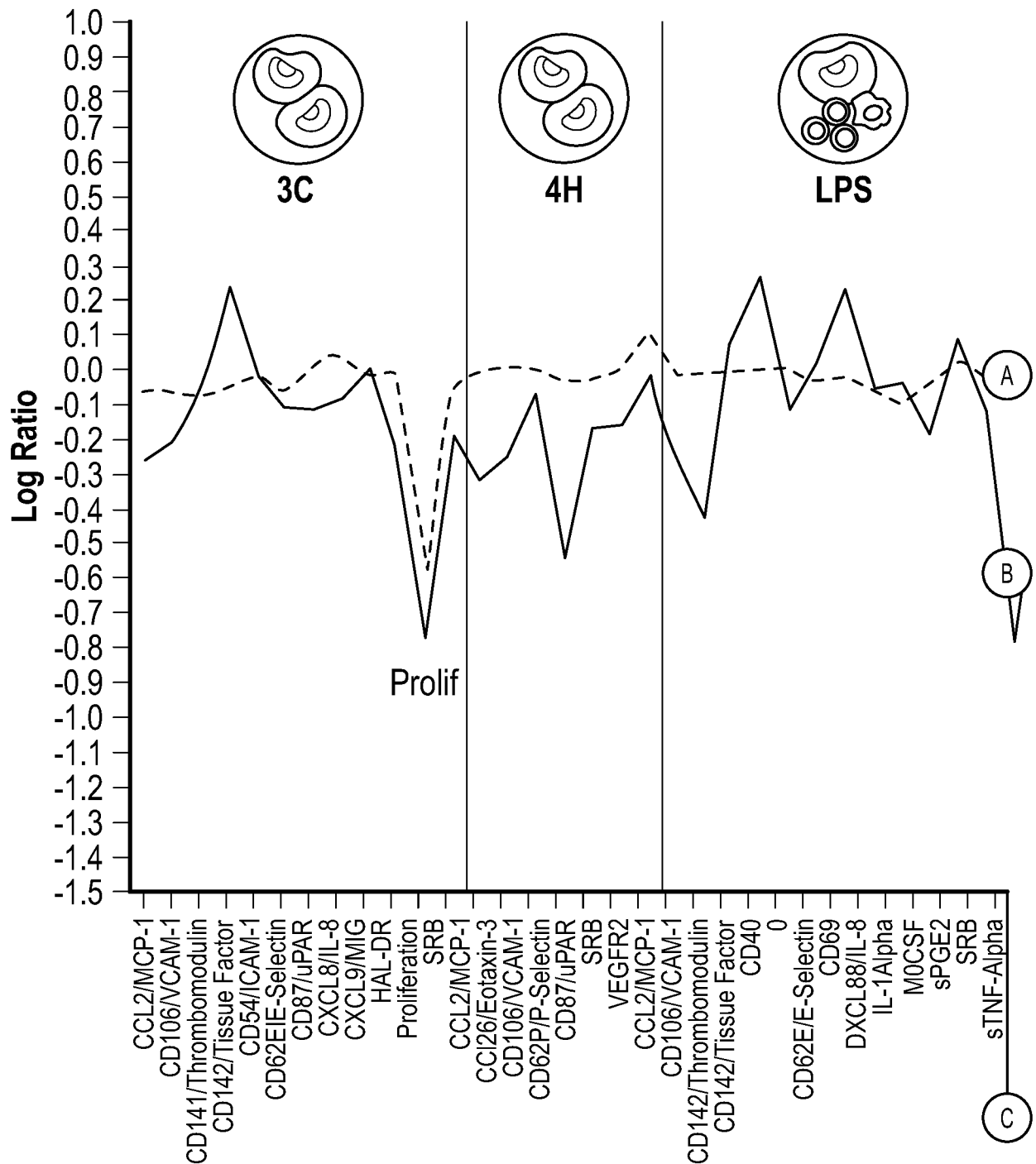


FIG. 69

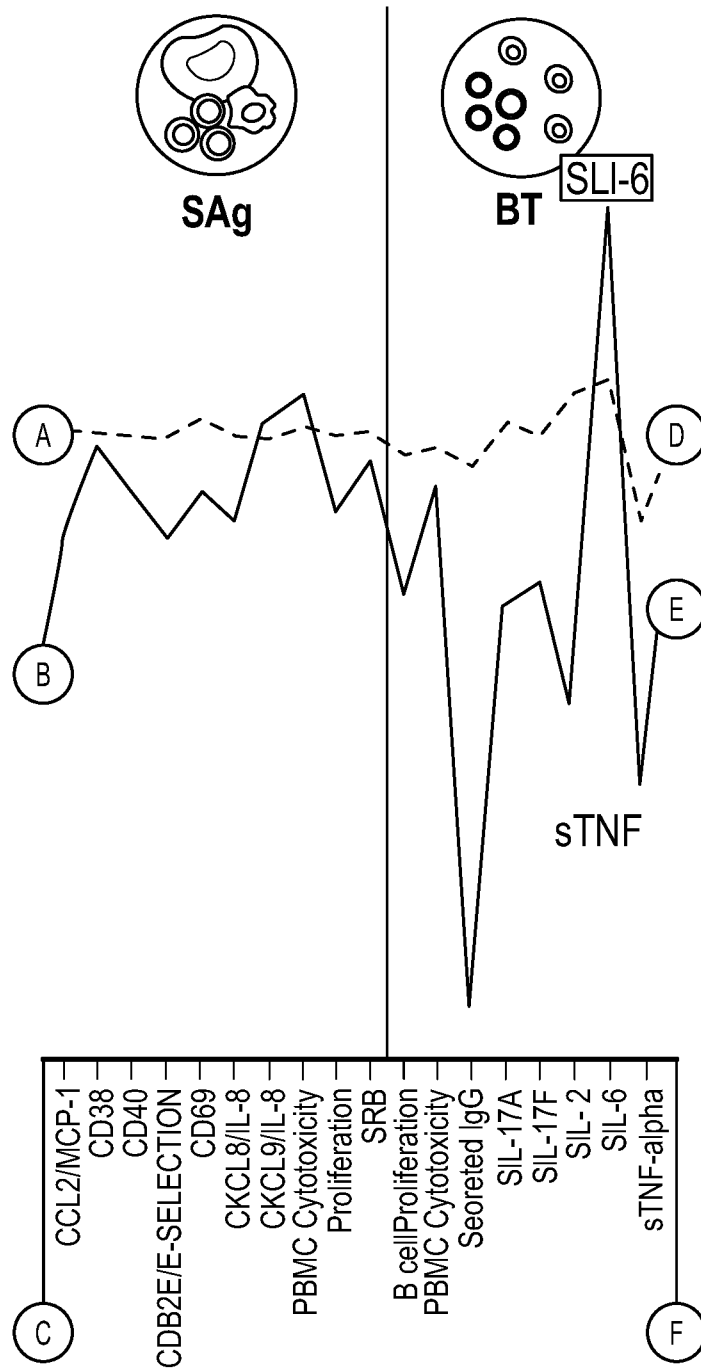


FIG. 69
(Continued)

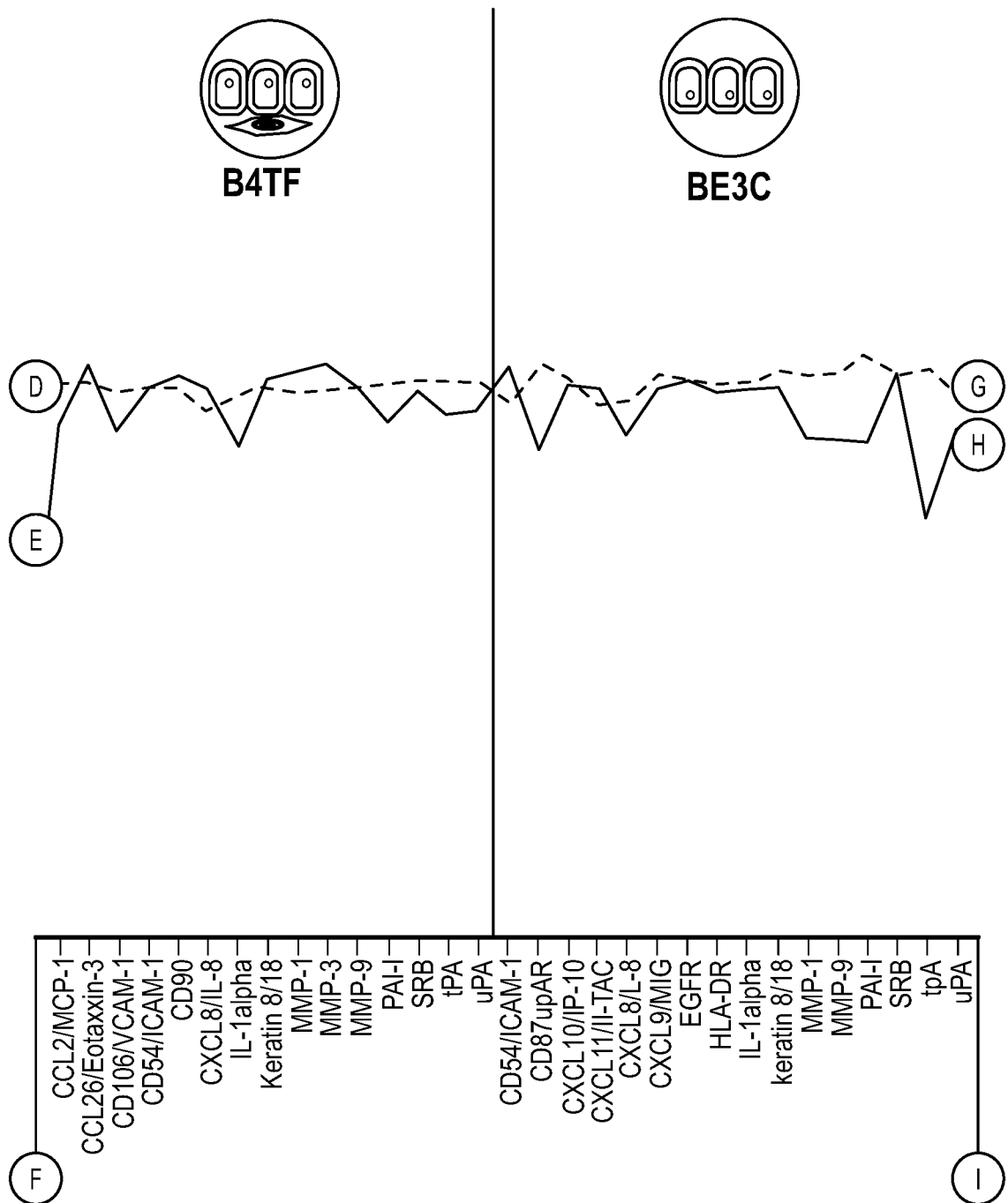


FIG. 69
(Continued)

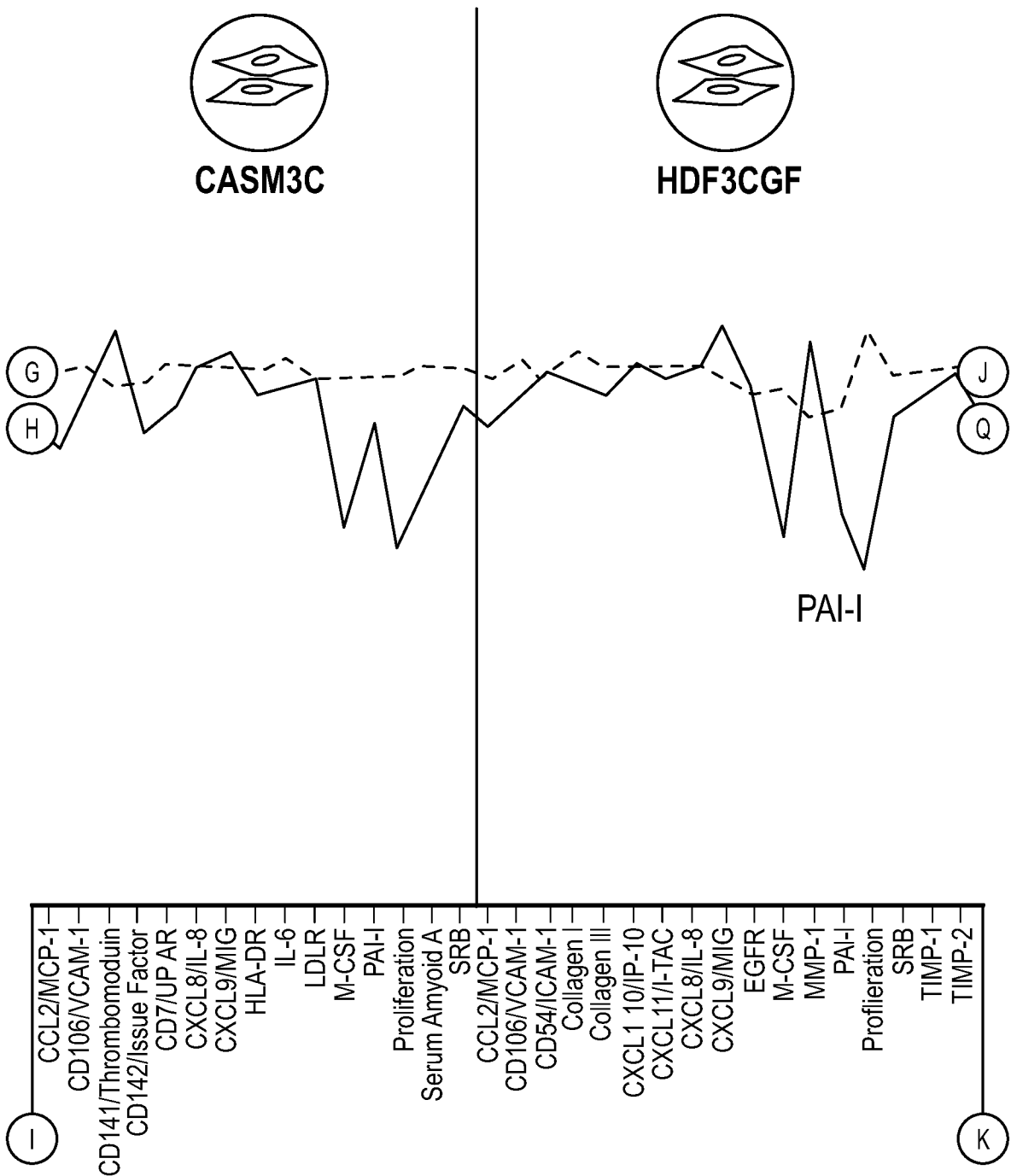


FIG. 69
(Continued)

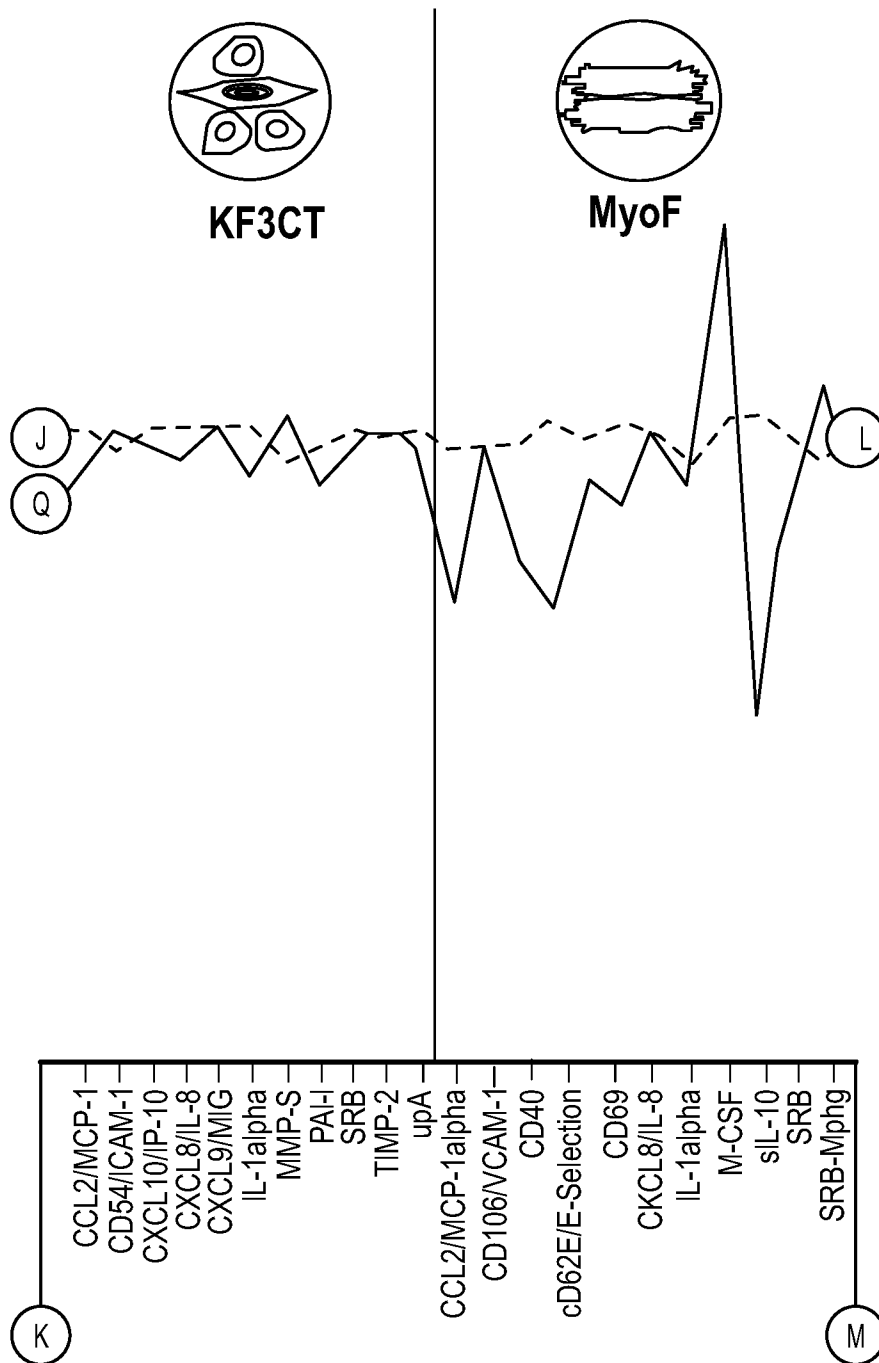


FIG. 69
(Continued)

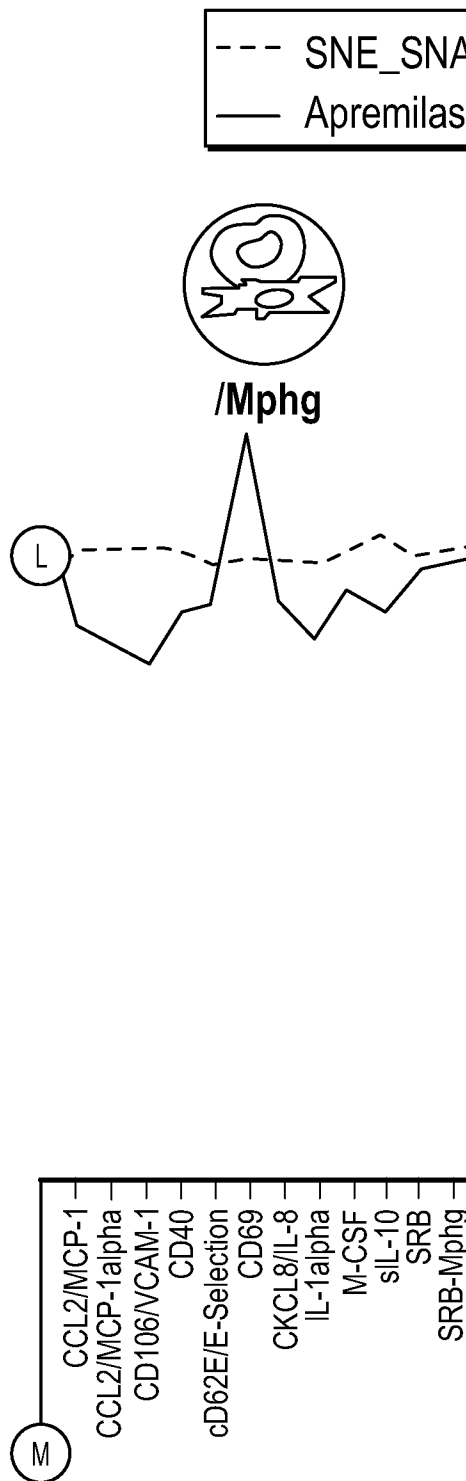


FIG. 69

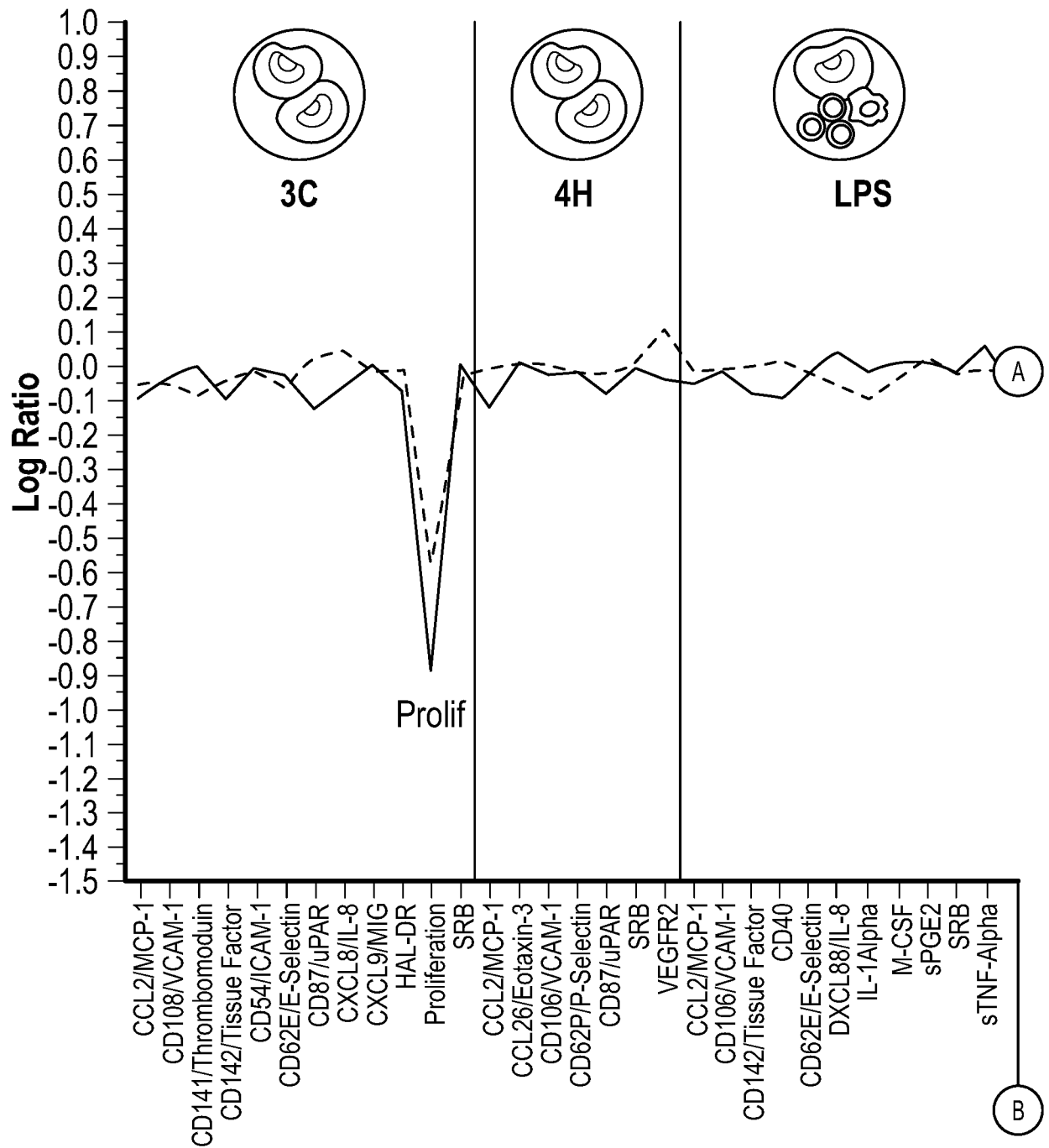


FIG. 70

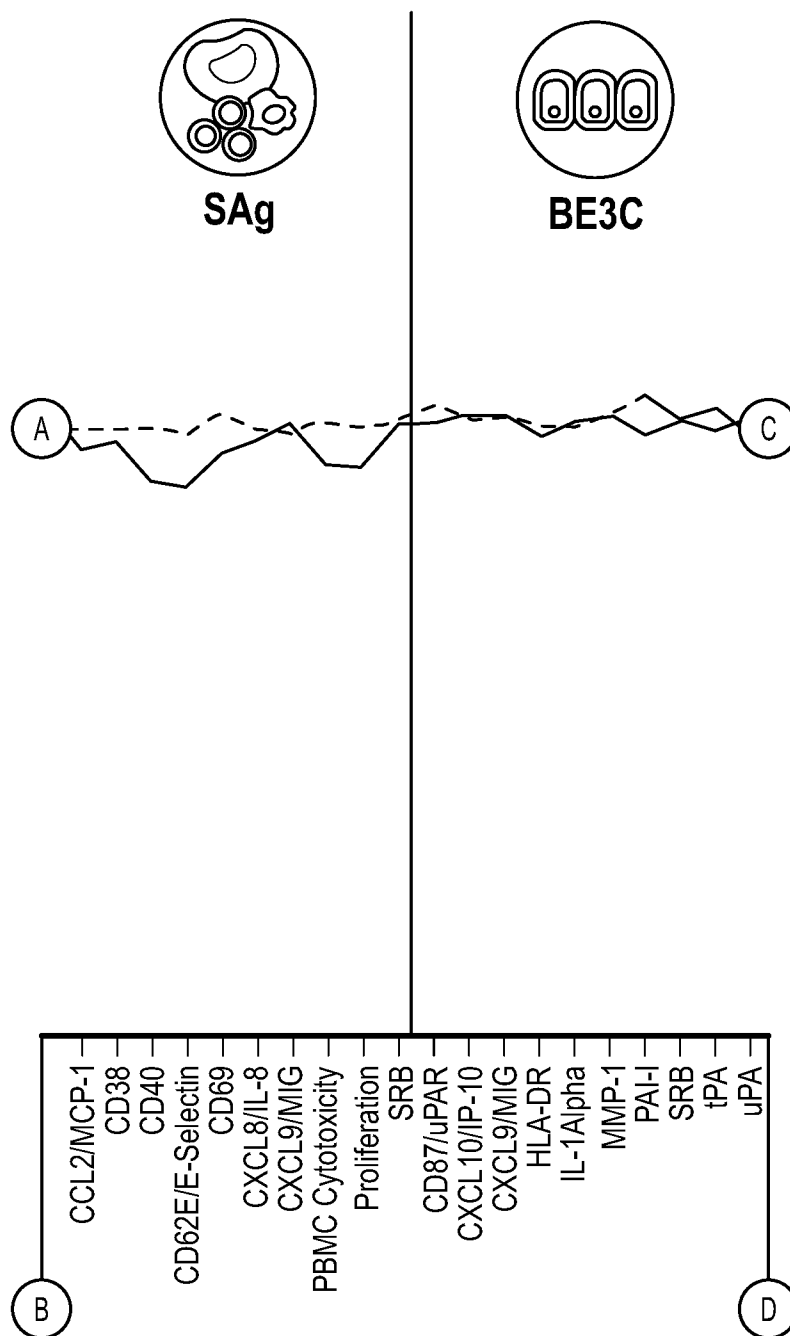


FIG. 70
(Continued)

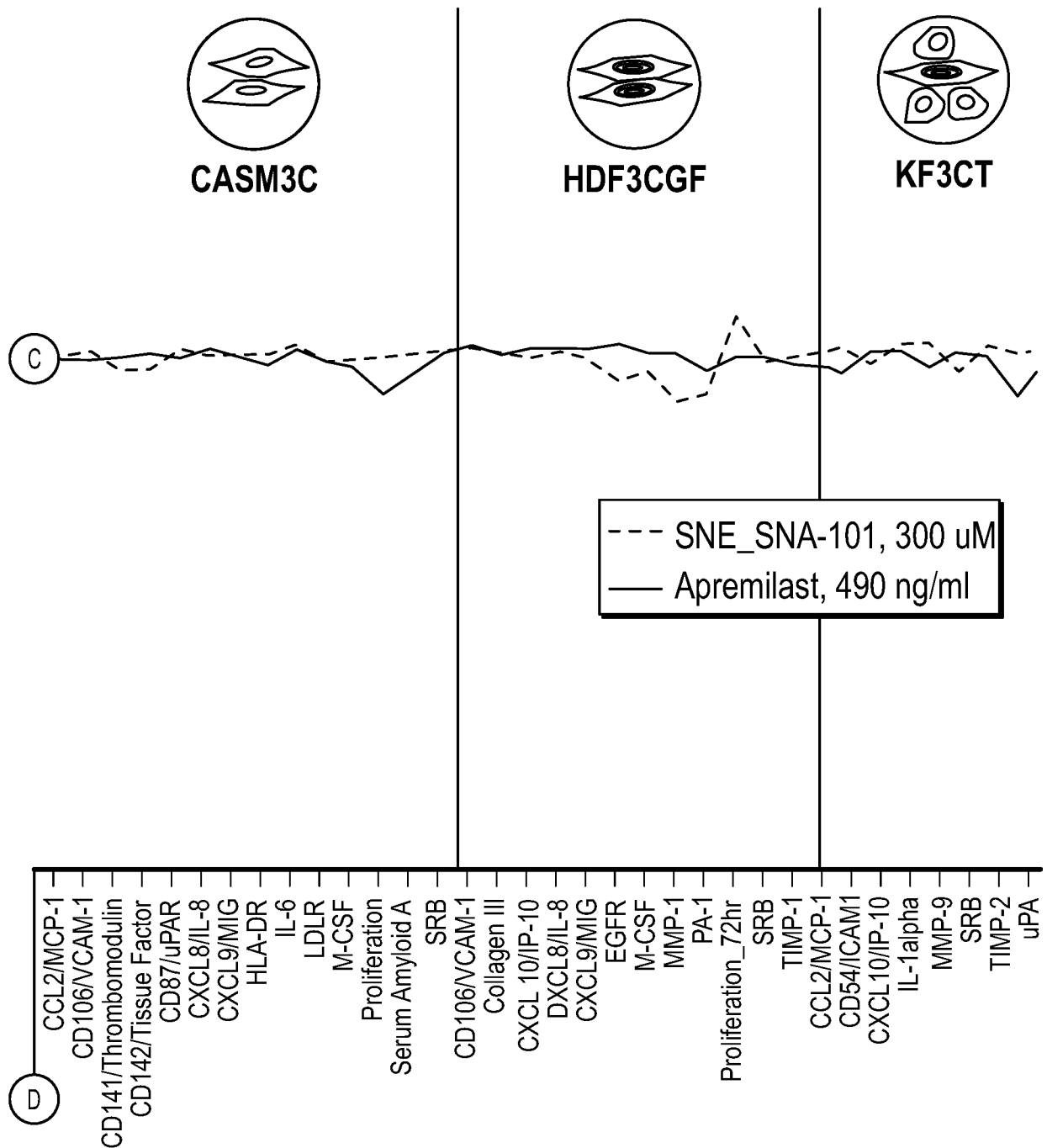


FIG. 70
(Continued)

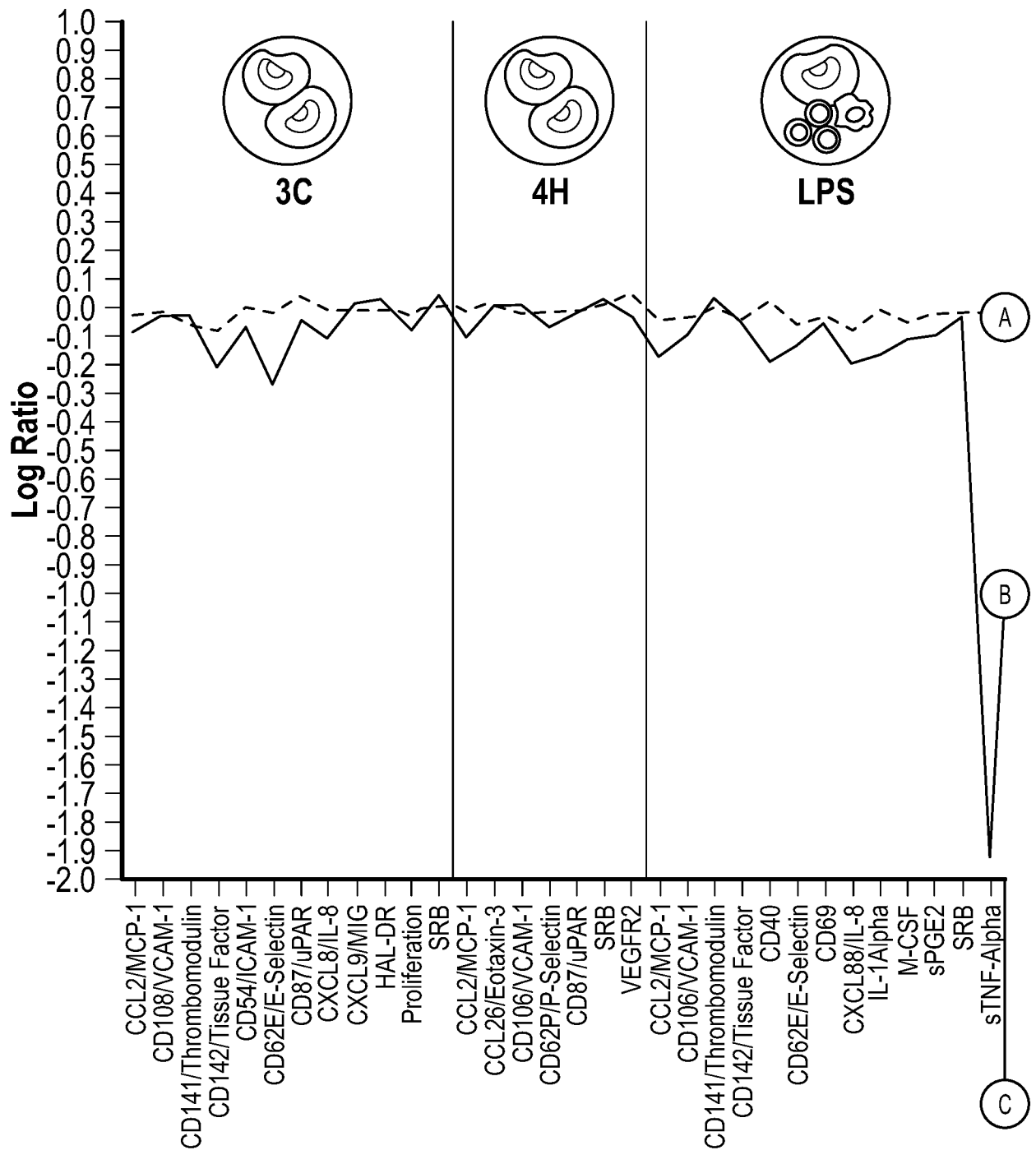


FIG. 71

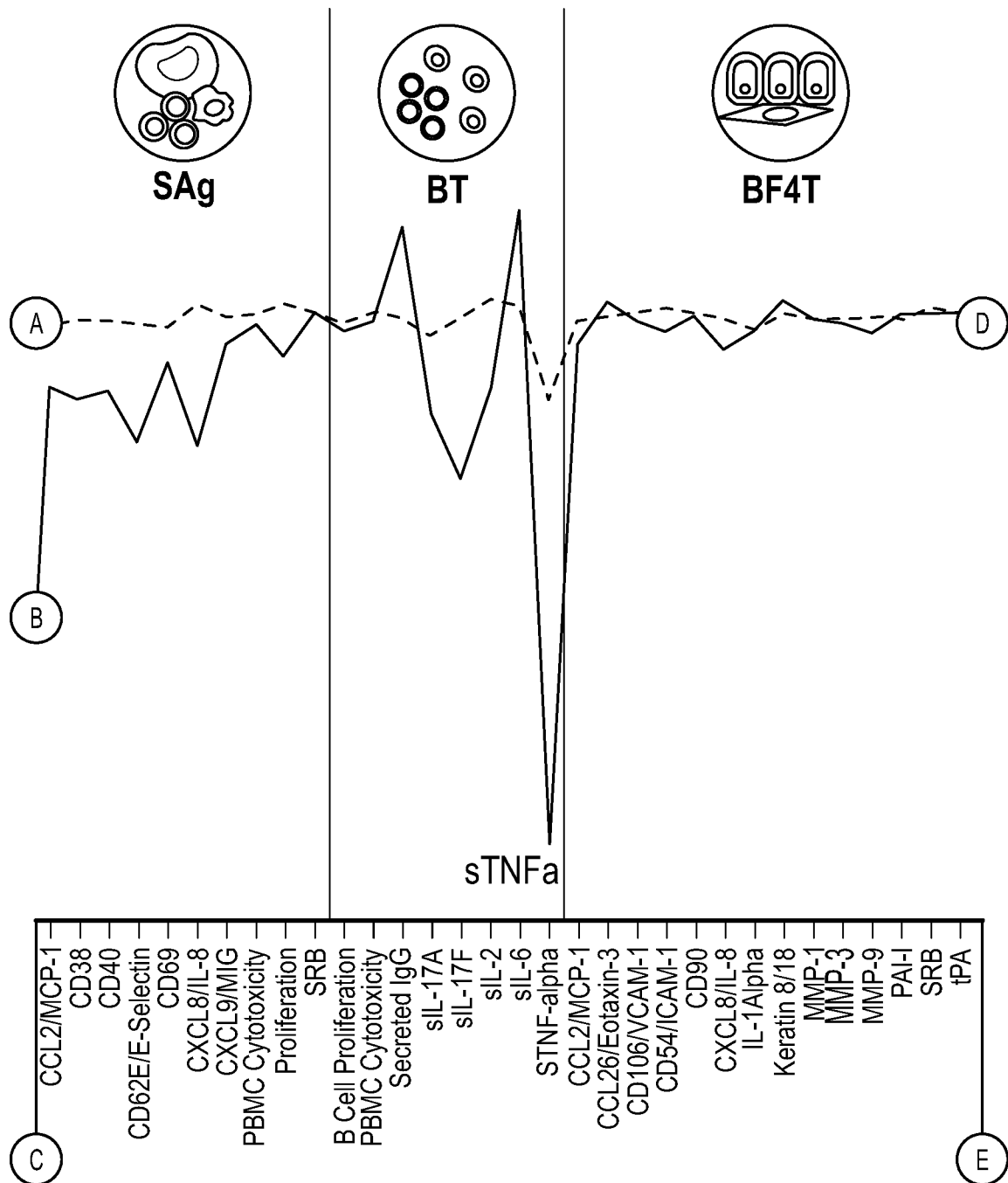


FIG. 71
(Continued)

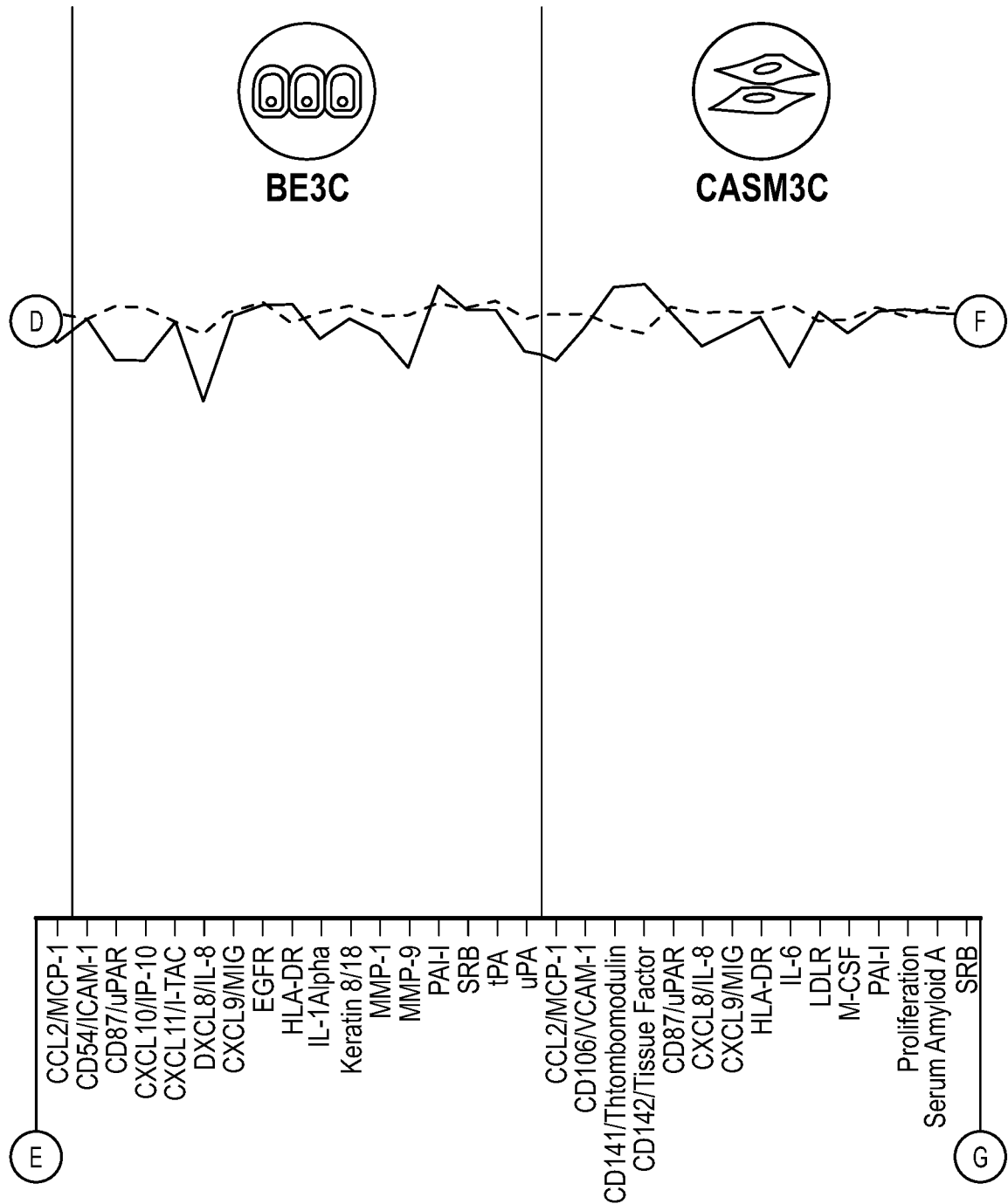


FIG. 71
(Continued)

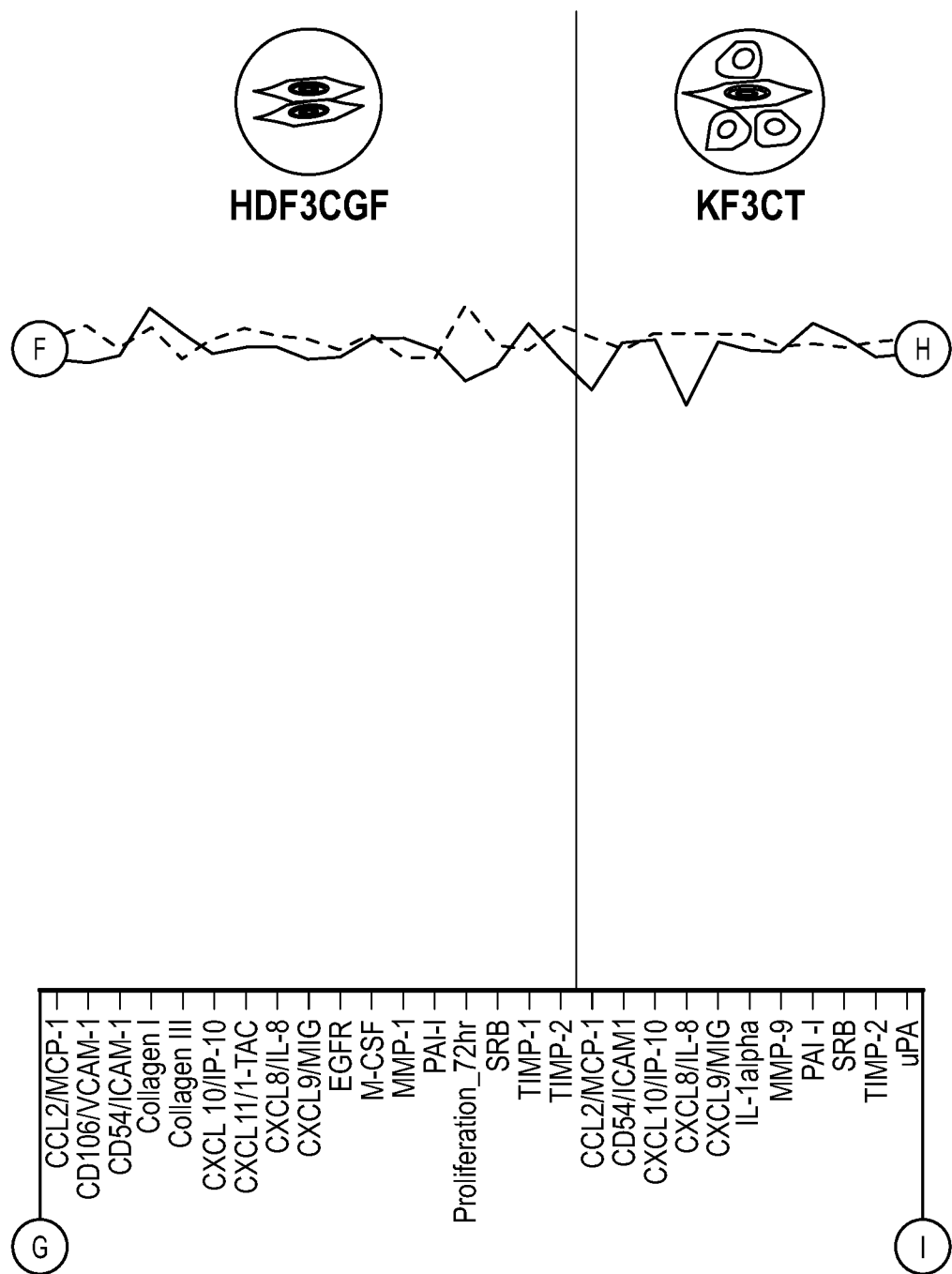


FIG. 71
(Continued)

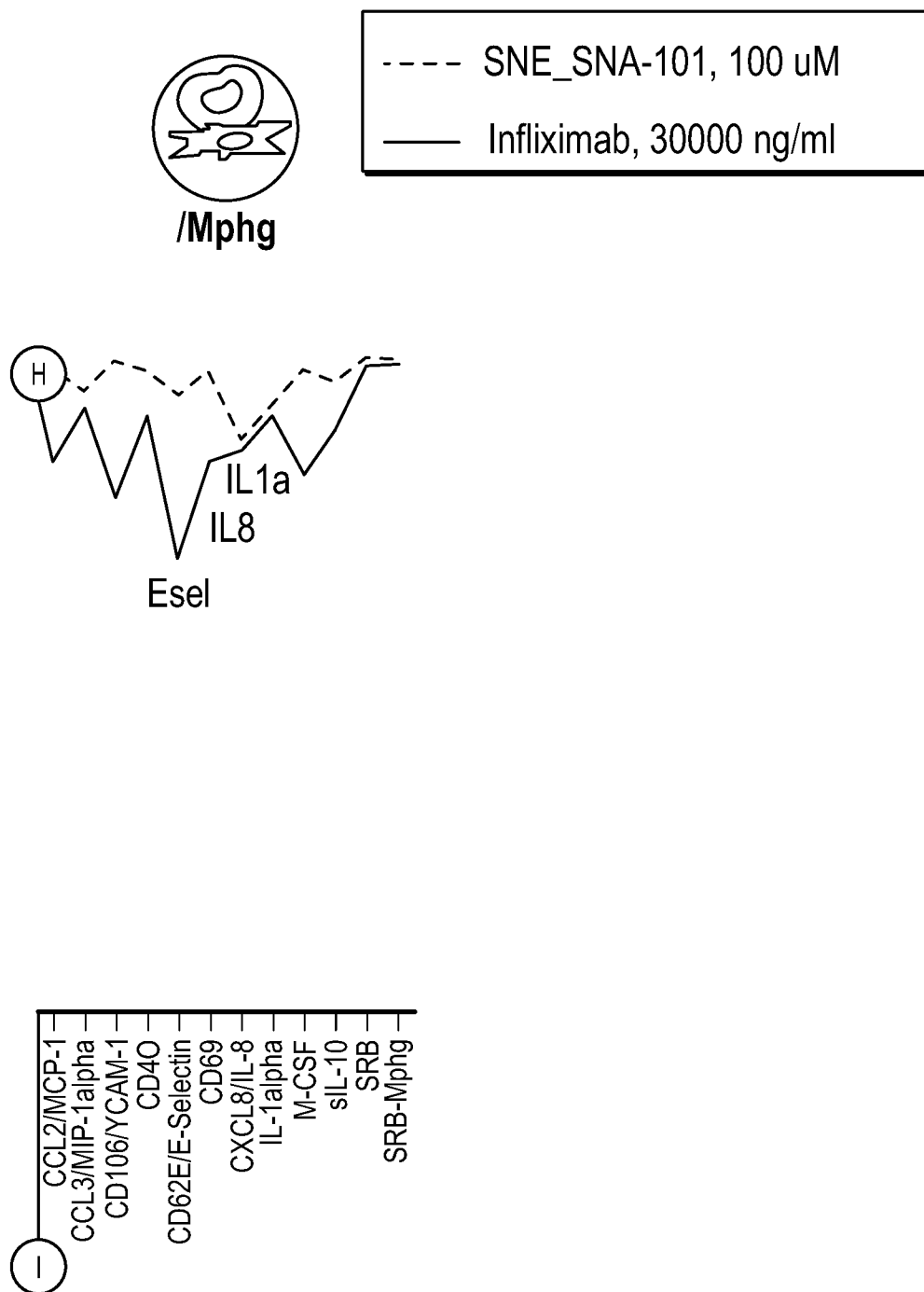


FIG. 71
(Continued)

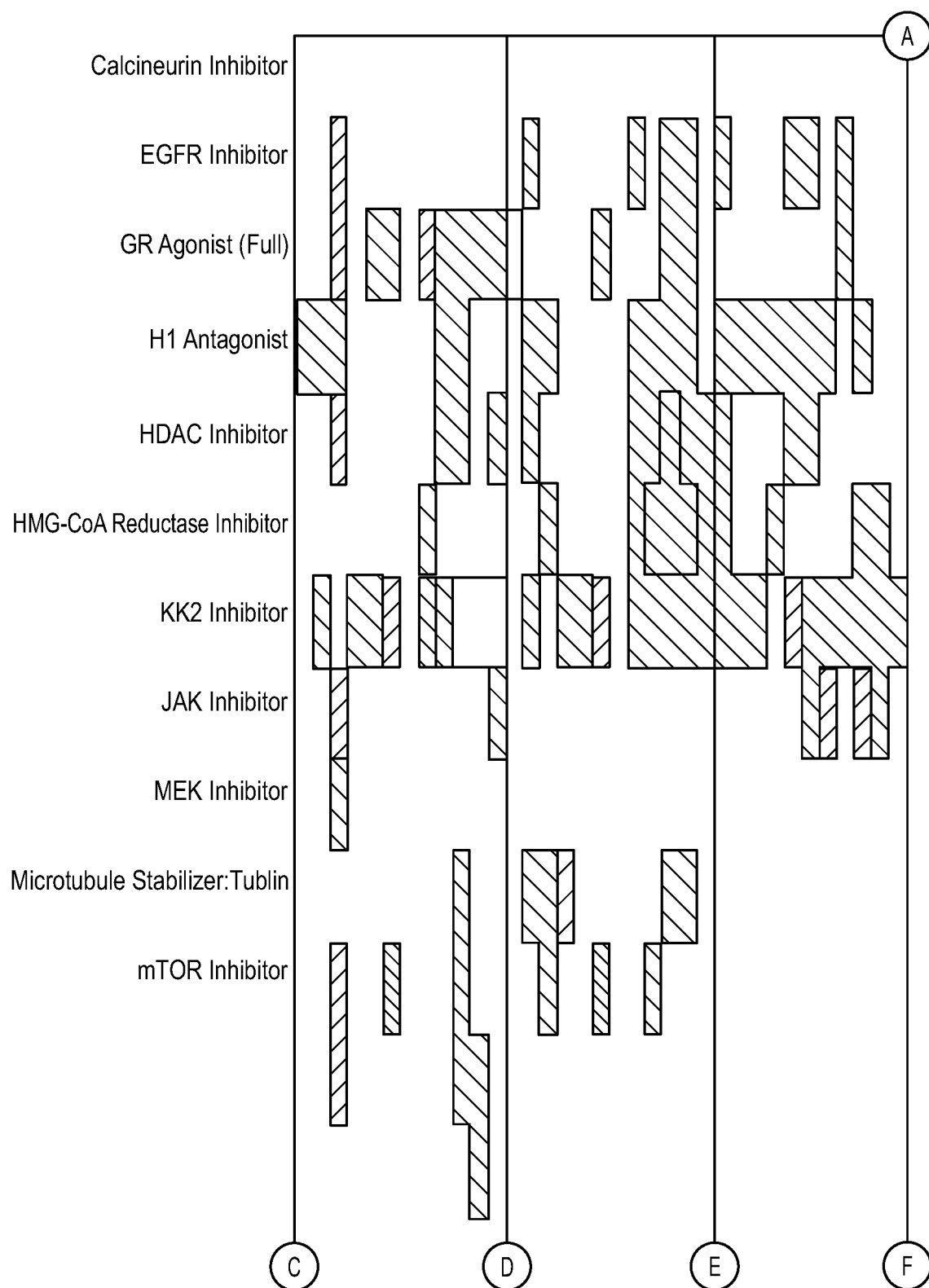


FIG. 72

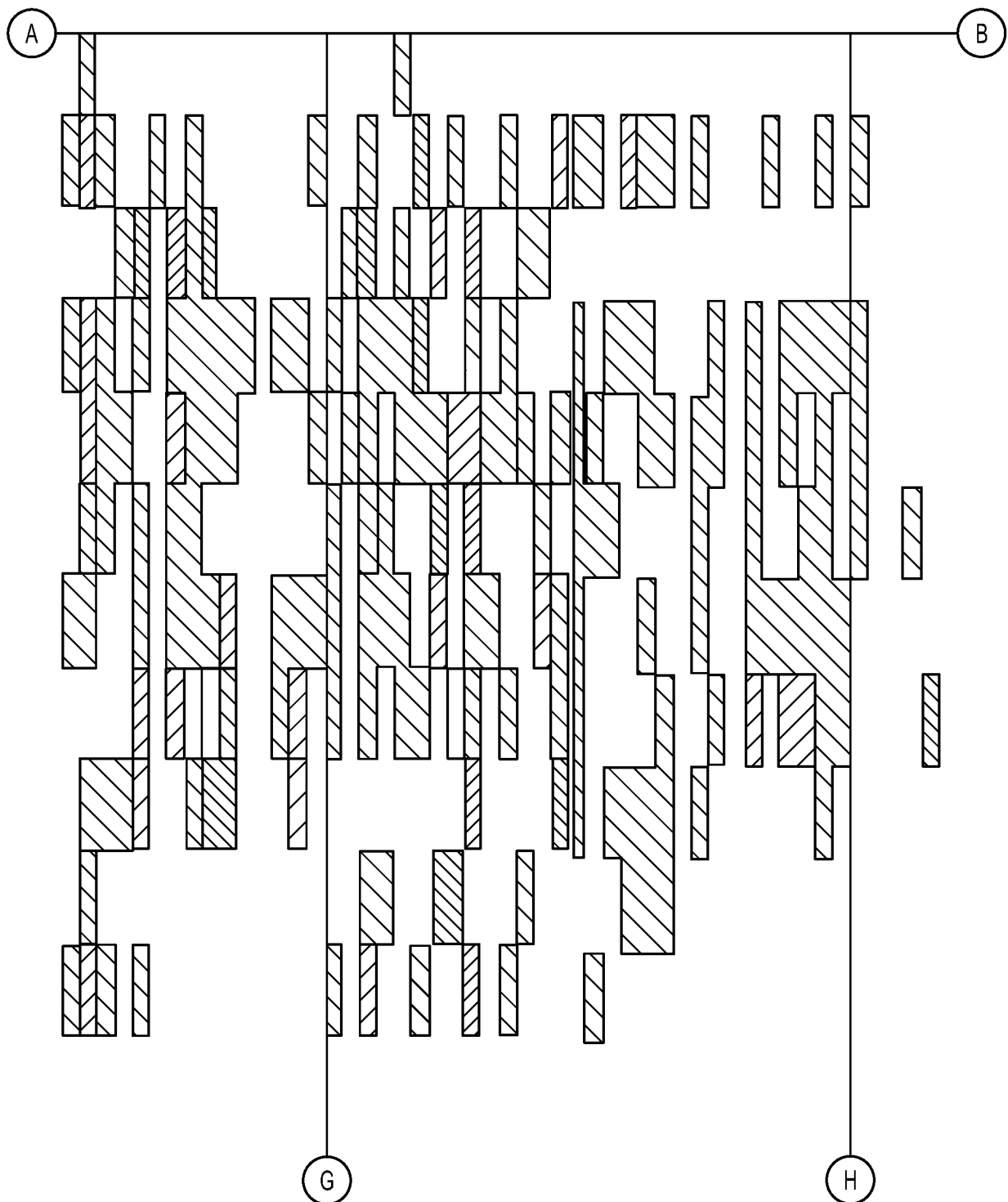


FIG. 72
(Continued)

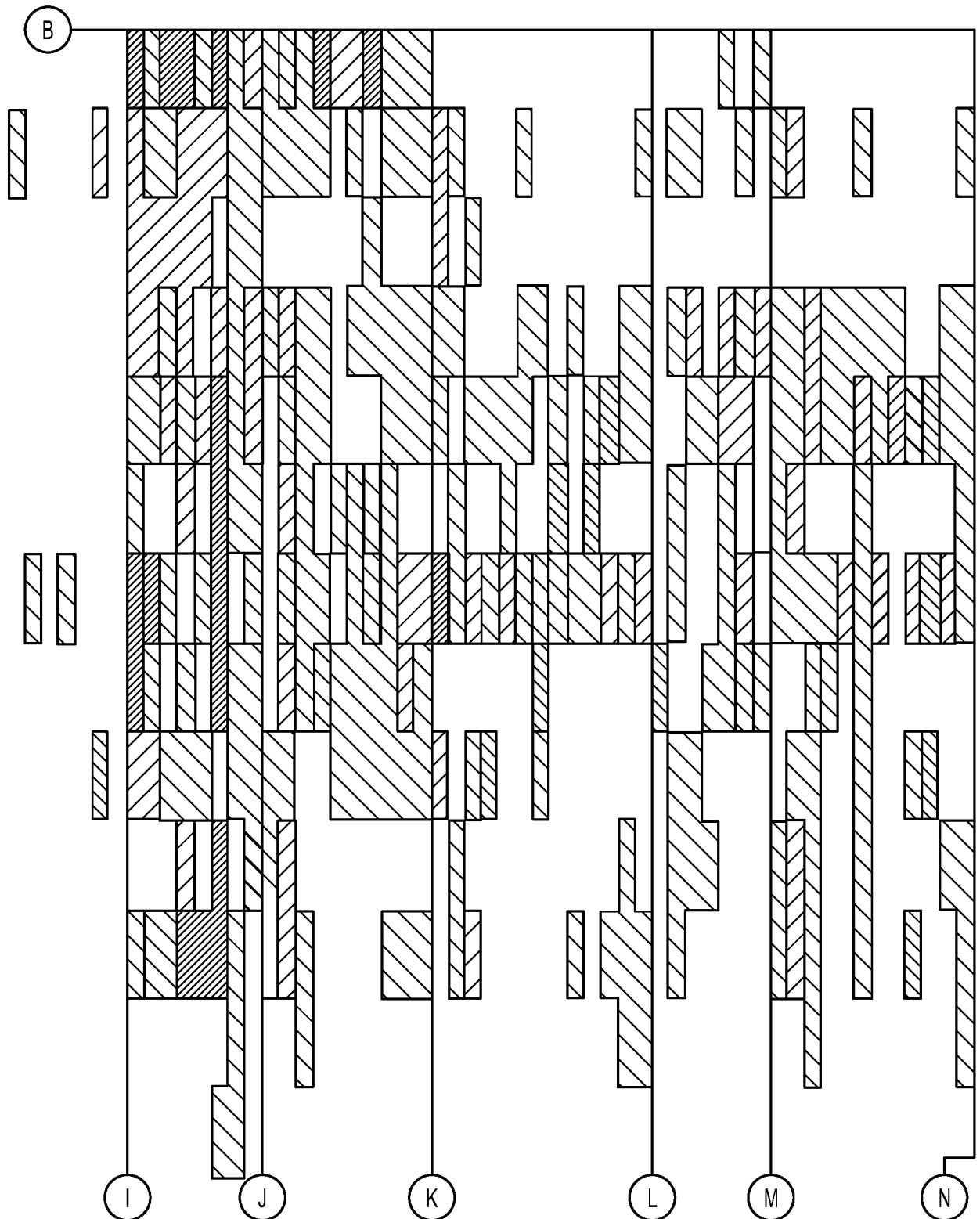


FIG. 72
(Continued)

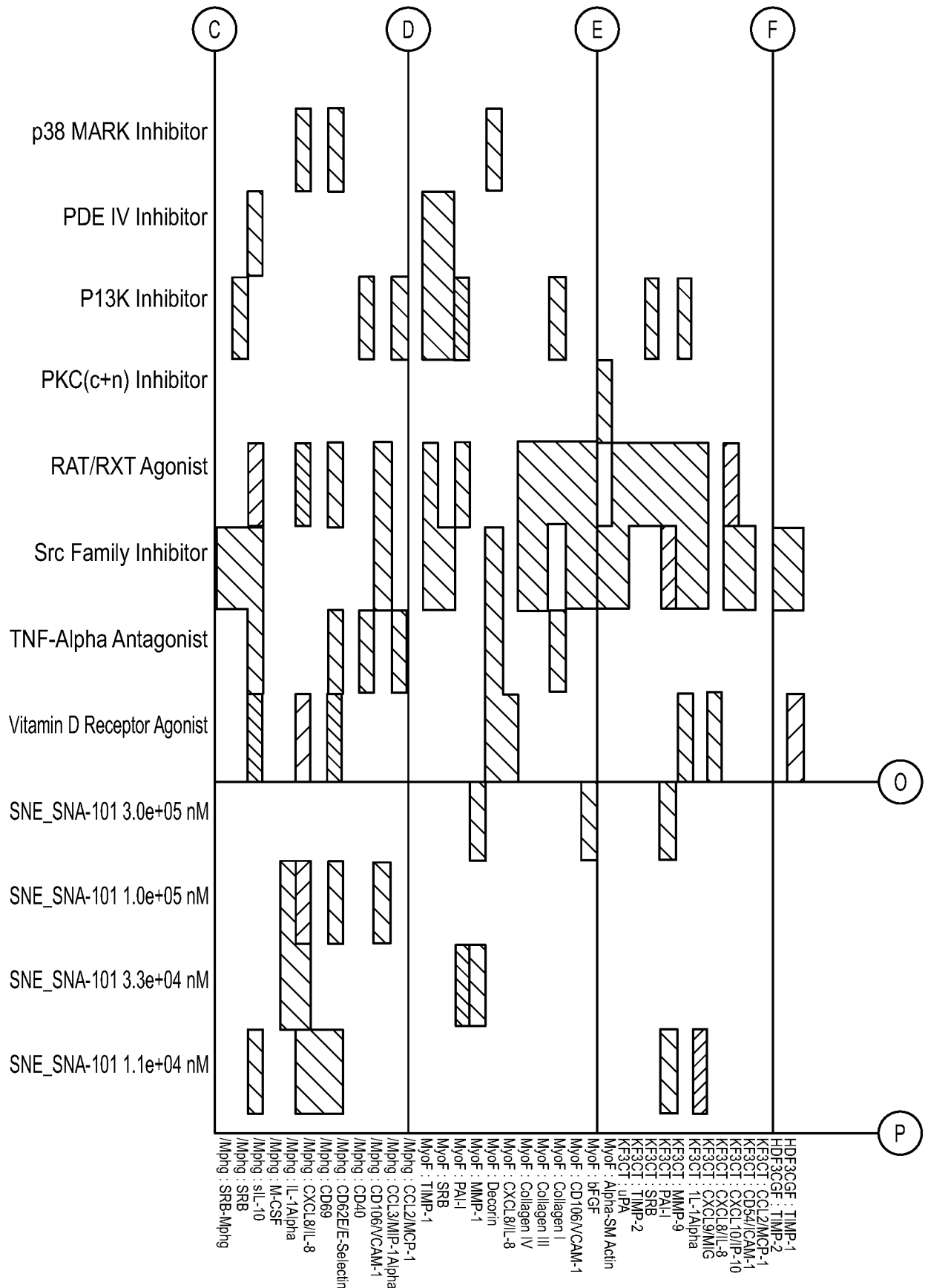


FIG. 72
(Continued)

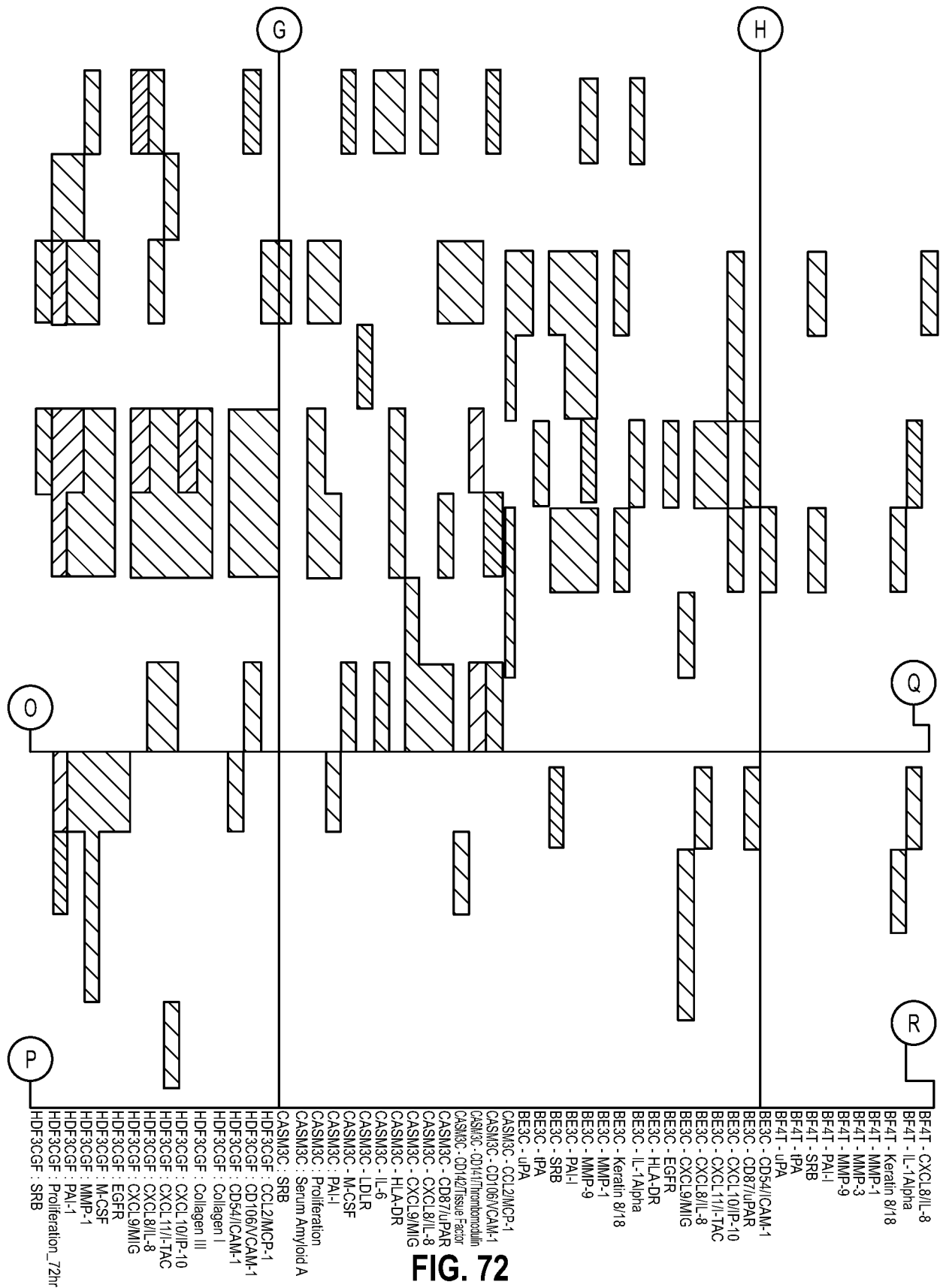


FIG. 72
(Continued)

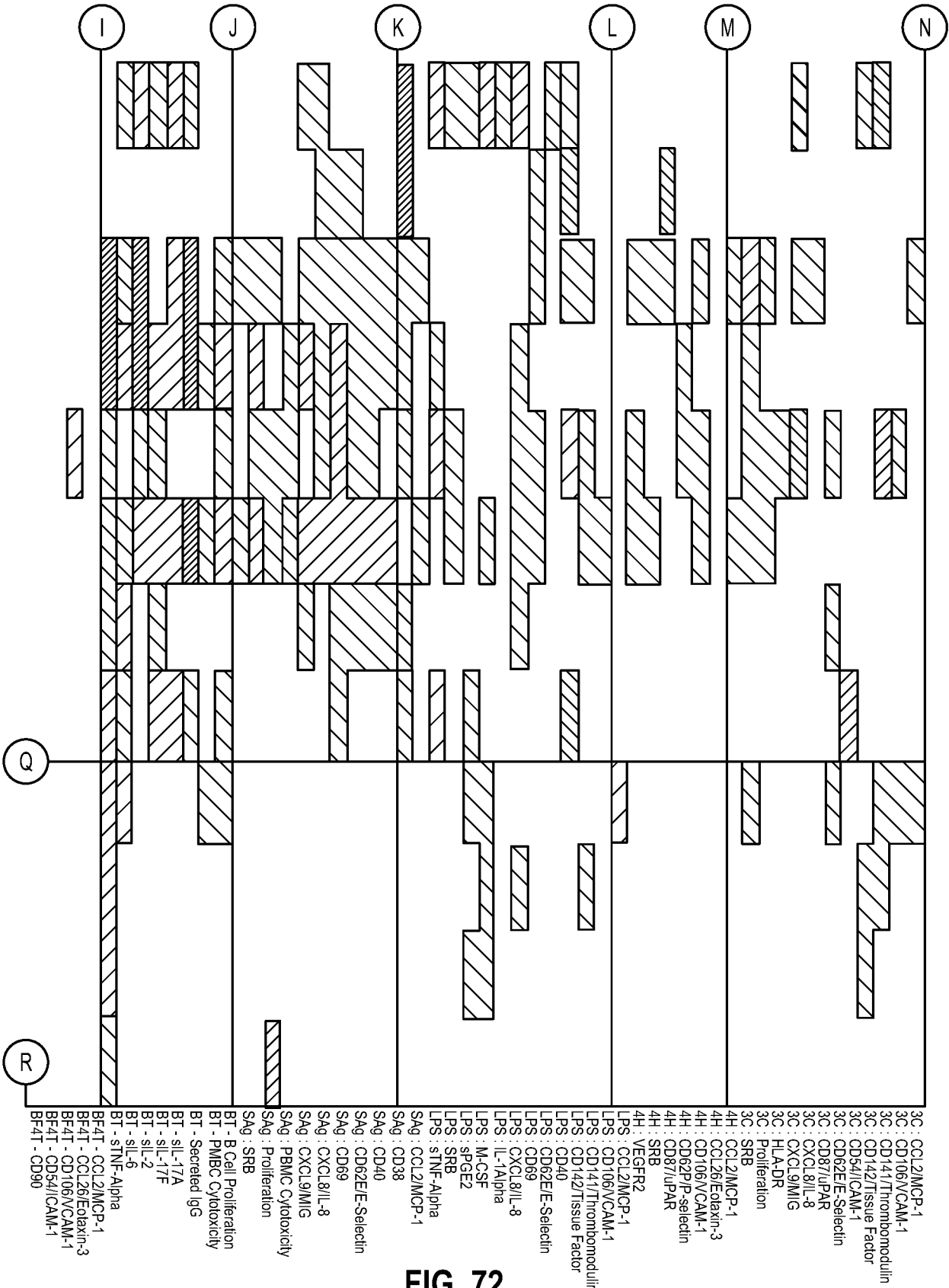


FIG. 72
(Continued)

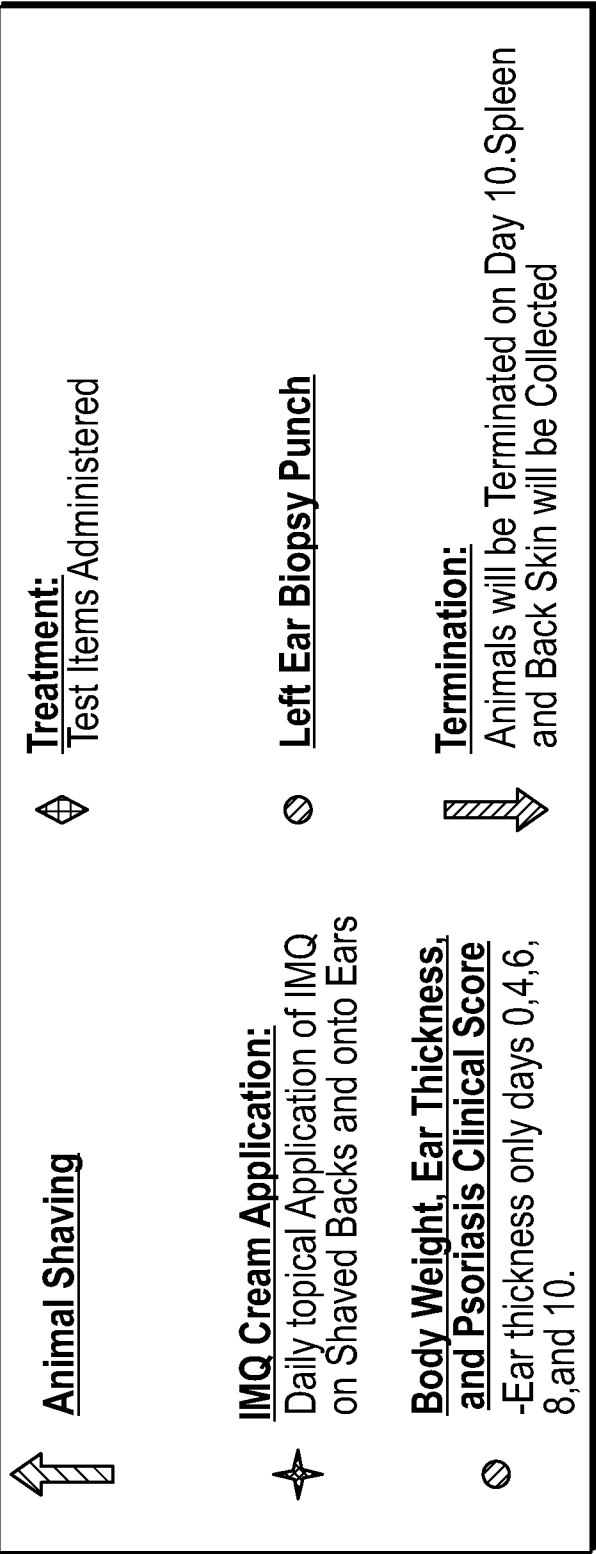
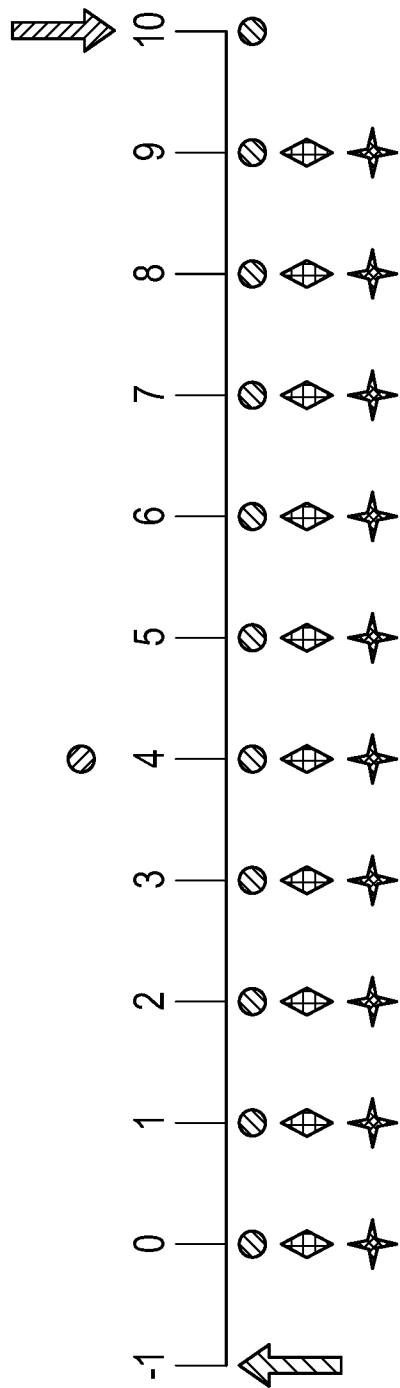


FIG. 73

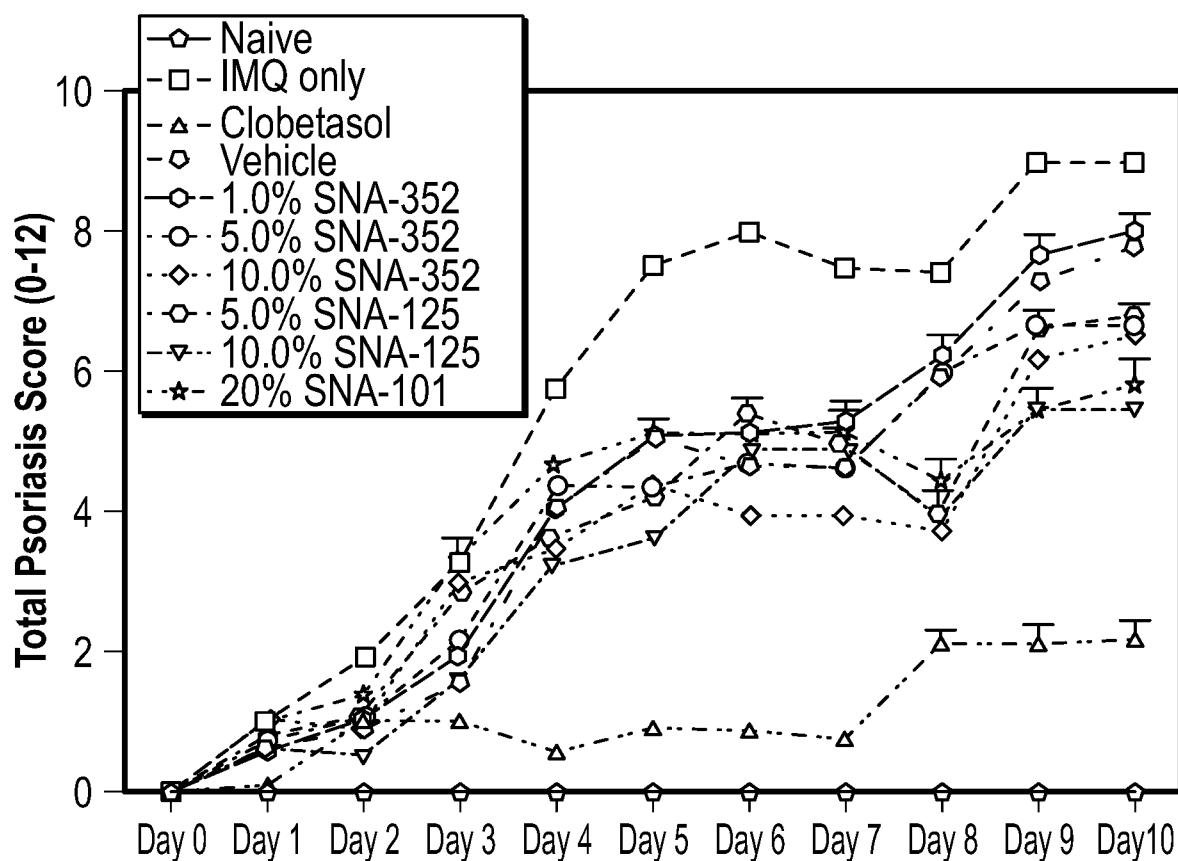


FIG. 74A

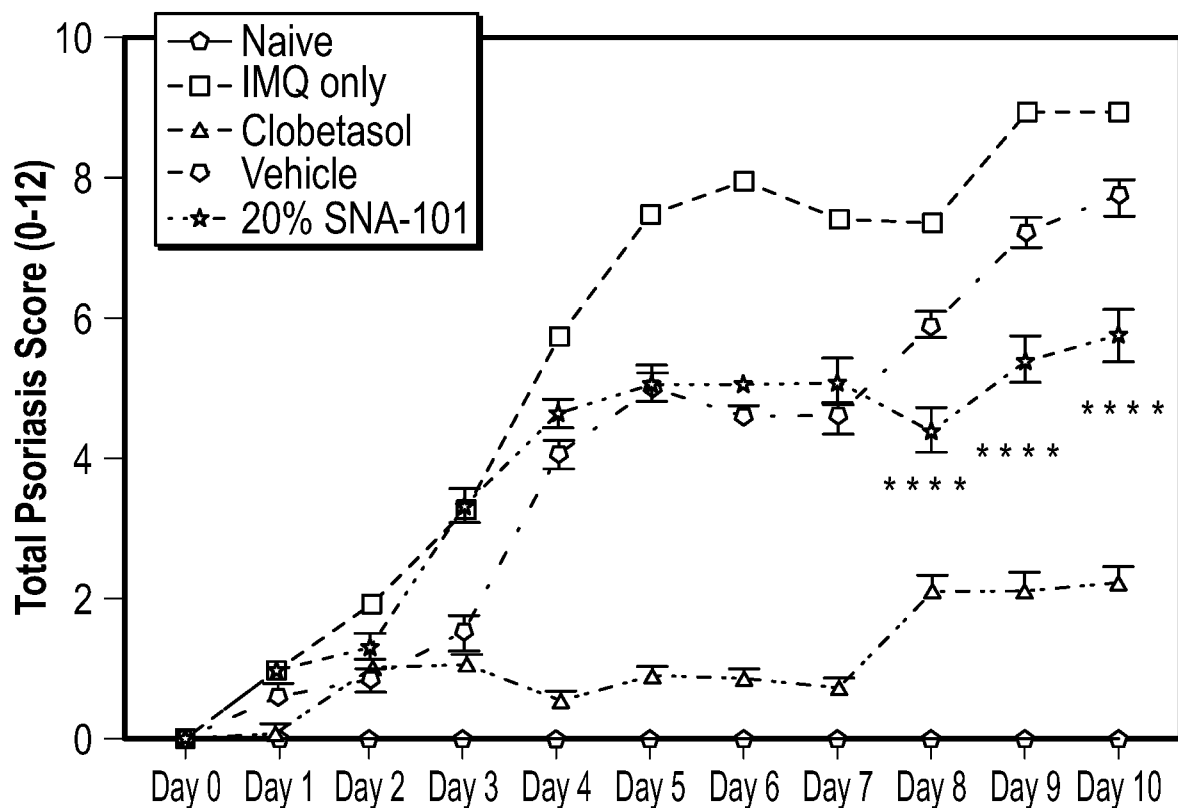


FIG. 74B

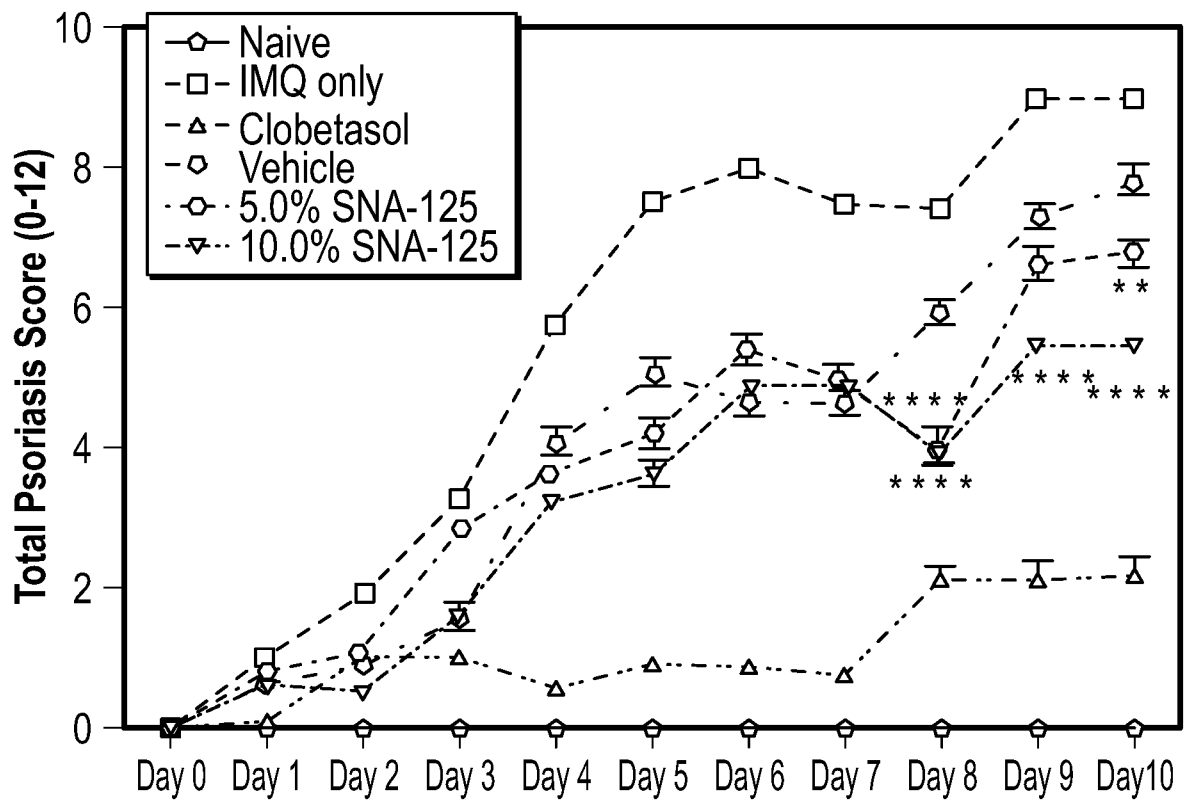


FIG. 74C

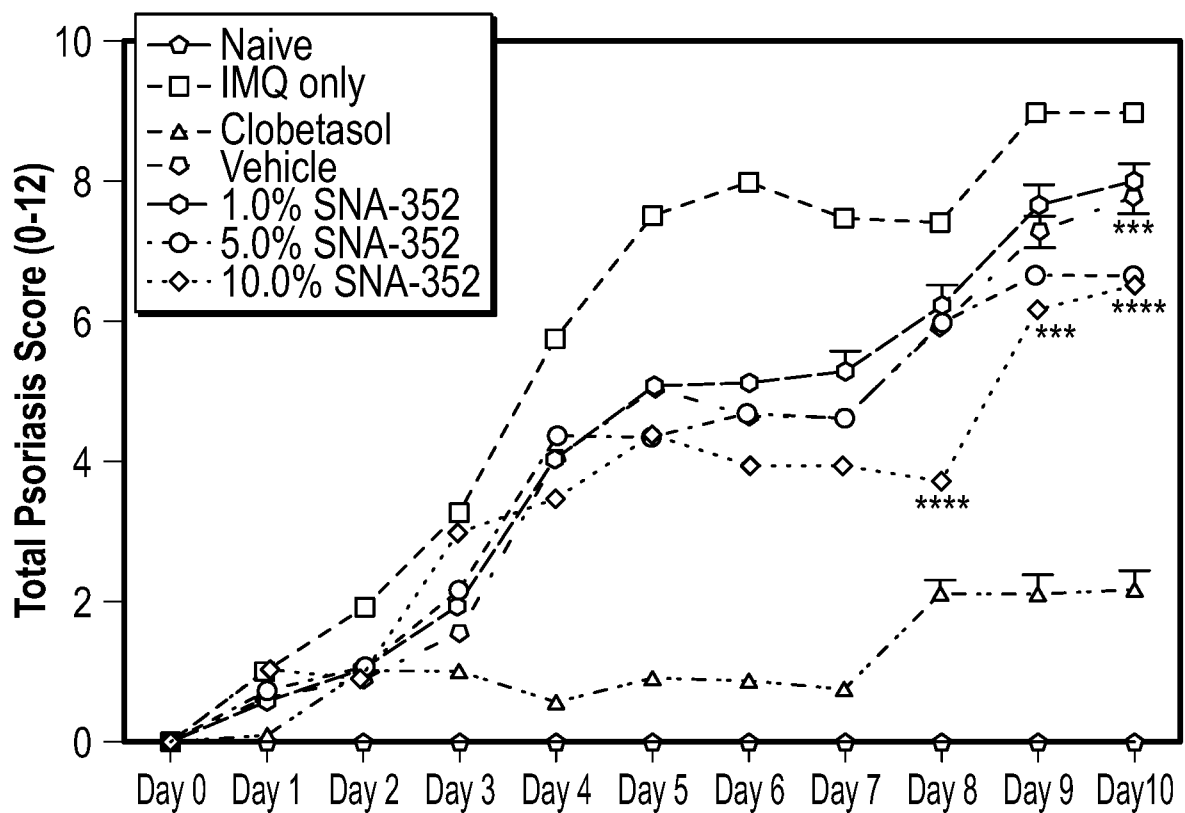


FIG. 74D

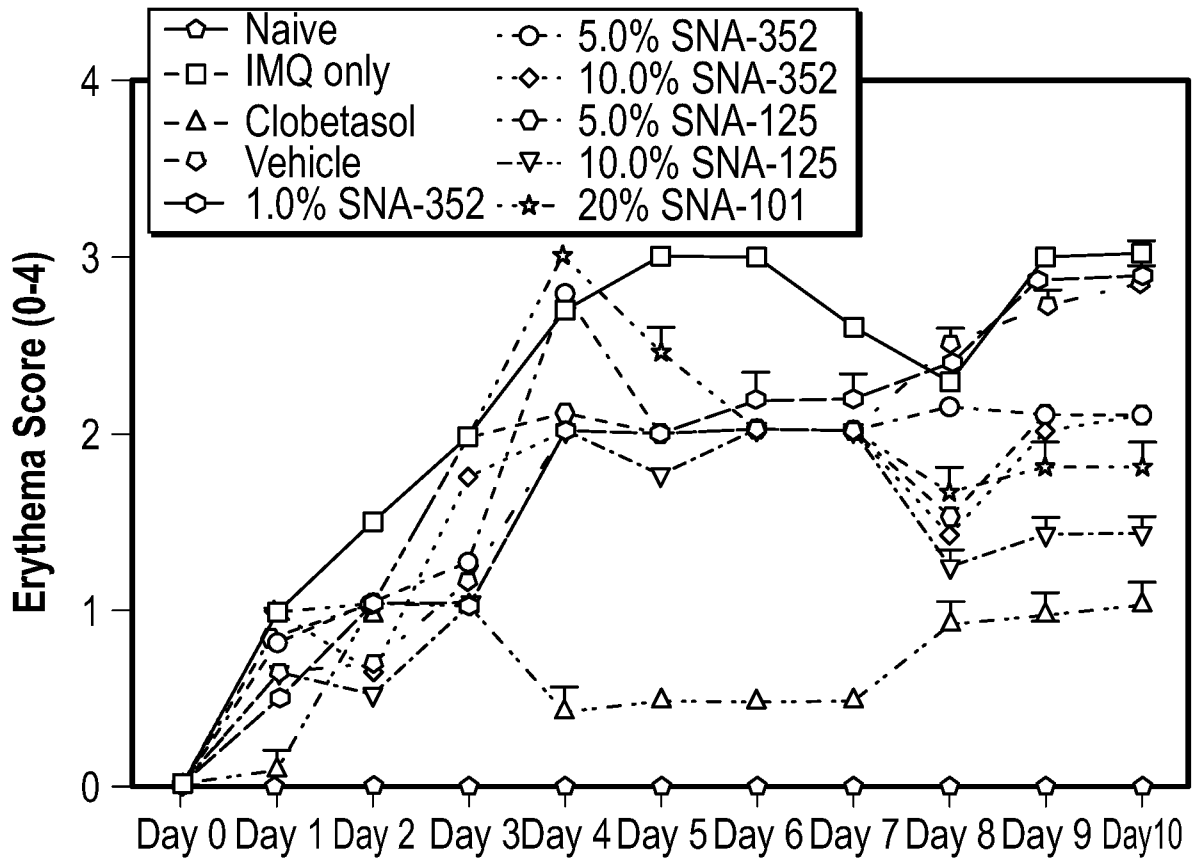


FIG. 75A

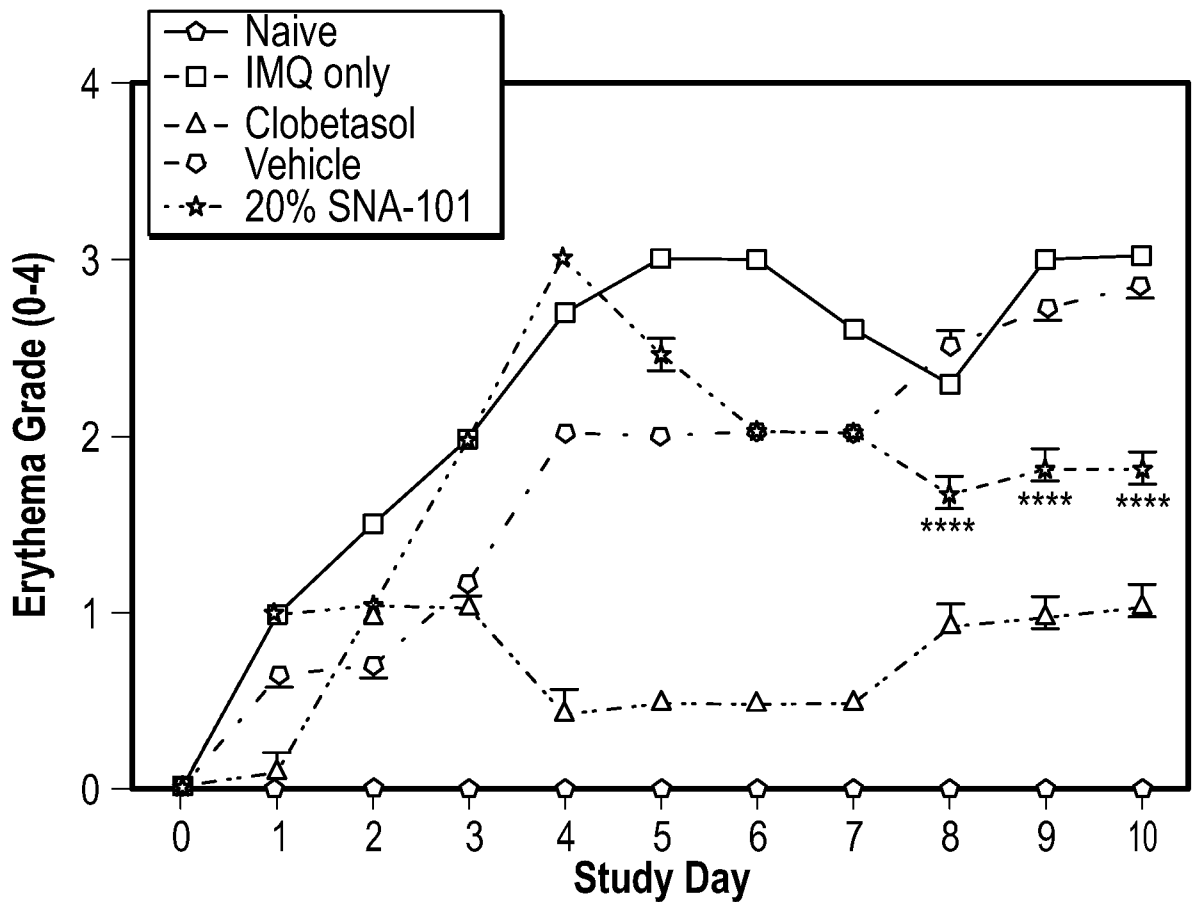
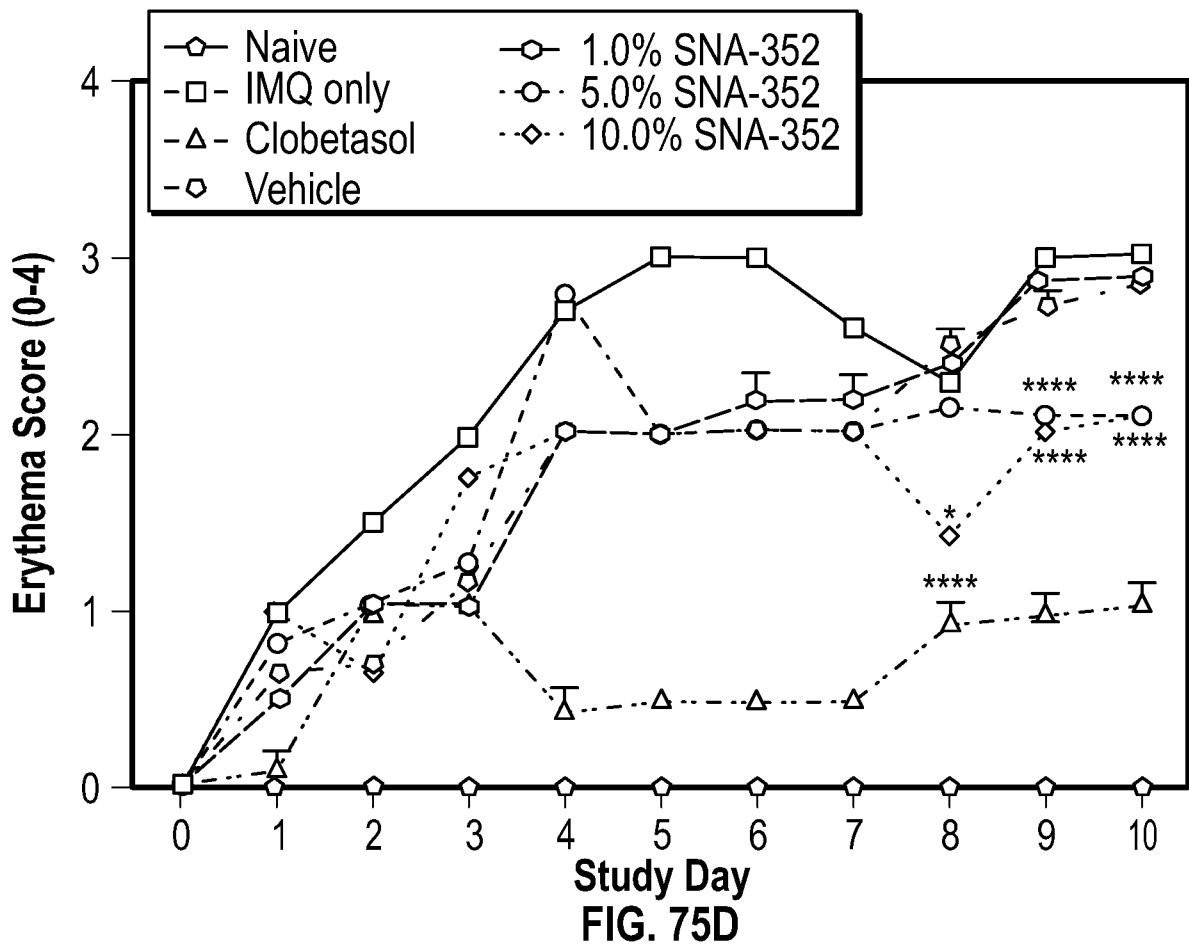
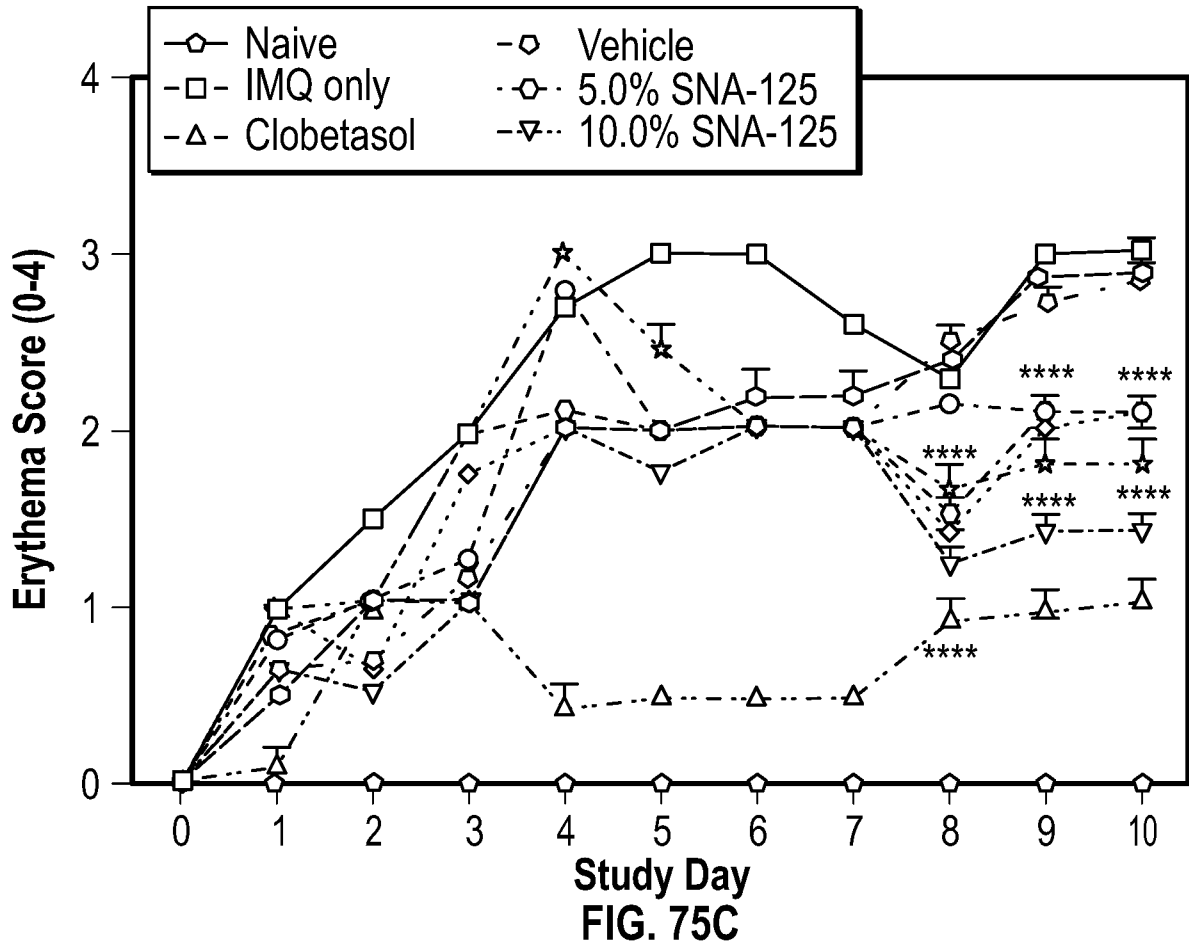


FIG. 75B



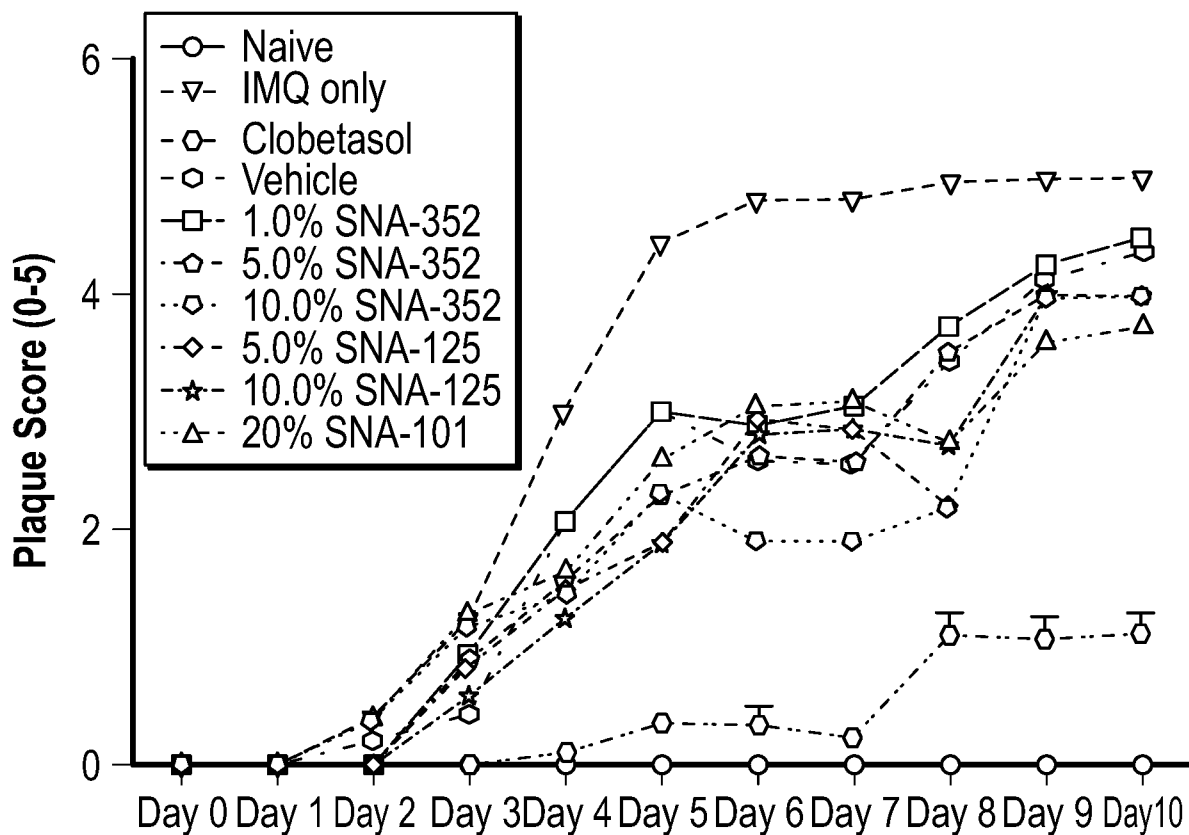


FIG. 76A

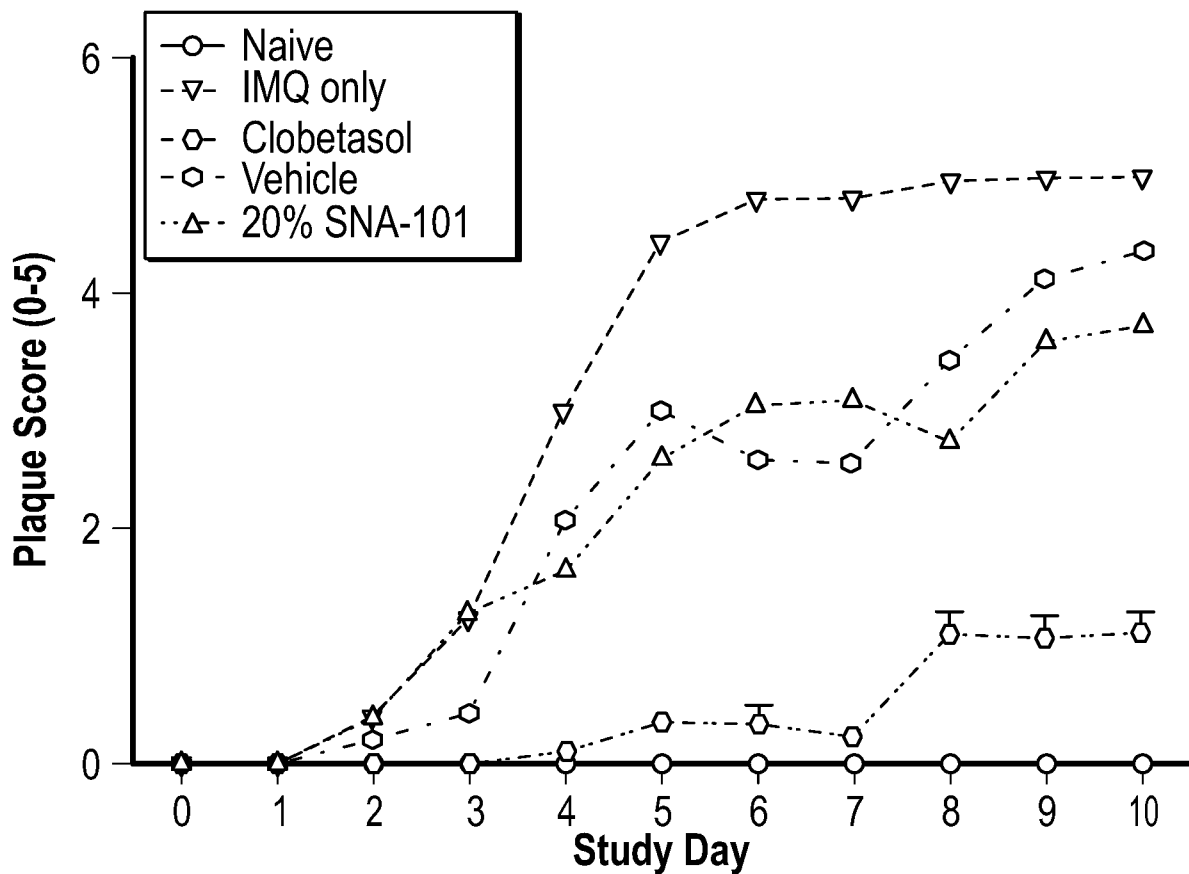


FIG. 76B

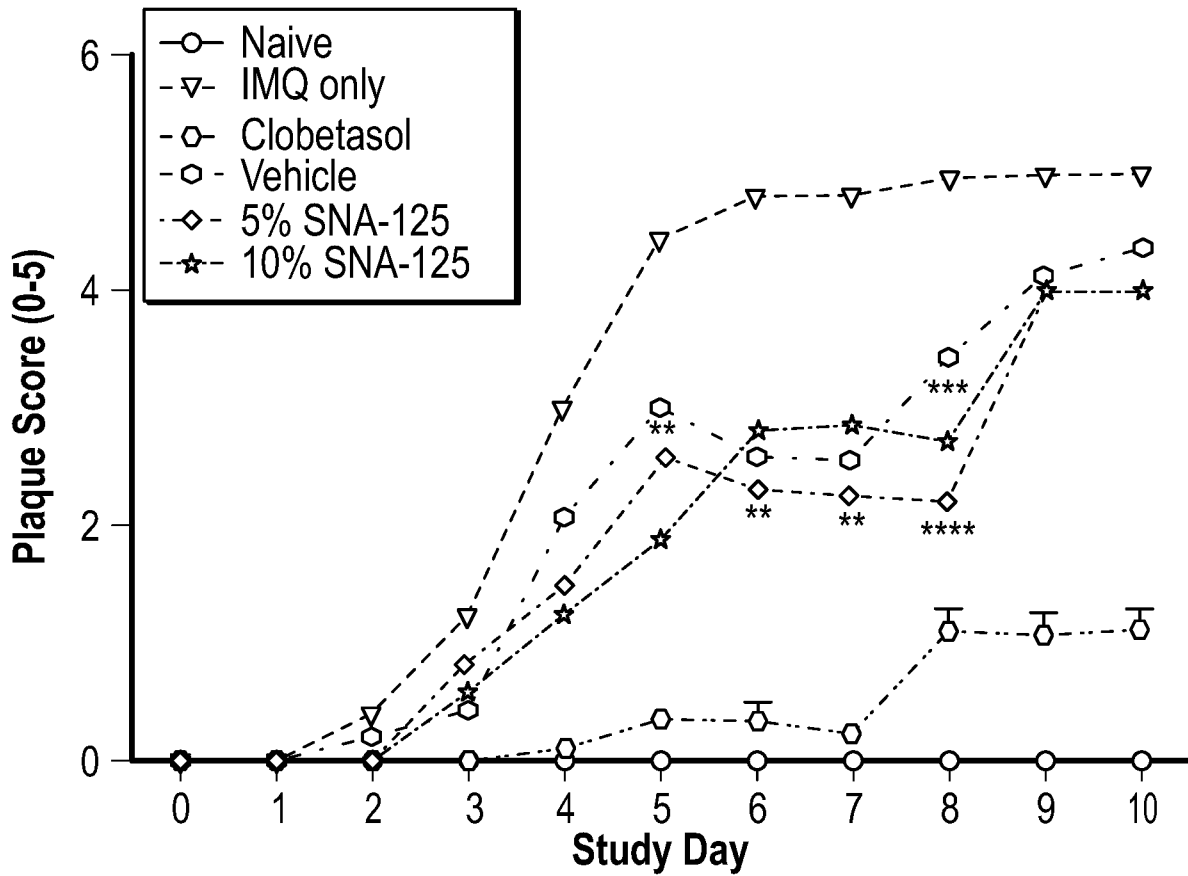


FIG. 76C

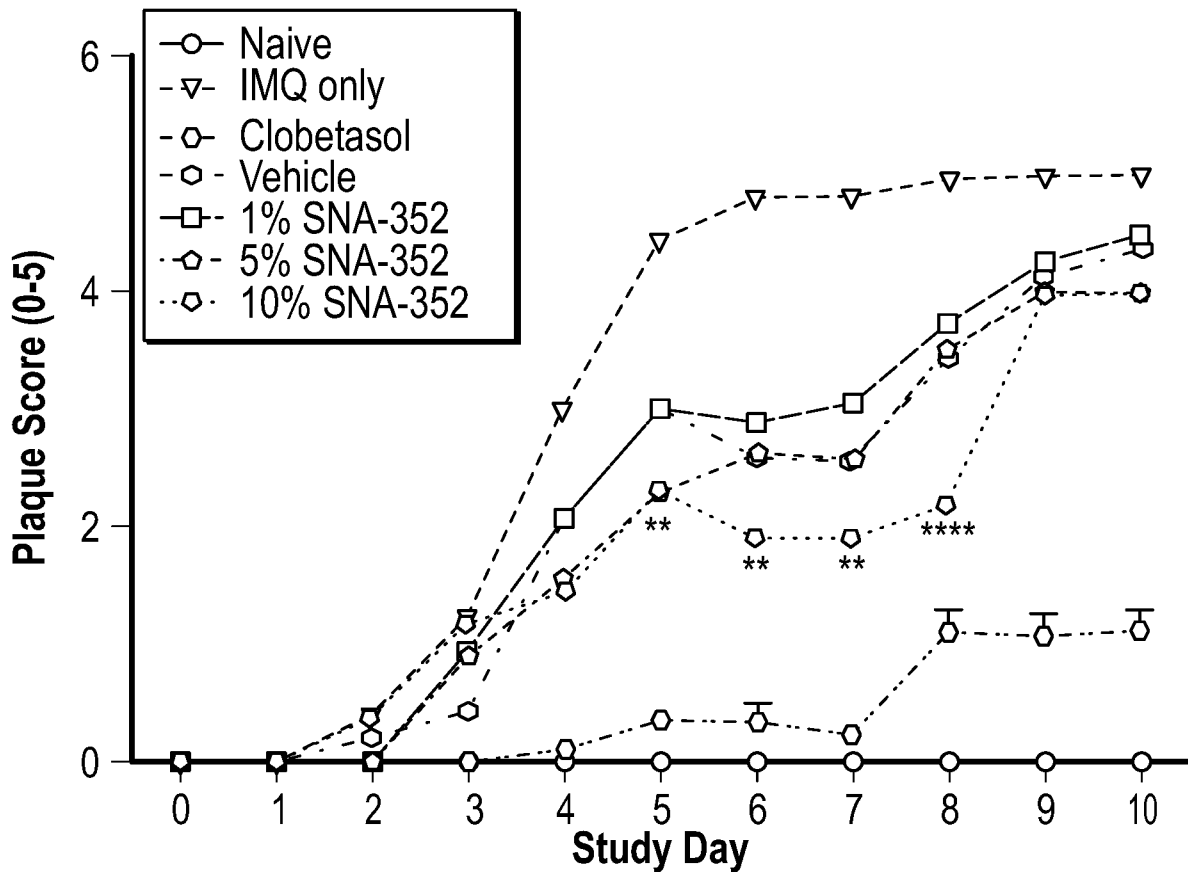


FIG. 76D

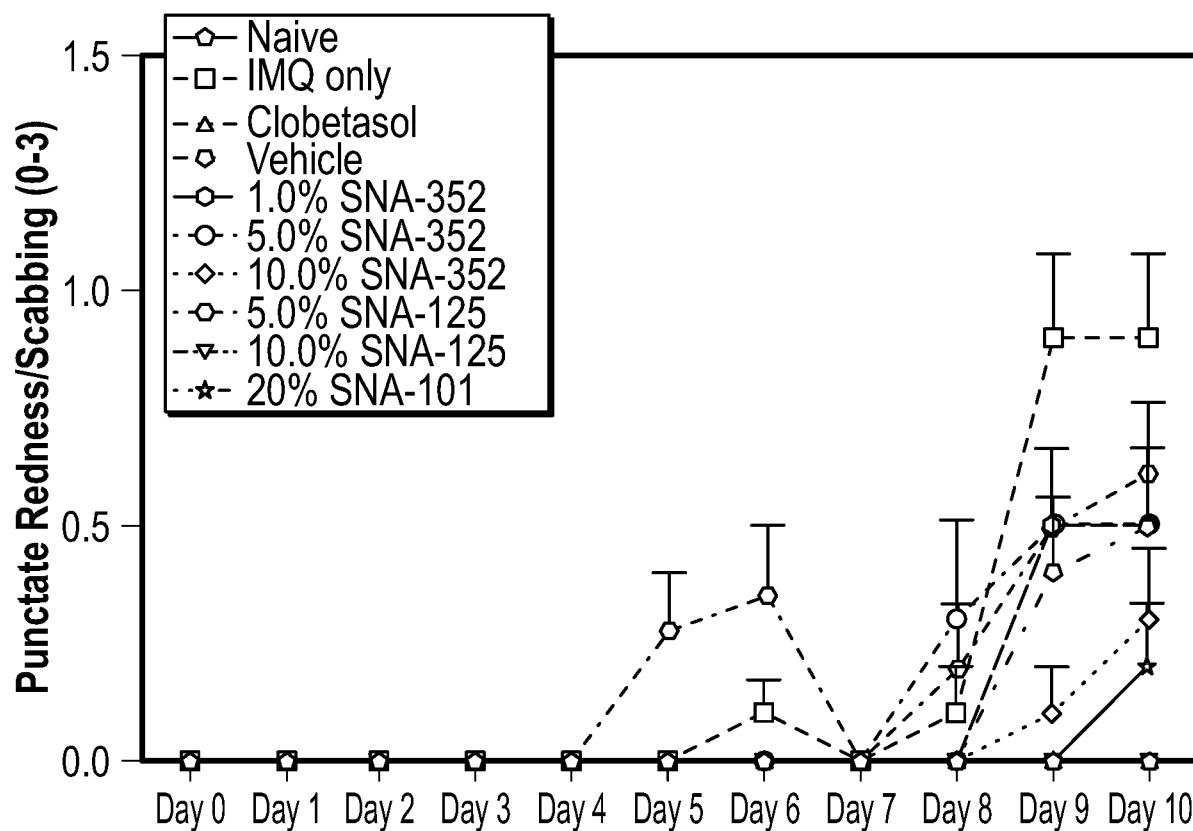


FIG. 77A

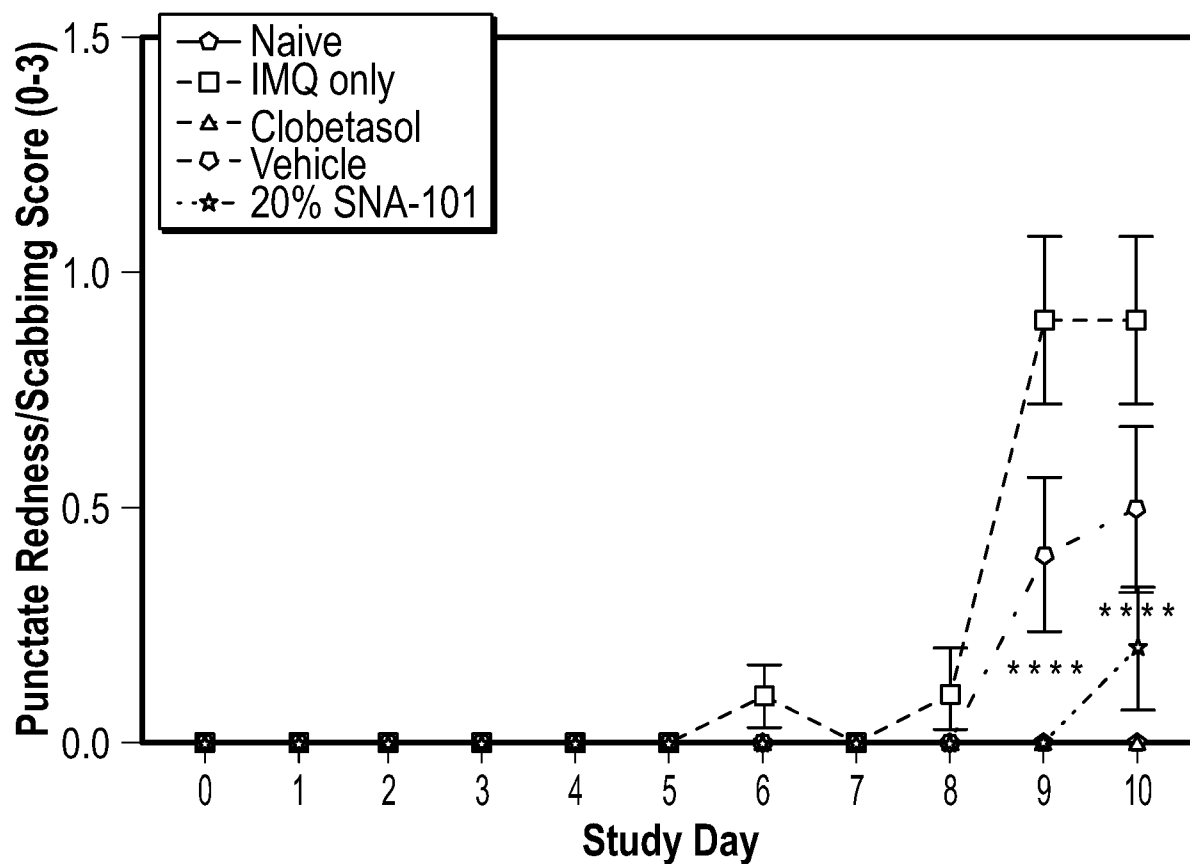


FIG. 77B

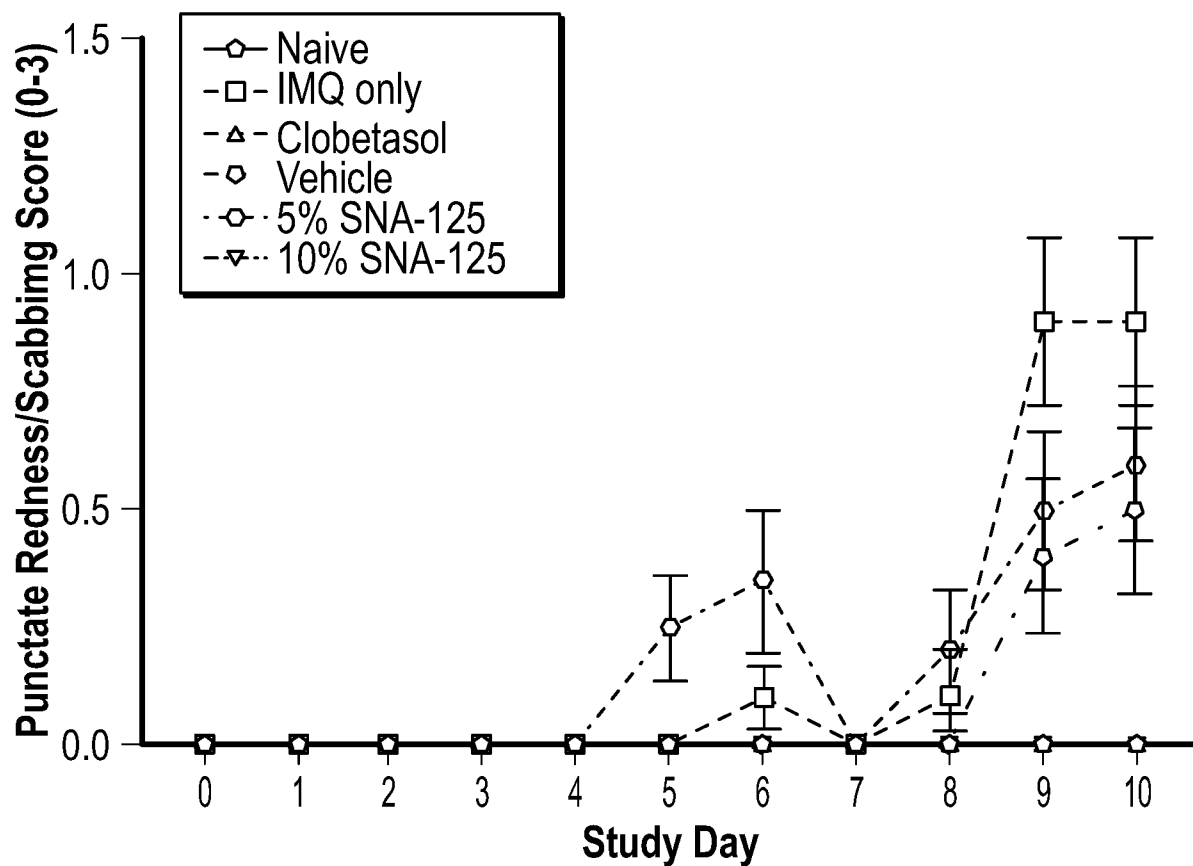


FIG. 77C

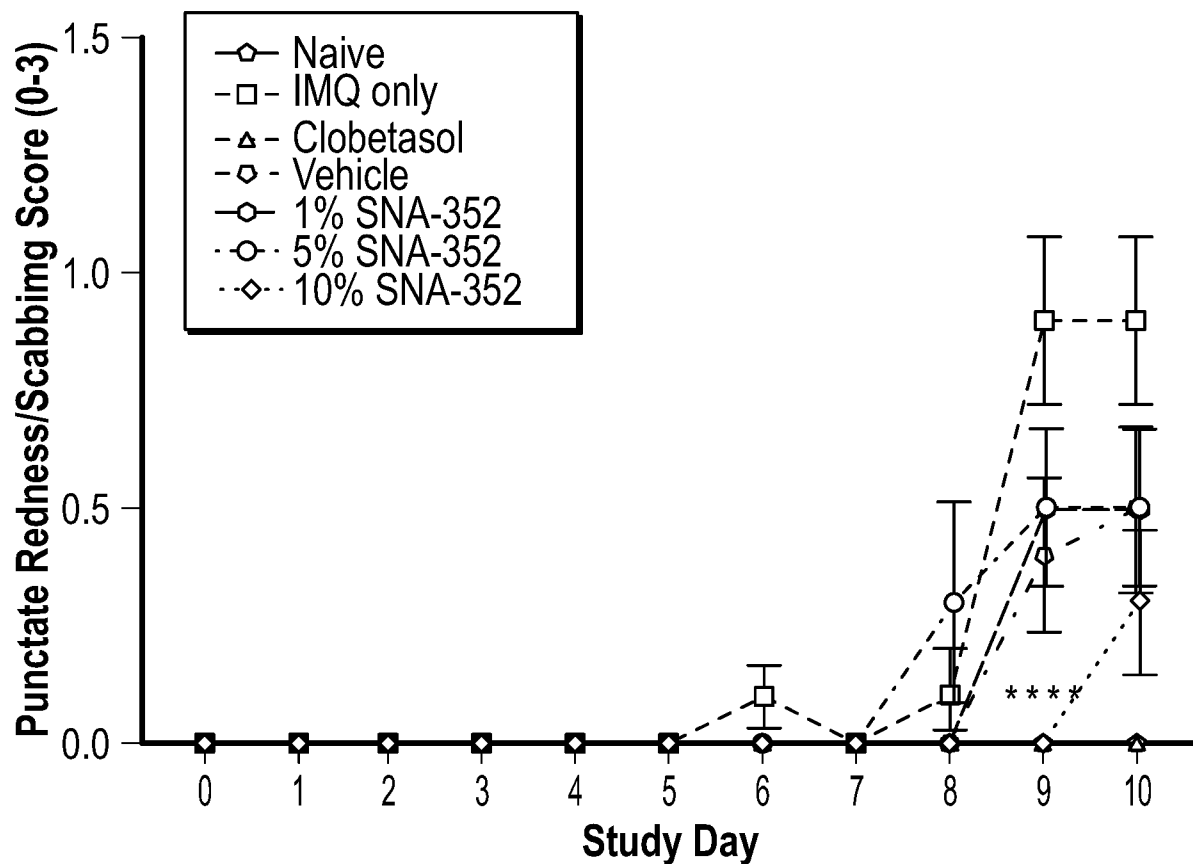
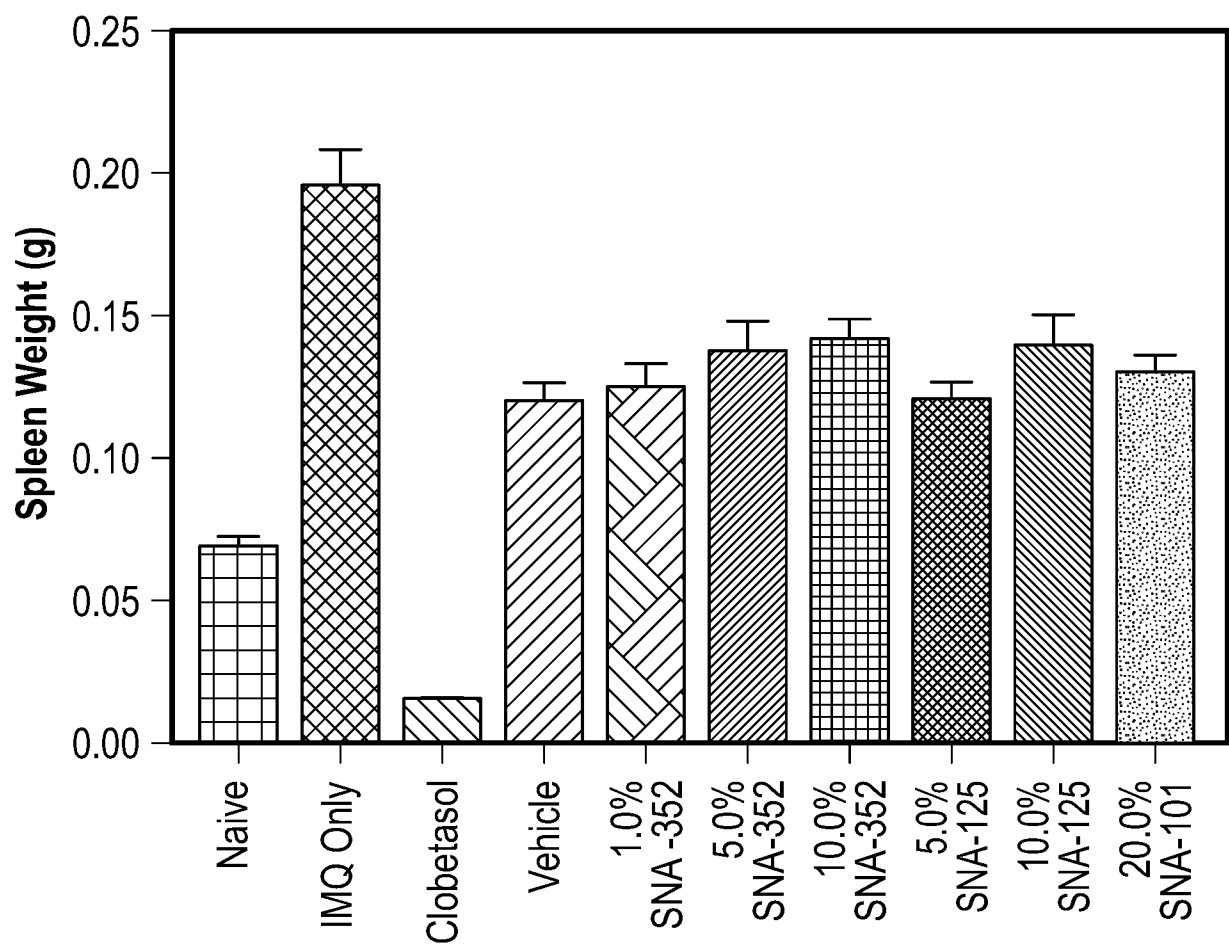


FIG. 77D

**FIG. 78A**

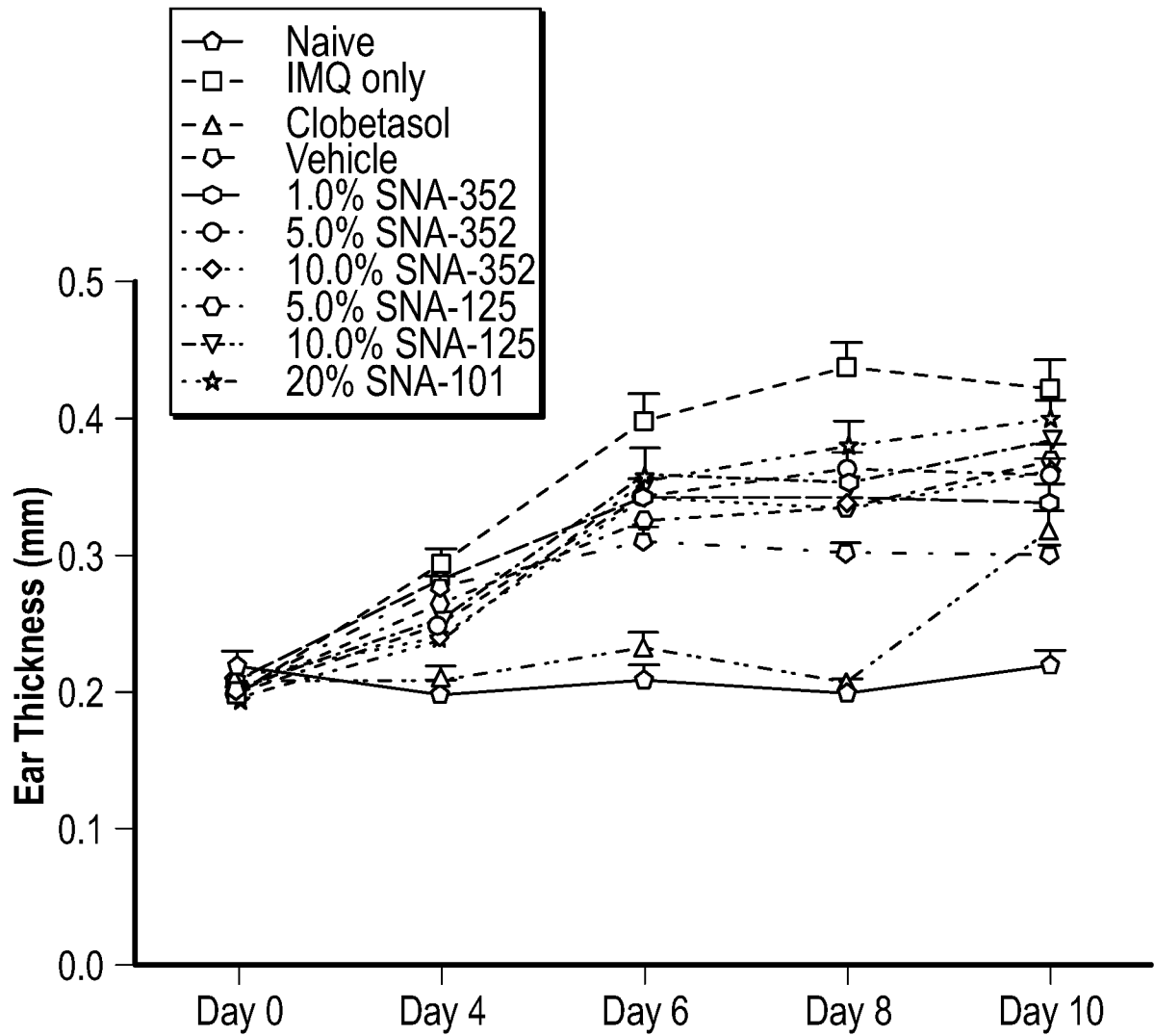


FIG. 78B

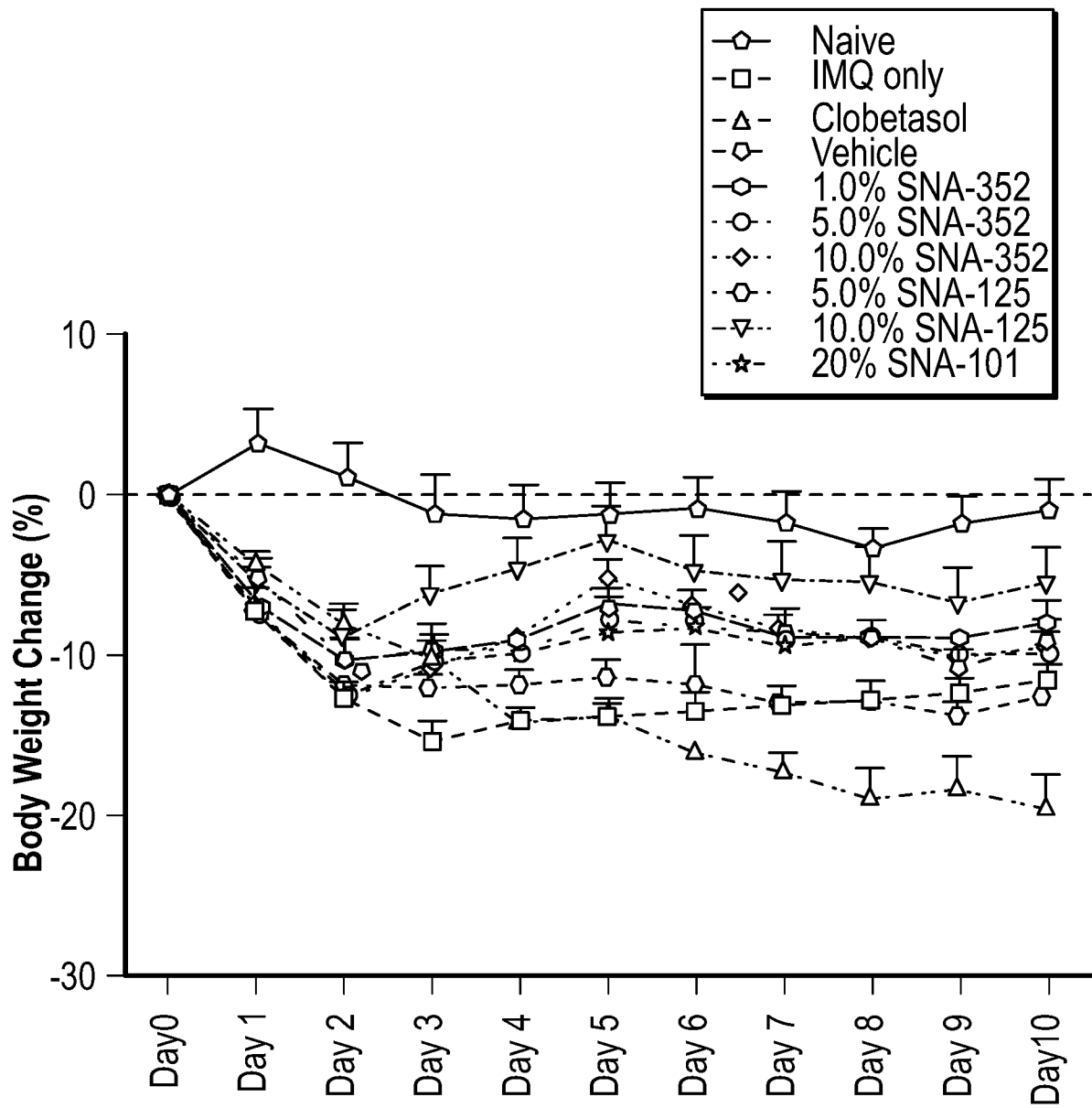


FIG. 78C

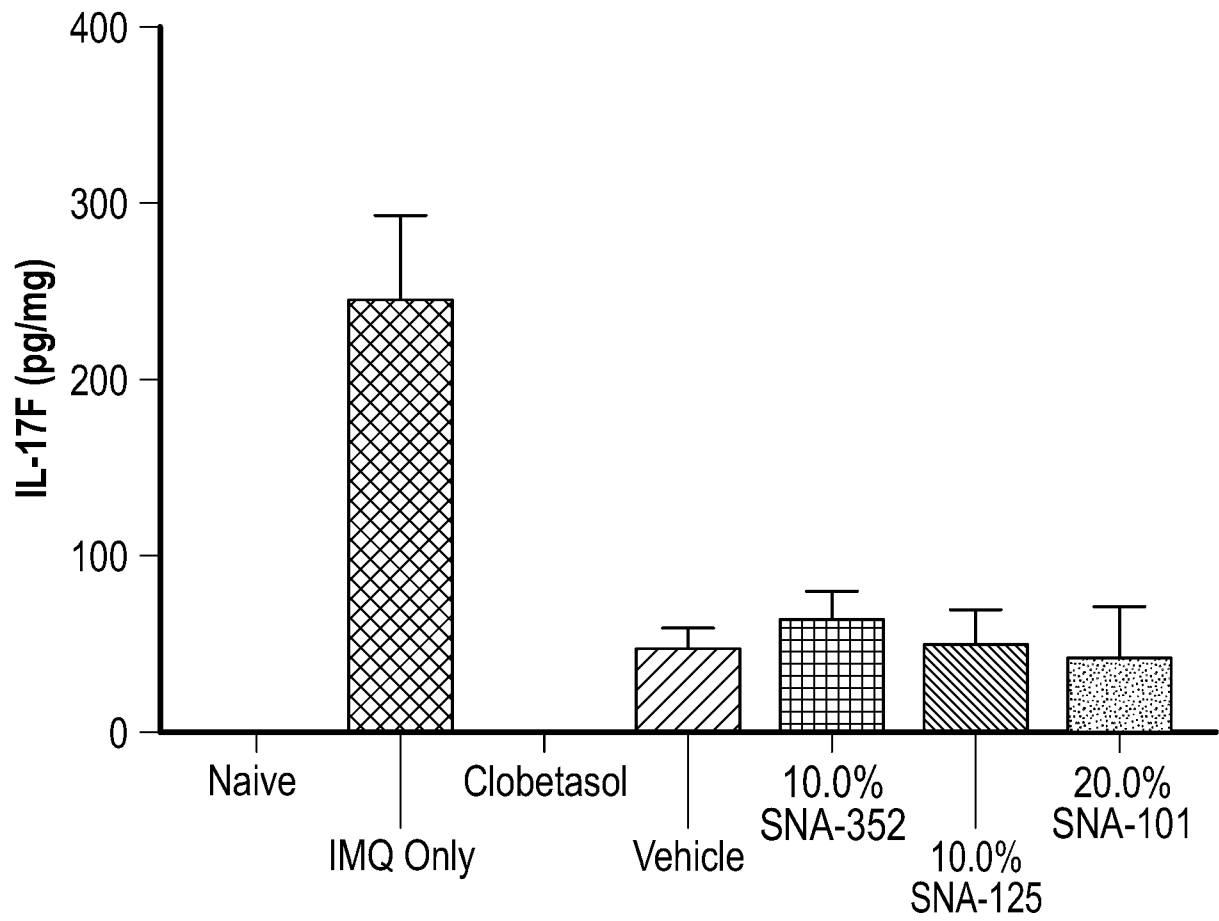


FIG. 79A

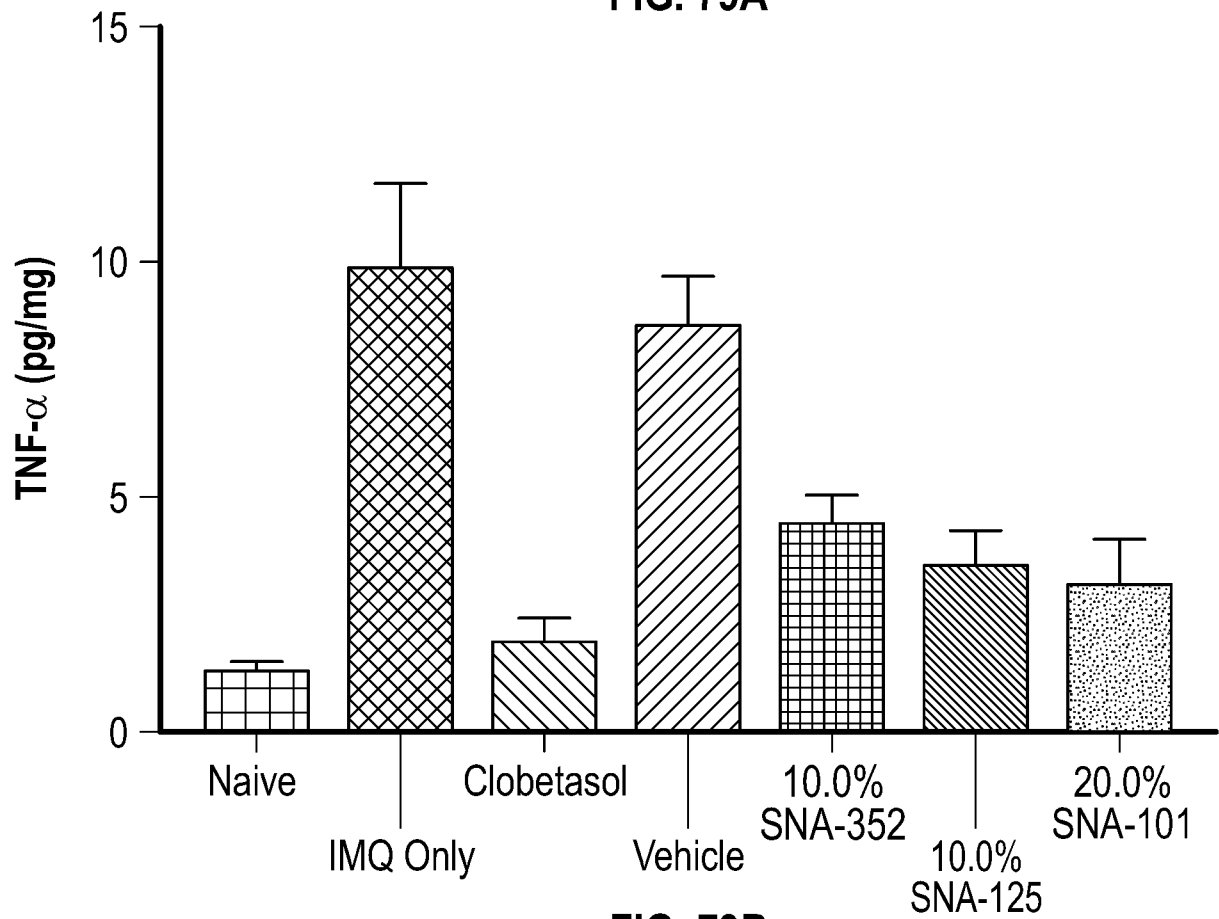


FIG. 79B

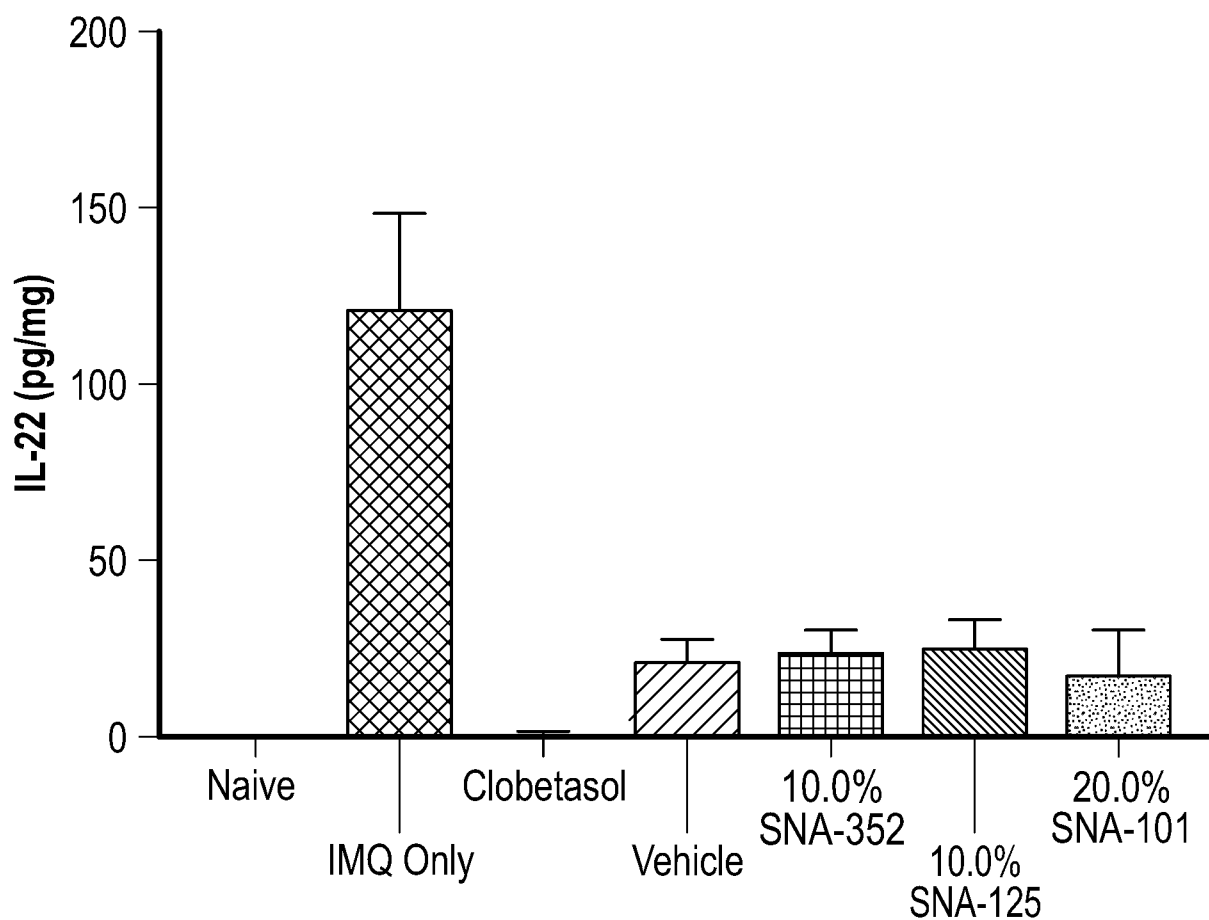


FIG. 79C

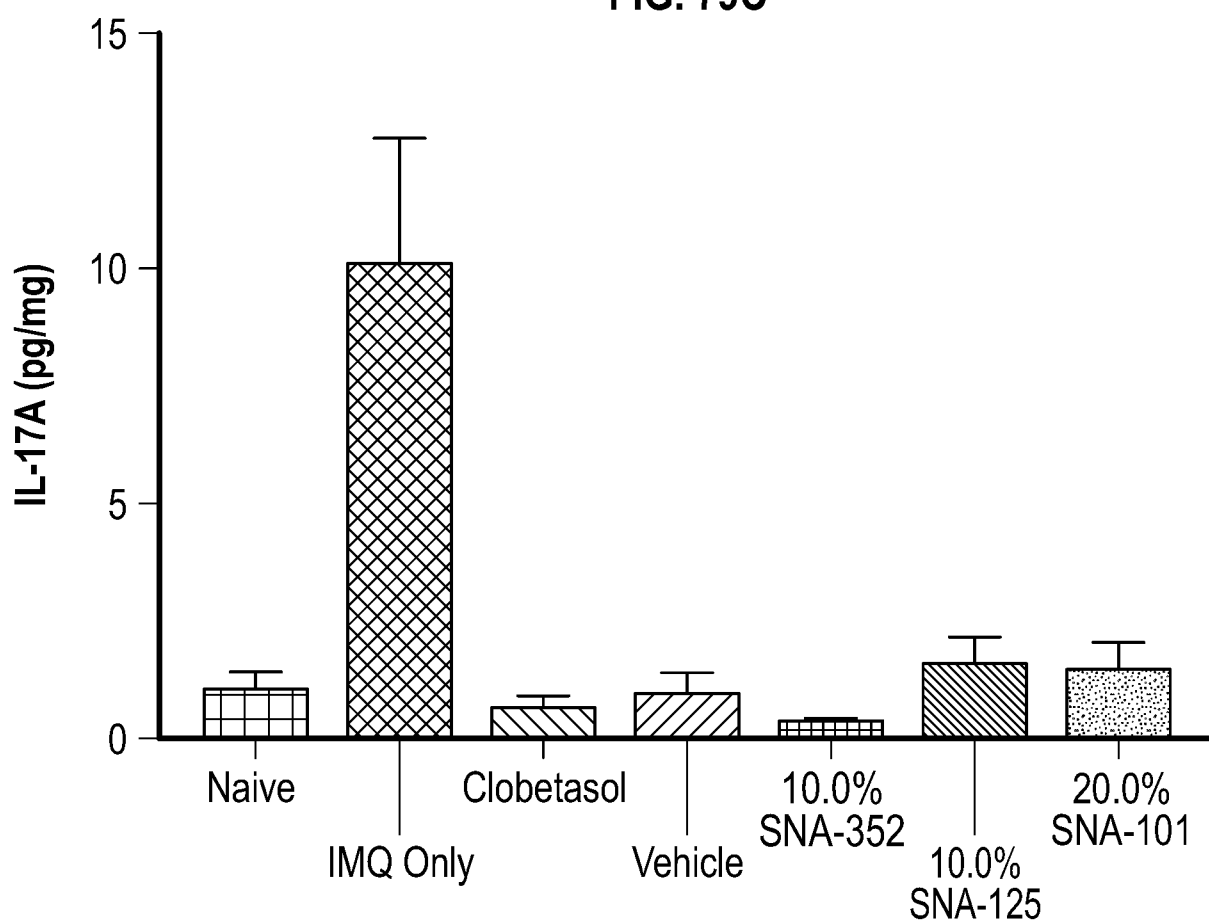


FIG. 79D

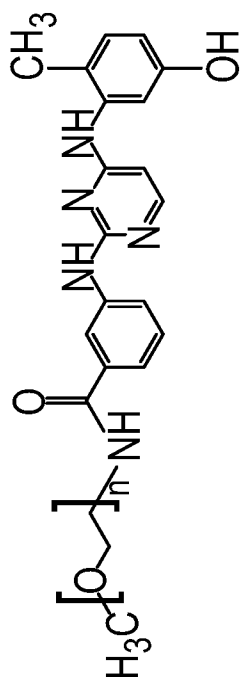
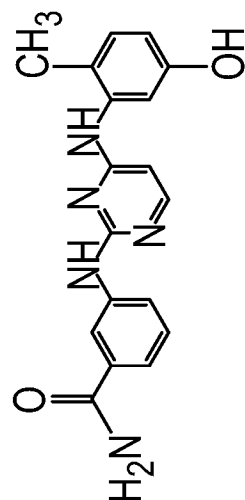
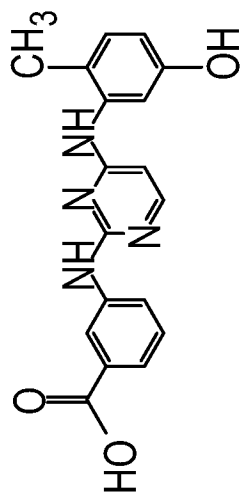


FIG. 80



Benzamide



Carboxylic Acid Intermediate

Hydrolysis

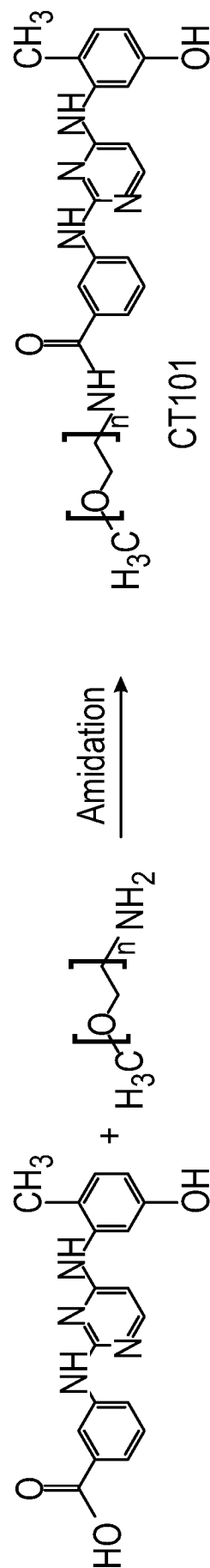


FIG. 81

CT101 Colonna Zorbax Eclipse XDB-C18 150x3.0 mm 5 micron P.N. 993967-302; S.N. USTD002295 AN-HPLC -77

* MWD1 A, Sig=270,4 Ref=450, 80 (CT101\17040408.D-CT101\17040403.D)

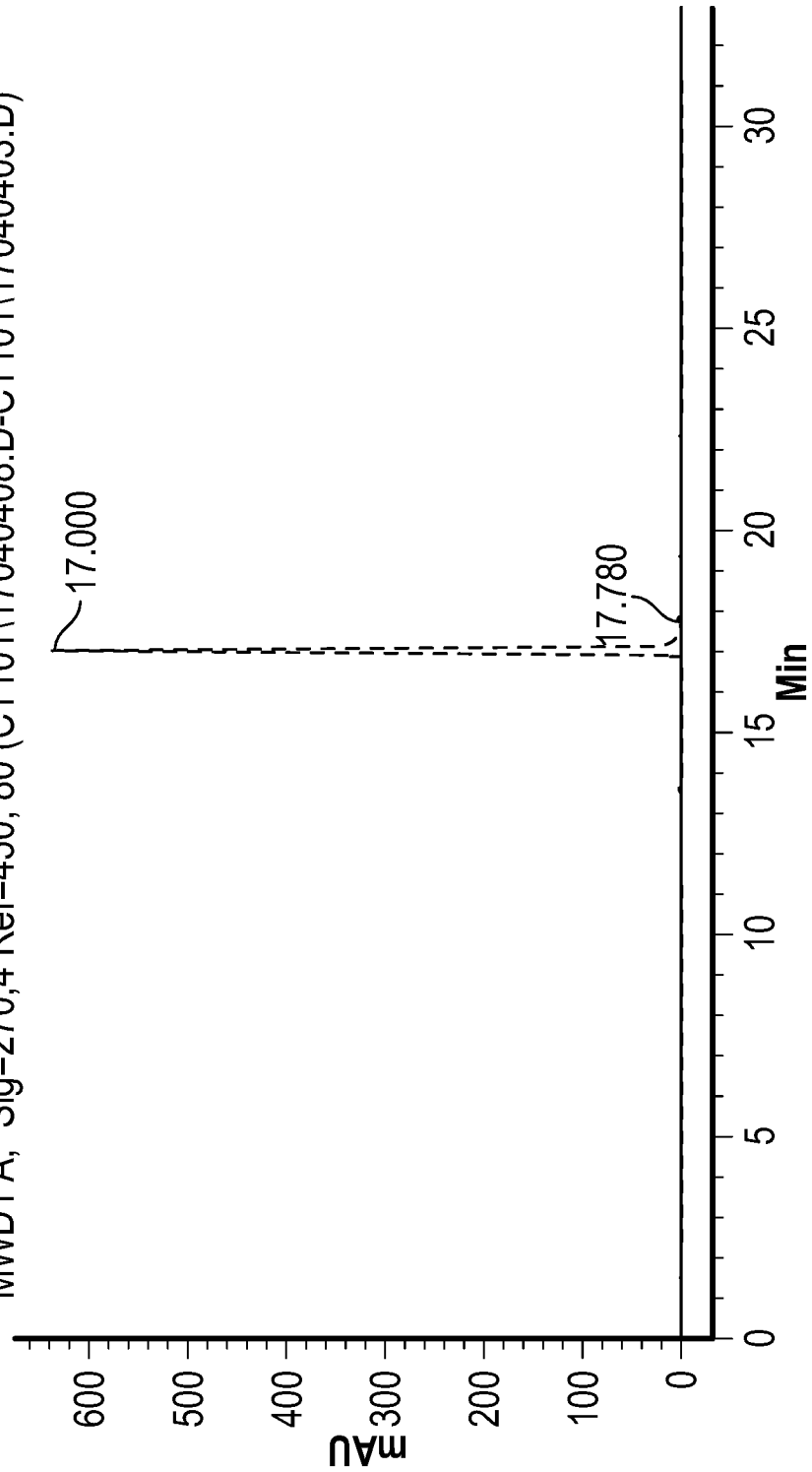


FIG. 82

Area Percent Report						
Sorted By : Signal						
Multiplier : 1.0000						
Dilution : 1.0000						
Use Multiplier & Dilution Factor with ISTDs						
Signal 1: MWD1 A, Sig=270, 4 Ref=450, 80						
Signal has been Modified After Loading from Rawdata File !						
Peak #	RetTime [min]	Type	Width [min]	Area [mAU* S]	Height [mAU]	Area %
1	17.000	PV	0.0950	4023.70142	642.74615	99.3328
2	17.780	VB	0.1527	27.02805	2.45000	0.6672
Totals :				4050.72946	645.19616	

FIG. 82
(Continued)

CT101 Colonna Zorbax Eclipse XDB-C18 150 x3.0 mm 5 micron P.N. 993967-302; S.N. USTD002295 AN-HPLC -77

*MWD1 A, Sig=270, 4 Ref=450, 80 (CT101\17042102.D-CT101\17042103.D)

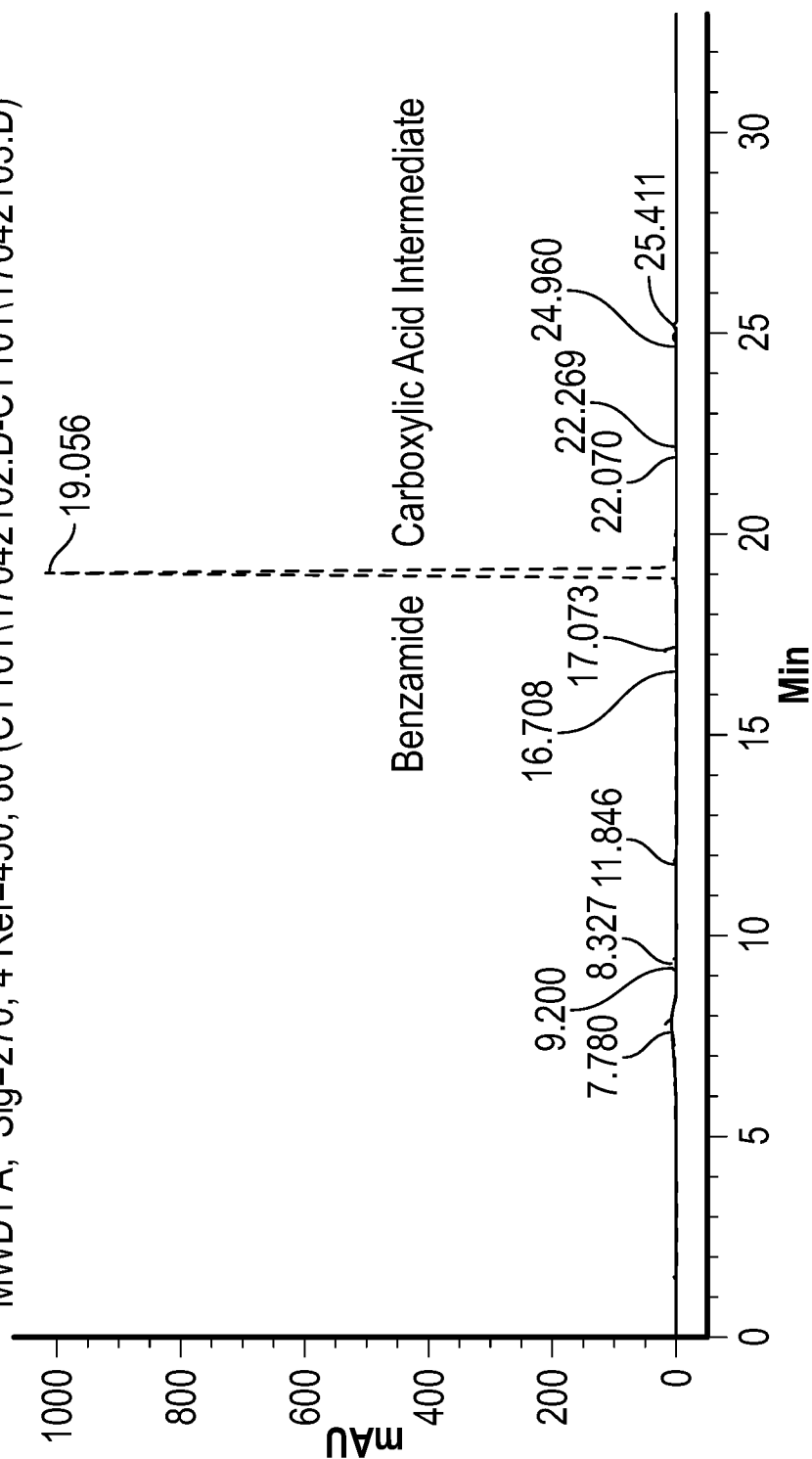


FIG. 83

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=270, 4 Ref=450, 80

Signal has been Modified After Loading from Rawdata File !

Peak #	RetTime [min]	Type	Width [min]	Area [mAU* S]	Height [mAU]	Area %
1	7.780	MM	0.1777	125.25410	11.74631	1.7150
2	9.208	BV	0.0765	41.57684	8.01511	0.5693
3	9.327	VB	0.1064	105.71587	13.95936	1.4475
4	11.846	PV	0.0728	7.17466	1.52831	0.0982
5	16.708	BB	0.1024	4.94176	7.00037e-1	0.0677
6	17.073	BB	0.0957	136.21542	21.57064	1.8651
7	19.056	PB	0.1019	6838.81592	1023.77240	93.6385
8	22.078	BV	0.1424	8.13235	8.44186e-1	0.1113
9	22.269	VB	0.2212	8.12716	4.66513e-1	0.1113
10	24.960	VV	0.1493	17.75022	1.73568	0.2430
11	25.411	VB	0.1829	9.716253	7.71840e-1	0.1330
Totals :				7303.42053	1085.11038	

FIG. 83
(Continued)

CT101 Colonna Zorbax Eclipse XDB-C18 150 x3.0 mm 5 micron P.N. 993967-302; S.N. USTD002295 AN-HPLC -77

* MWD1 A, Sig=270.4 Ref=450.80 (CT101\17042605.D-CT101\17042602.D)

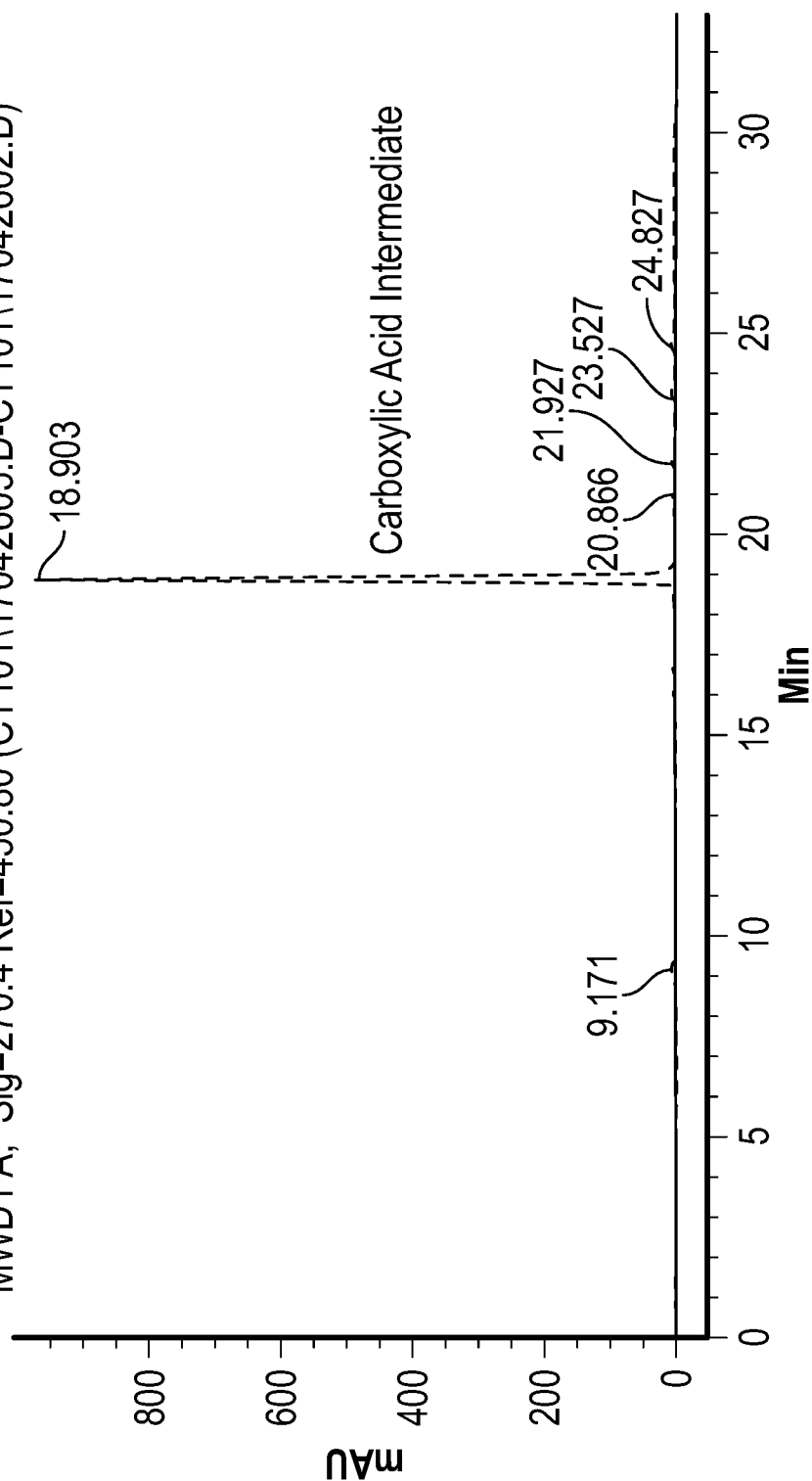


FIG. 84

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=270, 4 Ref=450, 80

Signal has been Modified After Loading from Rawdata File !

Peak #	RetTime [min]	Type	Width [min]	Area [mAU* S]	Height [mAU]	Area %
1	9.171	BB	0.0943	48.06380	7.35167	0.7213
2	18.903	BB	0.1040	6578.22266	959.35413	98.7210
3	20.866	VV	0.1467	7.18393	6.51057e-1	0.1078
4	21.927	BV	0.1545	7.80357	7.31477e-1	0.1171
5	23.527	BV	0.1233	11.48375	1.40576	0.1723
6	24.827	VB	0.1591	10.68862	9.65795e-1	0.1604
Totals :				6663.44634	970.45989	

FIG. 84
(Continued)

CT101 Colonna Zorbax Eclipse XDB-C18 150x3.0 mm 5 micron P.N. 993967-302; S.N. USTD002295 AN-HPLC -77

* MWD1 A, Sig=270, 4 Ref=450.80 (CT101\17050403.D-CT101\17050402.D)

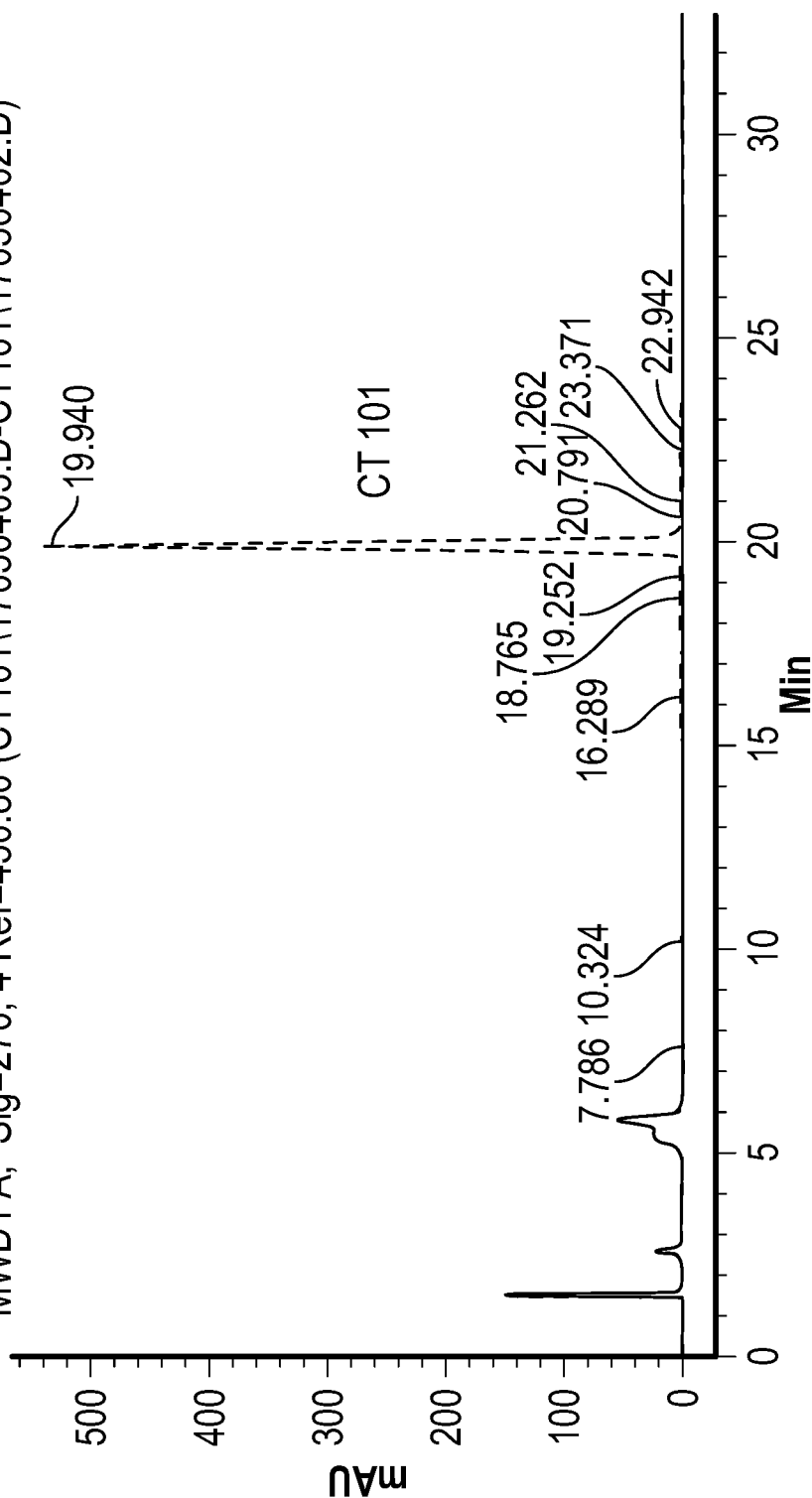


FIG. 85

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=270, 4 Ref=450, 80

Signal has been Modified After Loading from Rawdata File !

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*S]	Height [mAU]	Area %
1	7.786	BV	0.2061	13.85569	9.17872e-1	0.1900
2	10.324	BV	0.0906	9.89414	1.68312	0.1357
3	16.289	BV	0.6257	139.58029	2.61920	1.9137
4	18.765	BV	0.4822	95.96288	2.43282	1.3157
5	19.252	VB	0.1608	17.46975	1.46804	0.2395
6	19.940	BV	0.1985	6857.43359	538.90332	94.0190
7	20.791	VB	0.2150	37.69450	2.35175	0.5168
8	21.262	BB	0.3315	58.32554	2.33466	0.7997
9	22.371	BV	0.4514	47.07911	1.25560	0.6455
10	22.942	VB	0.1991	16.37336	1.10355	0.2245
Totals :				7293.66885	555.06993	

FIG. 85
(Continued)

CT101 Colonna Zorbax Eclipse XDB-C18 150x3.0 mm 5 micron P.N. 993967-302; S.N. USTD002295 AN-HPLC -77

* MWD1 A, Sig=270, 4 Ref=450.80 (CT101\17050406.D- CT101\17050402.D)

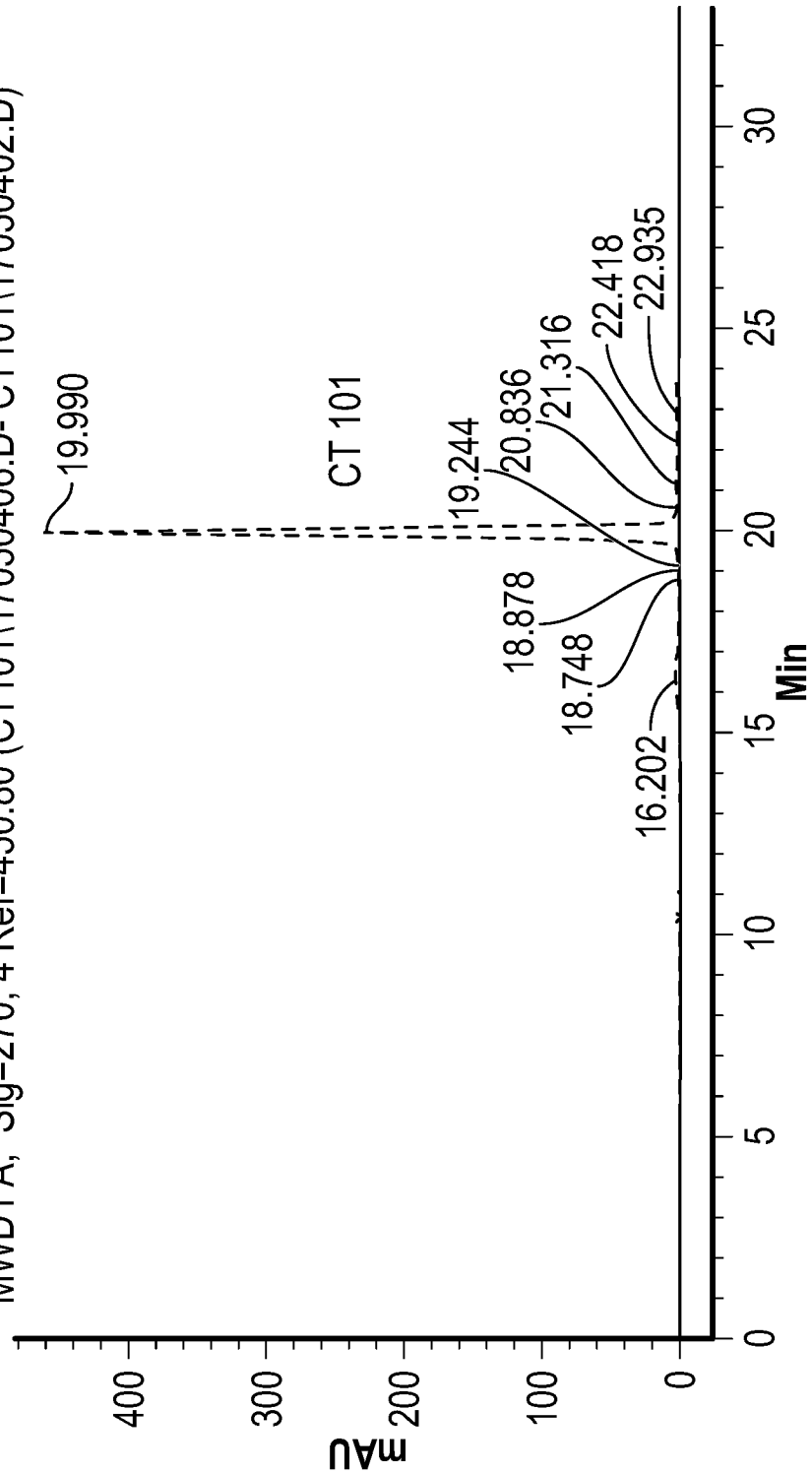


FIG. 86

Area Percent Report

Sorted By : Signal

Multiplier : 1.0000

Dilution : 1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=270, 4 Ref=450, 80

Signal has been Modified After Loading from Rawdata File !

Peak #	RetTime [min]	Type	Width [min]	Area [mAU* S]	Height [mAU]	Area %
1	16.202	BV	0.6090	59.21445	1.15325	0.9546
2	18.742	BV	0.3040	45.57183	1.91238	0.7347
3	18.873	VV	0.2102	29.72121	1.82313	0.4792
4	19.244	VB	0.1562	13.57882	1.18032	0.2189
5	19.990	BV	0.2016	5959.24365	458.66861	96.0723
6	20.836	VV	0.2269	28.82417	1.72497	0.4647
7	21.316	VB	0.2772	33.82668	1.73730	0.5453
8	22.418	BV	0.3318	23.15733	8.34316e-1	0.3733
9	22.935	VB	0.1614	9.73695	8.38850e-1	0.1570
Totals :				6202.87508	469.87313	

FIG. 86
(Continued)

Sample Name

Method

Project

Comment

Cartridge

Flowrate

Solvent A

Solvent B

Rack Type

Max Fraction Volume

Dispense Order

Initial Waste

2016CG07-S4 Pur4

SNAP C18 400g CT101 rev1

6 g Di Grezzo in ca. 21 ml di H2O

SNAP C18 400g

100 ml/min

Water

Acetonitrile

240 ml

150 ml

S

4.98CV

Detection Mode

UV1 (Collection)

UV2 (Collection)

UV1+UV2

210 nm

270 nm

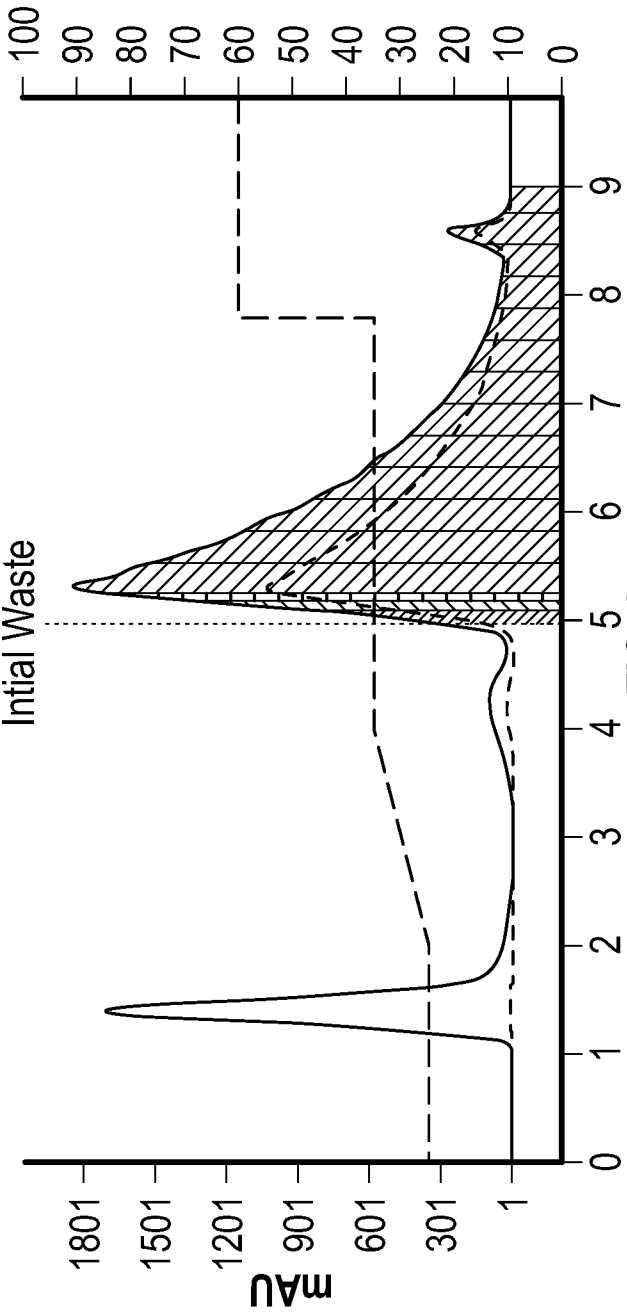


FIG. 87

Gradient	Flowrate 100 ml/min		
	Solvents	Mix	Length (CV)
Equil.	A/B	24%	3.0
1	A/B	24%	2.0
2	A/B	24% - 34%	2.0
3	A/B	34%	3.8
4	A/B	60%	2.0

FIG. 87
(Continued)

Metodo HPLC X CT101

Zobox Eclipse XBD - C18 Solvent Saver (3 x 150 mm x 5um)

P.N. 993967-302

S.N.USTD002293

* DAD1 A, Sig=270, 4 Ref=450.80 (CT 10.(1705106.D-CT 101\1705.D)

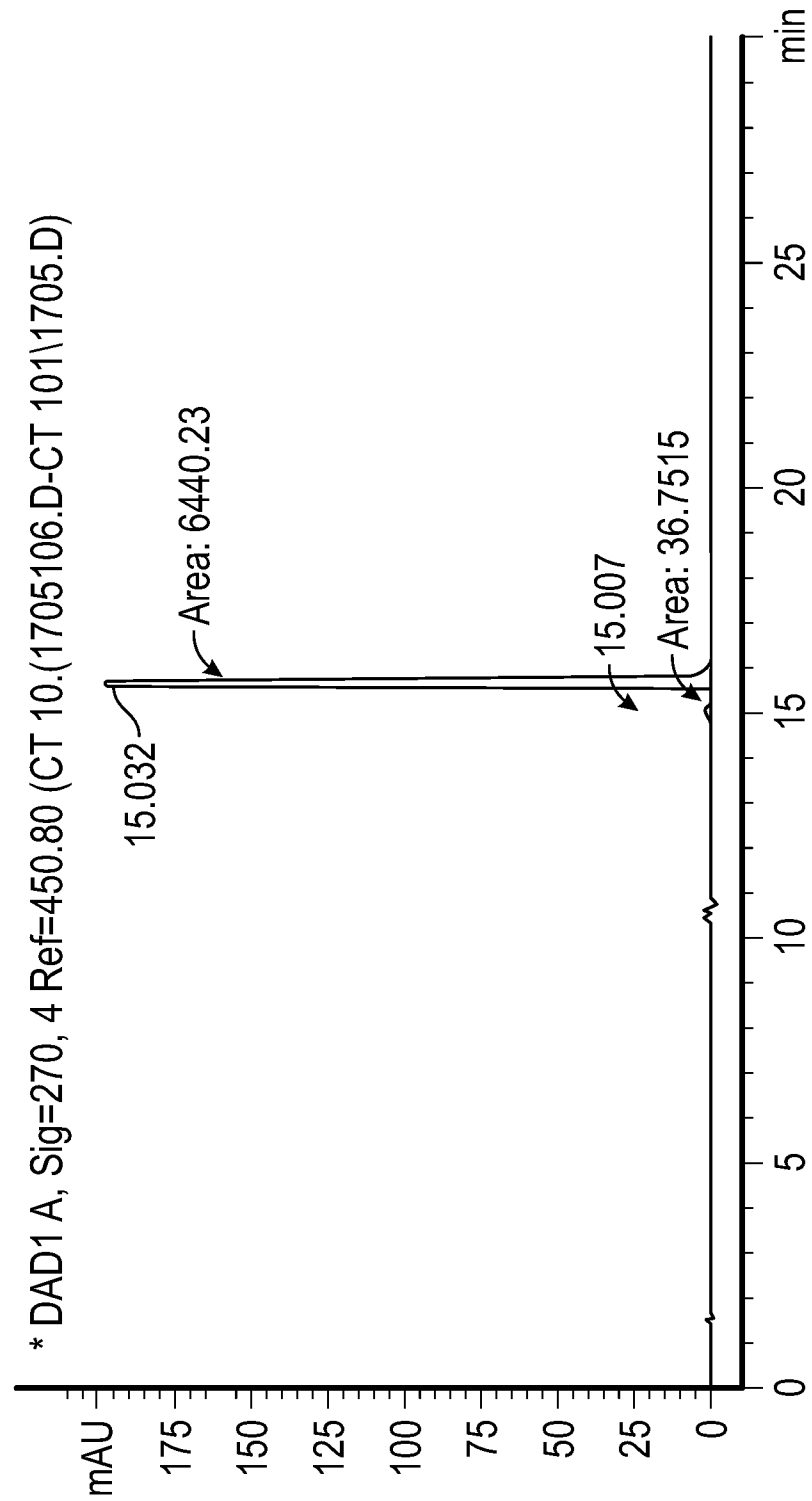


FIG. 88

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor With ISTDs

Signal 1 : DAD1 A, SIG=270 4 Ref=450,80
Signal has been Modified After Loading from Rawdata File !

Peak #	RetTime [min]	Type	Width [min]	Area [mAU* S]	Height [mAU]	Area %
1	15.007	MF	0.3692	36.75152	1.65888	0.5674
2	15.632	MF	0.0969	6440.22559	1108.7825	99.4326
Totals :				6476.97711	1109.73713	

FIG. 88
(Continued)

CT101 Lot 2017GC07/S4

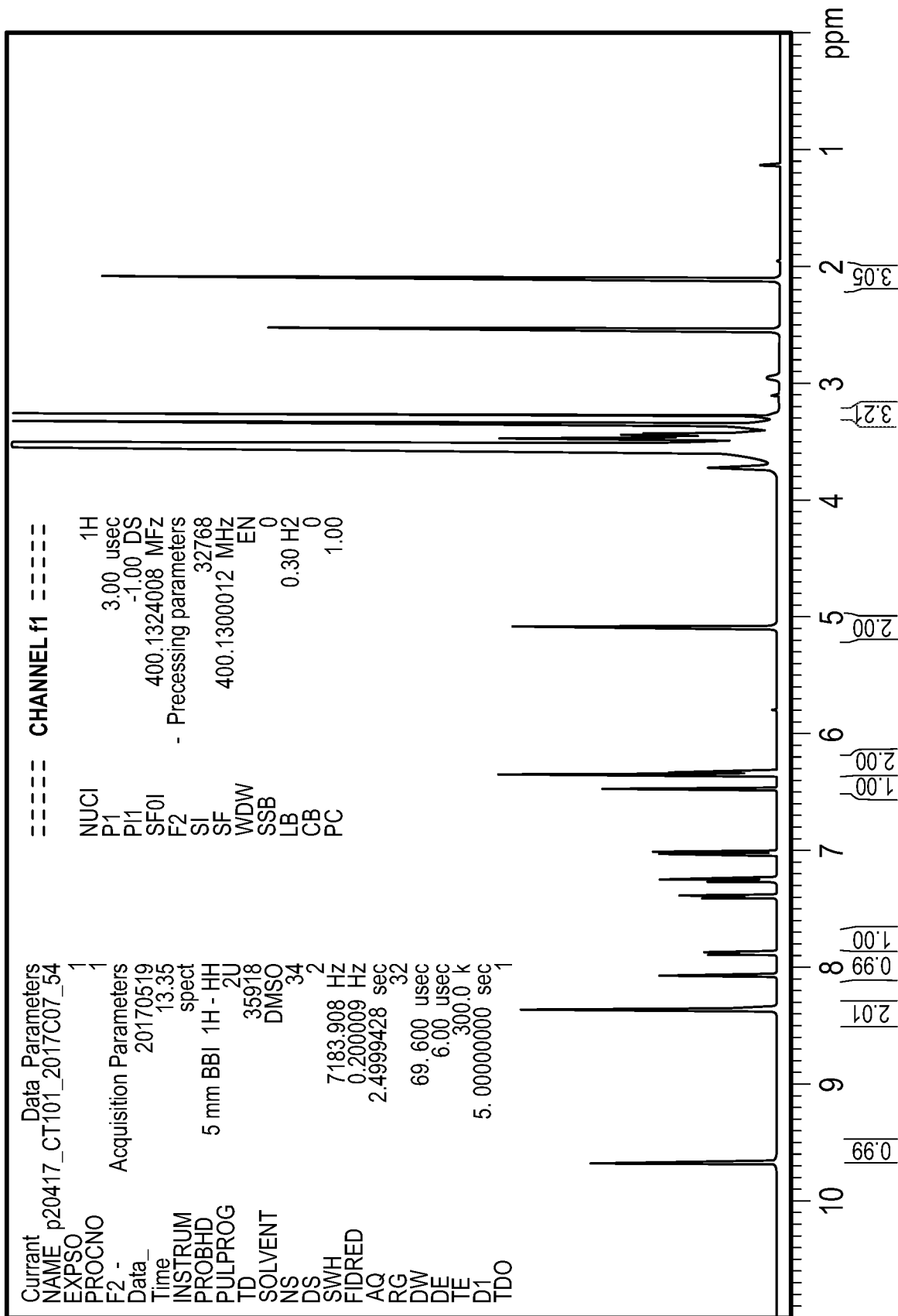


FIG. 89

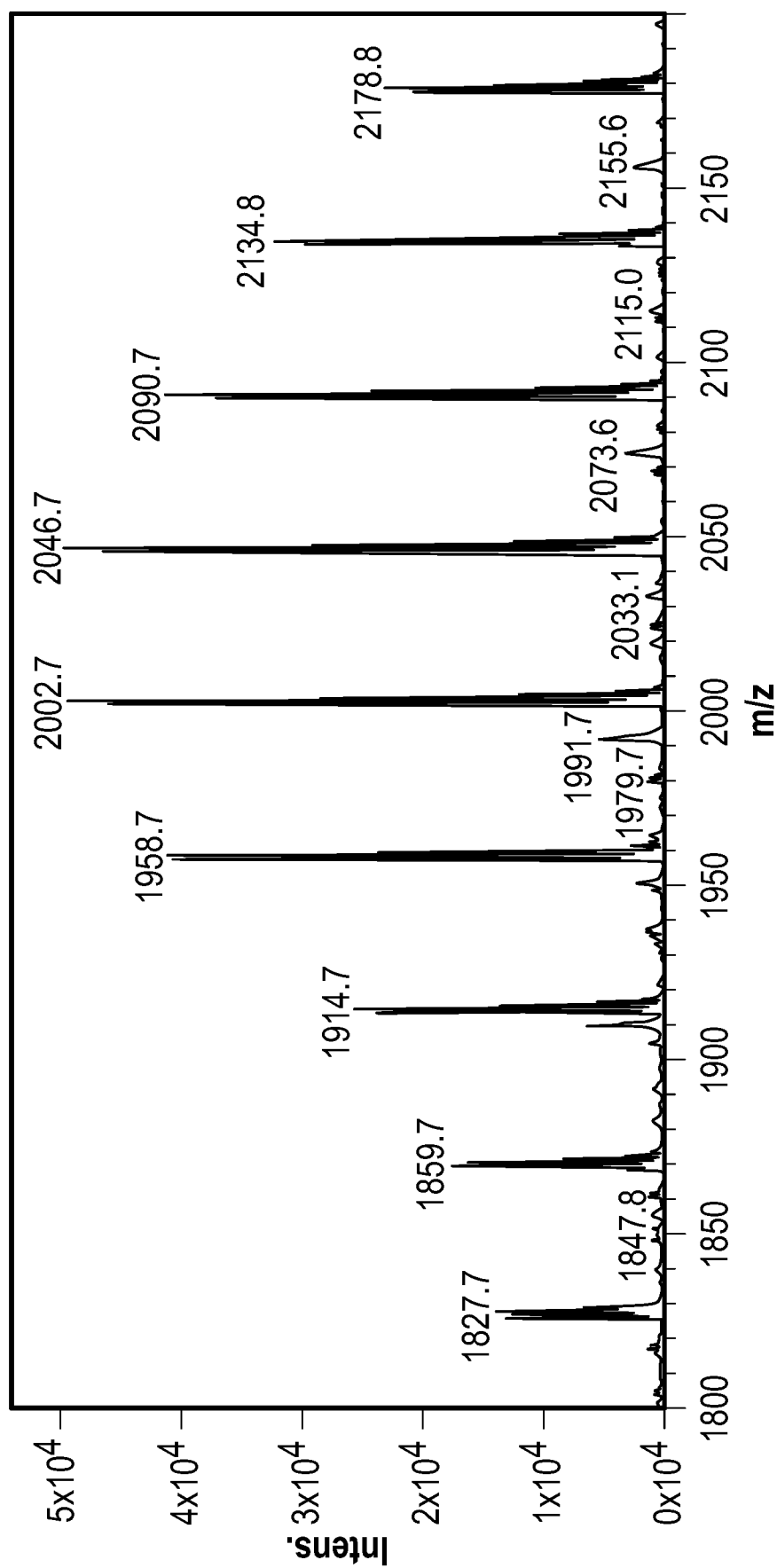


FIG. 90

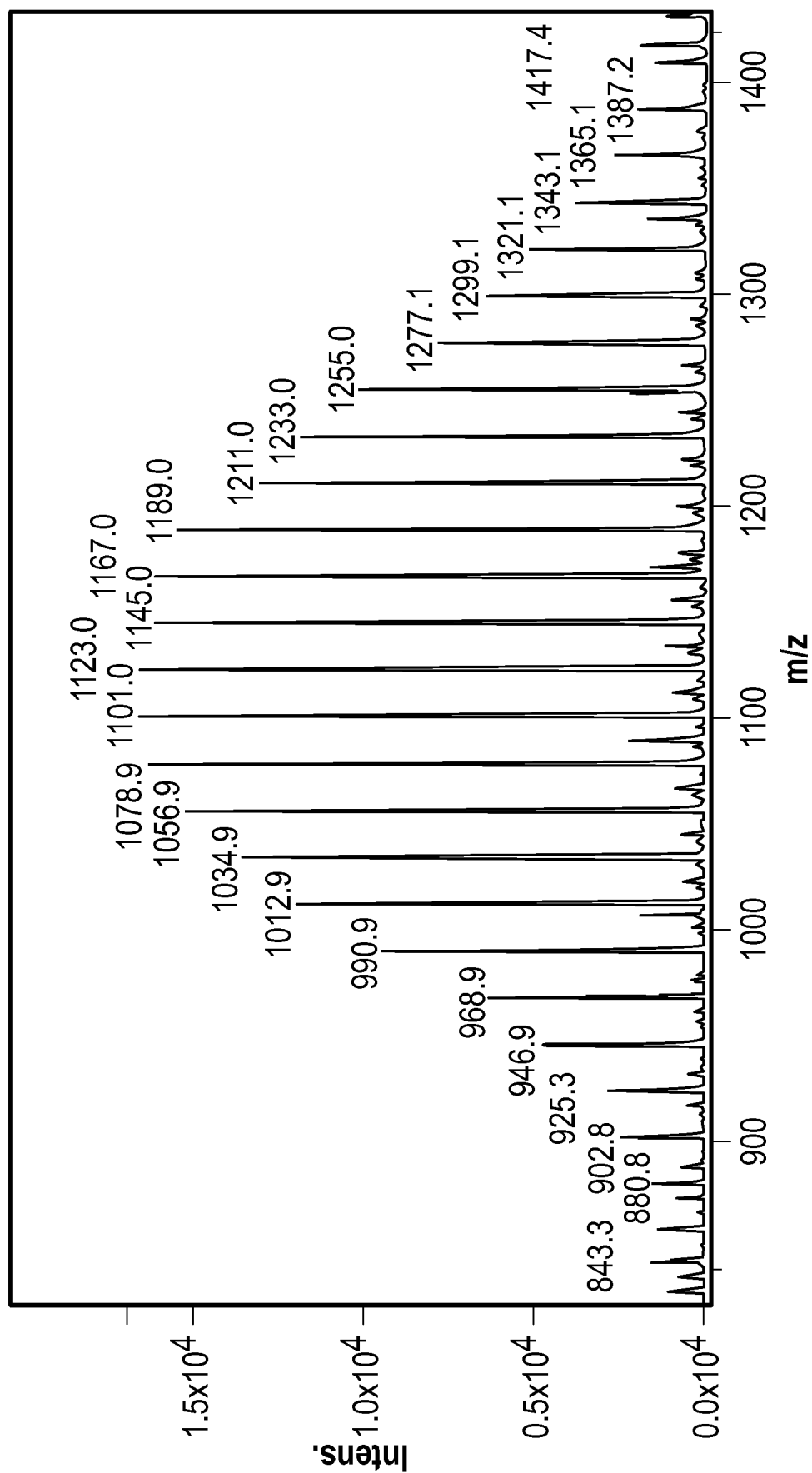


FIG. 91

Test Report n. 2017/022
CT101

Product : (3-({4-[(5-hydroxy-2-methylphenyl)amino]pyrimidin-2-yl}amino)-N-mPEG₂₀₀₀benzamide)

Formula : C₁₈H₁₆N₅O₂-[C₂H₄O]_n-CH₃ **Mol. Weight:** 2282 (*)

Cas n°: ***** **Quantity:** 15,25 g

Lot number: 2017CG07/S4

Parameter	Unit	Results	Anal. Meth.
Aspect		Off white Crystalline Powder	Visual
NMR Spectrum		Conform to Structure	
Purity (HPLC,270 nm)	% Area	99,43	MI CT101 002
Impurities (HPLC,270 nm)			MI CT101 002
Unknown Rrt 0,90	% Area	0,04	

(*) Based on M_w = 1964 Da of MeO-PEG-NH₂

Note: Purity Chromatogram and ¹H-NMR Spectrum Enclosed

FIG. 92

Metodo HPLC X CT101
Zobox ECLIPCE XBD-C18 Solvent Saver (3 x 150 mm x 5µm)
P.N. 993967-302
S.N.USTD002293

* DAD1 A, Sig=270, 4 Ref=450, 80 (CT101\17042105.D-CT101\17042105.D

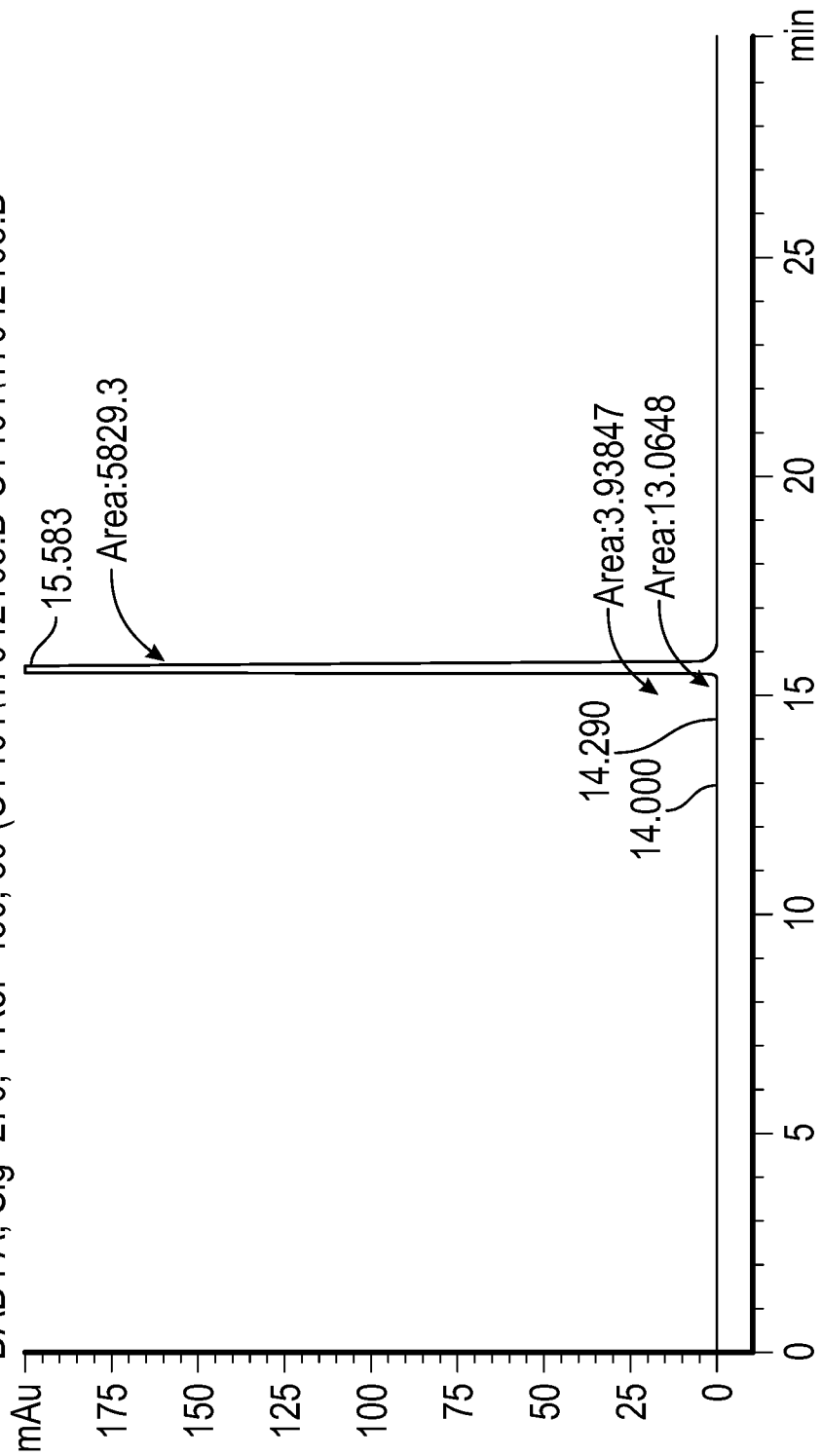


FIG. 93

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor With ISTDs

Signal 1 : DAD1 A, Sig=270 4 Ref=450, 80
Signal has been modified after loading from Rawdata file

Peak #	RetTime [min]	Type	Width [min]	Area [mAU* S]	Height [mAU]	Area %
1	14.000	MF	0.1147	2.40518	3.48348e-1	0.0411
2	14.290	MF	0.0870	3.93847	7.54502e-1	0.0673
3	14.974	MF	0.3245	13.06479	6.70968e-1	0.2234
4	15.583	FM	0.0973	5829.30078	998.9289	99.6682
Totals :				5848.70922	1000.67771	

FIG. 93
(Continued)

CT101 1ot 2017GC07/S2A

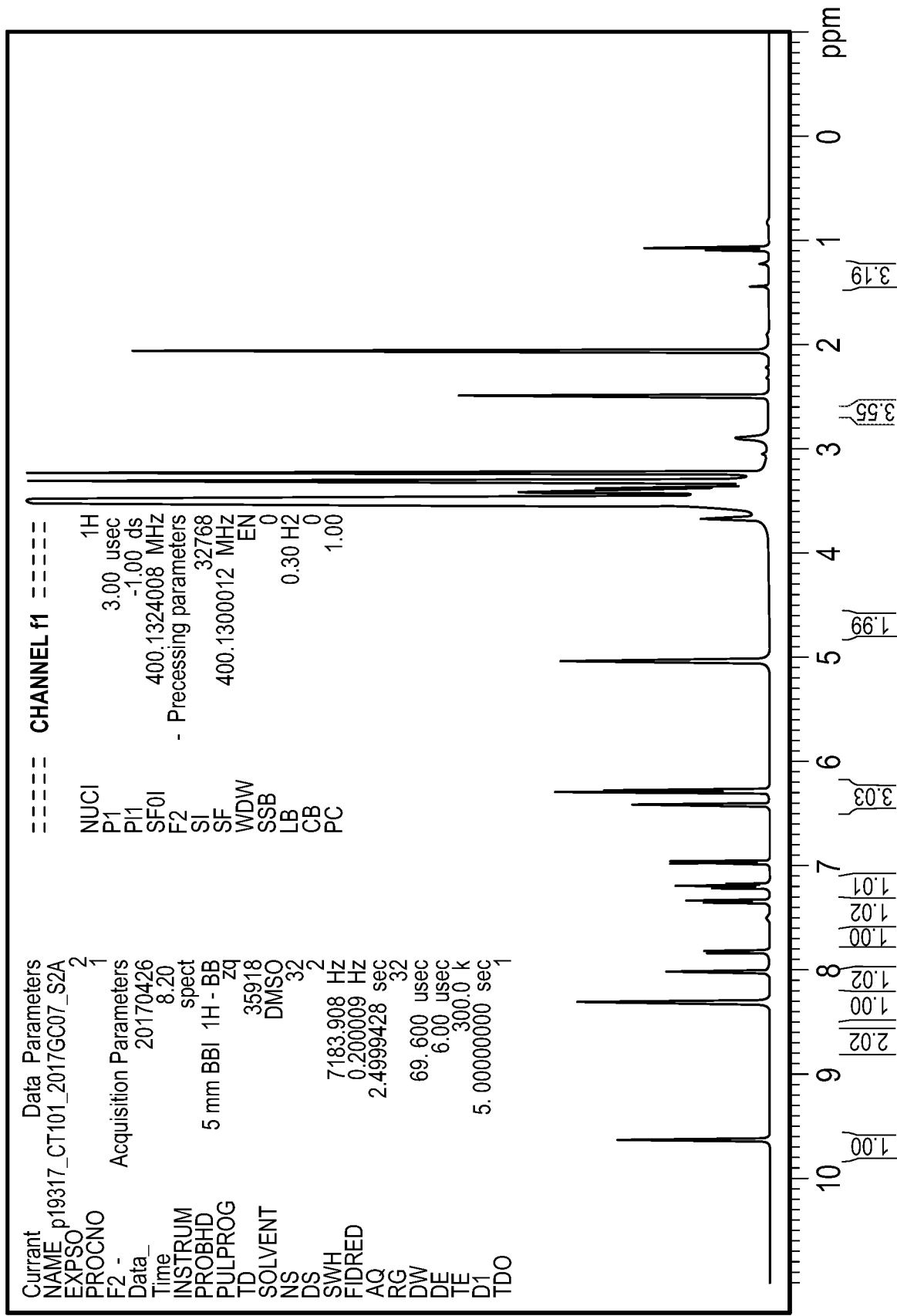


FIG. 94

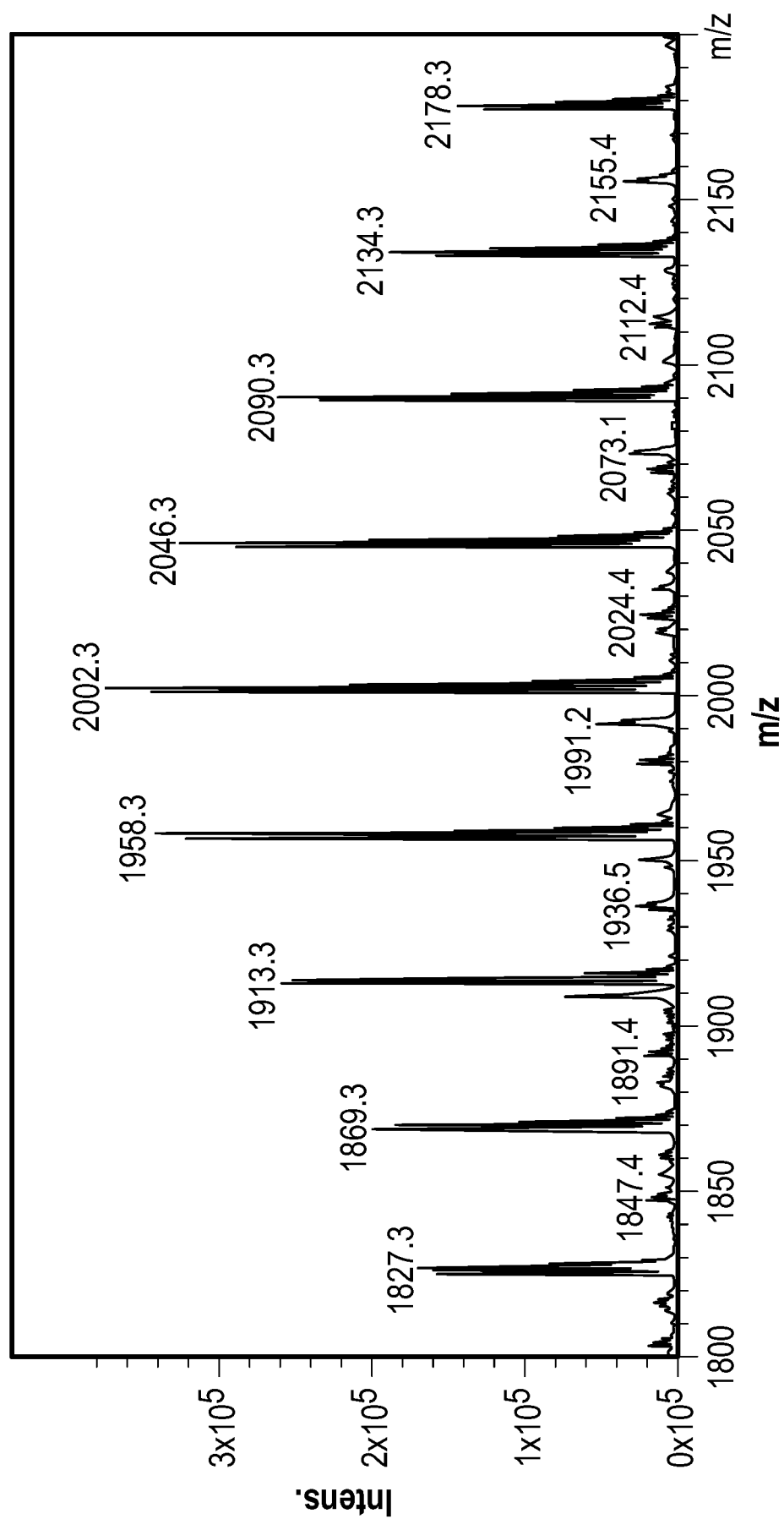


FIG. 95

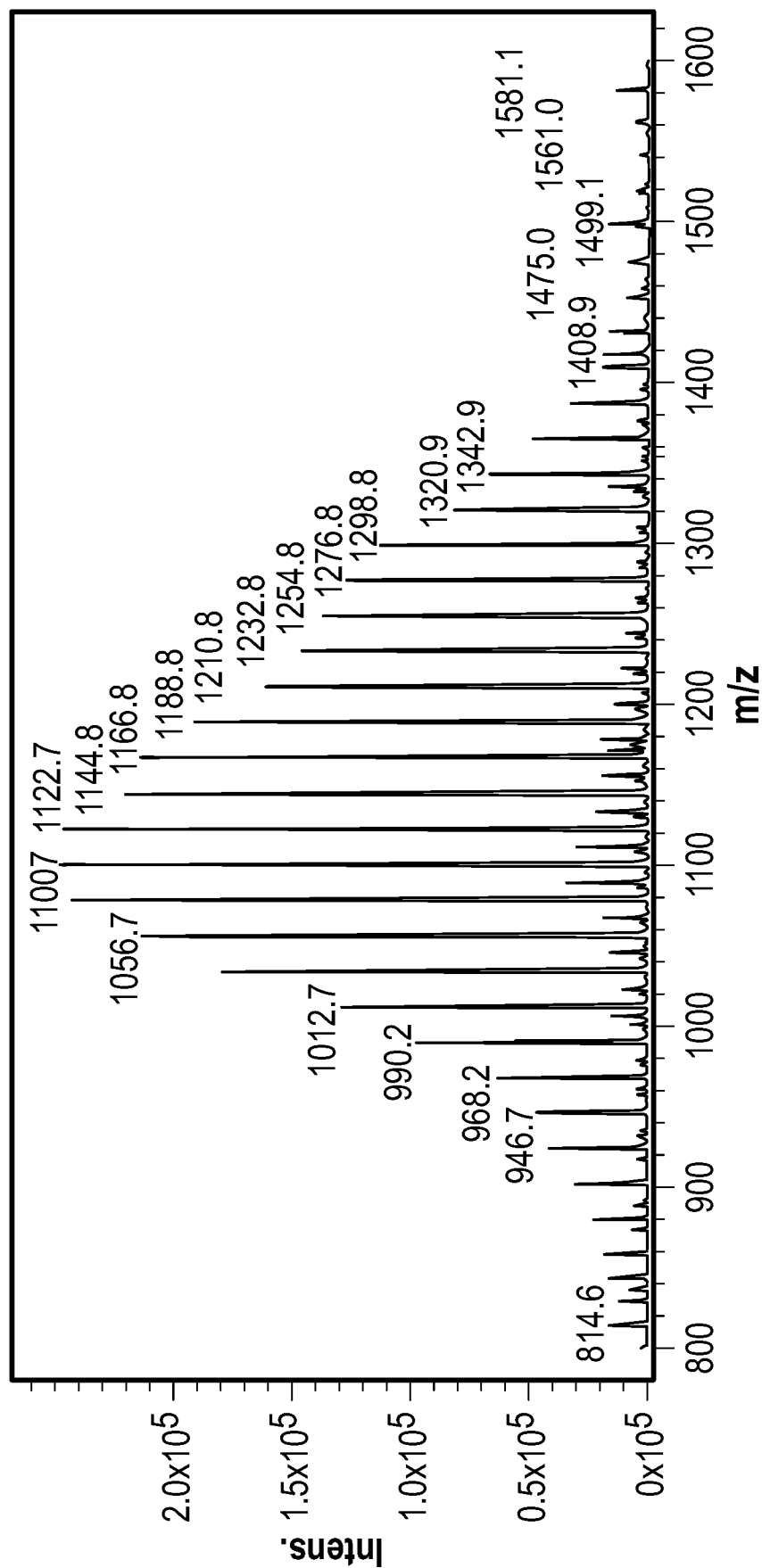


FIG. 96

Test Report n. 2017/022
CT101**Product :** (3-({4-[(5-hydroxy-2-methylphenyl)amino]pyrimidin-2-yl}amino)-N-mPEG₂₀₀₀benzamide)**Formula :** $C_{18}H_{16}N_5O_2-[C_2H_4O]_n-CH_3$ **Mol. Weight:** 2282 (*)**Cas n°:** ***** **Quantity:** 1,20 g**Lot number:** 2017CG07/S2A

Parameter	Unit	Results	Anal. Meth.
Aspect		Off white Crystalline Powder	Visual
NMR Spectrum		Conform to Structure	
Purity (HPLC,270 nm)	% Area	99,67	MI CT101 002
Impurities (HPLC,270 nm)			MI CT101 002
Unknown Rrt 0,90	% Area	0,04	
Unknown Rrt 0,92	% Area	0,07	
Unknown Rrt 0,96	% Area	0,22	

(*) Based on $M_w = 1964$ Da of MeO-PEG-NH₂Note: Purity Chromatogram and ¹H-NMR Spectrum Enclosed**FIG. 97**

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/023109

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/60 A61P11/00 A61P1/00 A61P17/00 A61P27/02
ADD.

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)

A61K

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

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

EP0-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BAMBOROUGH ET AL: "N-4-Pyrimidinyl-1H-indazol-4-amine inhibitors of Lck: Indazoles as phenol isosteres with improved pharmacokinetics", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 17, no. 15, 1 August 2007 (2007-08-01), pages 4363-4368, XP022144705, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2007.04.029	1-38,42, 44,47, 48,52, 54,57
A	page 4361; table 1; compound 2 ----- -/--	39-41, 43,45, 46, 49-51, 53,55, 56,58



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" 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)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" 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

"X" 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

"Y" 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

Date of the actual completion of the international search

28 May 2018

Date of mailing of the international search report

02/08/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Trifilieff-Riolo, S

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2018/023109

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	POLI ET AL.: "IDENTIFICATION OF NEW FYN KINASE INHIBITORS USING A FLAP-BASED APPROACH", CHEMICAL INFORMATION AND MODELING, vol. 53, 2013, pages 2538-2547, XP002781399,	1-38,42, 44,47, 48,52, 54,57
A	page 2539 - page 2540; figure 2; compound 5	39-41, 43,45, 46, 49-51, 53,55, 56,58
Y	----- MISHRA ET AL: "PEGYLATION IN ANTI-CANCER THERAPY: AN OVERVIEW", ASIAN JOURNAL OF PHARMACEUTICAL SCIENCES,, vol. 11, no. 3, 1 January 2016 (2016-01-01), pages 337-348, XP002781148,	1-38,42, 44,47, 48,52, 54,57
A	the whole document	39-41, 43,45, 46, 49-51, 53,55, 56,58

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/023109

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 2, 5, 6(completely); 3, 4, 7-58(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US2018/ 023109

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 2, 5, 6(completely); 3, 4, 7-58(partially)

compound SNA-101 (= CT 101), its use to treat following diseases: skin, lung, gastrointestinal system, eye, joint, autoimmune, inflammatory condition

2. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 2 as active entity to treat "a cell" or skin, lung, gastrointestinal, eye, joint, autoimmune or inflammatory diseases

3. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 3 as active entity to treat "a cell" or skin, lung, gastrointestinal, eye, joint, autoimmune or inflammatory diseases

4. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 4 as active entity to treat "a cell" or skin, lung, gastrointestinal, eye, joint, autoimmune or inflammatory diseases

5. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 5 as active entity to treat "a cell" or skin, lung, gastrointestinal, eye, joint, autoimmune or inflammatory diseases

6. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 6 as active entity to treat "a cell" or skin, lung, gastrointestinal, eye, joint, autoimmune or inflammatory diseases

7. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 7 as active entity to treat "a cell" or skin, lung, gastrointestinal, eye, joint, autoimmune or inflammatory diseases

8. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 8 as active entity to treat "a

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US2018/ 023109

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

9. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 9 as active entity to treat "a
cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

10. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 10 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

11. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 11 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

12. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 12 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

13. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 13 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

14. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 14 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

15. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 15 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

16. claims: 3, 4, 7-58(all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

conjugate comprising compound 16 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

17. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 17 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

18. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 18 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

19. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 19 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

20. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 20 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

21. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 21 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

22. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 22 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

23. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 23 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

24. claims: 3, 4, 7-58(all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

conjugate comprising compound 24 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

25. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 25 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

26. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 26 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

27. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 27 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

28. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 28 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

29. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 29 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

30. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 30 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

31. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 31 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

32. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 32 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

33. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 33 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

34. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 34 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

35. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 35 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

36. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 36 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

37. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 37 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

38. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 38 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

39. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 39 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

40. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 40 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

41. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 41 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

42. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 42 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

43. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 43 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

44. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 44 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

45. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 45 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

46. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 46 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

47. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 47 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

48. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 48 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

49. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 49 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

50. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 50 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

51. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 51 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

52. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 52 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

53. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 53 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

54. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 54 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

55. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 55 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

autoimmune or inflammatory diseases

56. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 56 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

57. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 57 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

58. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 58 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

59. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 59 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

60. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 60 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

61. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 61 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

62. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 62 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

63. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 63 as active entity to treat

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US2018/ 023109

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

64. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 64 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

65. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 65 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

66. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 66 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

67. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 67 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

68. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 68 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

69. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 69 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

70. claims: 3, 4, 7-58(all partially)

conjugate comprising compound 70 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases

71. claims: 3, 4, 7-58(all partially)

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US2018/ 023109

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

conjugate comprising compound 71 as active entity to treat
"a cell" or skin, lung, gastrointestinal, eye, joint,
autoimmune or inflammatory diseases
