(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 November 2017 (23.11.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/201296 A1

(51) International Patent Classification:
A61K 47/14 (2017.01) *A61K 8/38* (2006.01)
A61K 47/44 (2017.01)

(21) International Application Number:
PCT/US2017/033346

(22) International Filing Date:
18 May 2017 (18.05.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/338,334 18 May 2016 (18.05.2016) US
62/470,130 10 March 2017 (10.03.2017) US

(71) Applicant: **DERMALA INC.** [US/US]; J-Labs, 3210 Merryfield Row, San Diego, CA 92121 (US).

(72) Inventors: **RASOCHOVA, Lada**; 2529 Via Pisa, Del Mar, CA 92014 (US). **KEM, Michelle**; 7525 Charmant Drive, Unit 106, San Diego, CA 92122 (US).

(74) Agent: **BUCKLEY, Kevin, W.**; Torrey Pines Law Group, P.C., 12636 High Bluff Drive, Suite 400, Torrey Pines Law Group, P.C., San Diego, CA 92130 (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, 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, 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,

(54) Title: COMPOSITIONS AND METHODS FOR TREATING ACNE VULGARIS

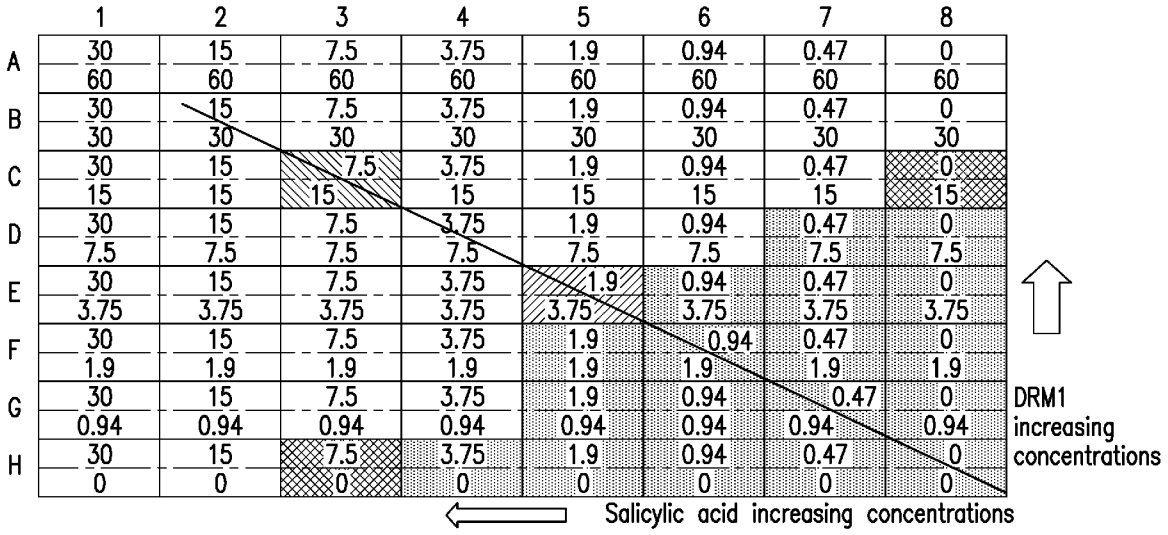


FIG. 1

(57) Abstract: Disclosed are compositions, methods of treatment using the compositions and methods of preparing the compositions for the treatment of acne vulgaris. The compositions include succinic acid and an API selected from the group consisting of salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, antibiotic, retinoid and combinations thereof in a pharmaceutically acceptable preparation. The compositions that include the combination of succinic acid and another API produce improved efficacy in treating acne vulgaris.

WO 2017/201296 A1

[Continued on next page]

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

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))*

COMPOSITIONS AND METHODS FOR TREATING ACNE VULGARIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application Serial No. 62/338,334 filed on May 18, 2016, and U.S. Provisional Application Serial No. 62/470,130 filed on March 10, 2017, each of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

FIELD

[0003] The present invention is related to compositions and methods for the treatment of acne vulgaris and, in particular, to topical compositions and methods based upon compositions that are combinations of succinic acid and one or more of topical API including retinoid, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, or antibiotic, in a pharmaceutically acceptable preparation.

INTRODUCTION

[0004] Acne is a significant inflammatory skin disorder and it is considered the most common skin disorder. There are four disease factors associated with acne vulgaris. They include sebum overproduction, follicular hyperkeratinization, *Propionibacterium acnes* (*P. acnes*) proliferation in hair follicles, and inflammation. Acne affects about 50 million people in the US. It is highly common among adolescents, affecting up to 95% of men and 83% of women in that age group. In approximately 10% of cases, acne has been reported to become severe nodulocystic acne with the potential of producing lifelong disfiguring scars. Acne lesions typically recur for years. Therefore, acne is considered a chronic disease.

[0005] The psychosocial morbidity associated with acne is important and includes depression and suicidal thoughts. In addition to impact on patient's quality of life, the economic burden of acne treatment is significant.

[0006] Currently available treatments for acne are insufficient. Treatment options against acne vulgaris typically only address one or two disease factors - *P. acnes* proliferation and follicular hyperkeratinization. Treatment options for acne vulgaris suffer from significant side effects and none provides complete protection. Potential side effects include skin irritation including burning, erythema, peeling, dryness, and contact allergy. Antibiotics pose various side effects that range from colitis, vaginal candidiasis, and photosensitivity to the development of bacterial resistance and cross-resistance.

[0007] Thus, there is a need to develop new therapies with reduced side effects and therapies that address multiple acne vulgaris disease factors – *P. acnes* proliferation, follicular hyperkeratinization, and inflammation.

SUMMARY

[0008] Accordingly, the applicants herein have succeeded in devising new formulations and methods for treating acne vulgaris.

[0009] Thus, in various embodiments, the present invention includes a topical composition for treating acne vulgaris in which the composition includes a combination of succinic acid and salicylic acid in a pharmaceutically acceptable preparation. The combination of succinic acid and salicylic acid acts in a synergistic manner in killing *Propionibacterium acnes* (*P. acnes*) and treating acne vulgaris, requires ¼ of the dose of succinic acid and salicylic acid to be effective in killing *P. acnes* and treat acne vulgaris and it is faster acting compared to succinic acid or salicylic acid alone. In various other embodiments, the present invention includes a topical composition for treating acne vulgaris in which the composition includes a combination of succinic acid and an API that may be topical retinoid, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, antibiotics or combinations thereof, in a pharmaceutically acceptable preparation. The combination of succinic acid and BPO, succinic acid and azelaic acid, and succinic acid and picolinic acid acts in additive manner against *P. acnes* and requires ½ of the dose of individual components to kill *P. acnes* and treat acne vulgaris. The combination of succinic acid and antibiotic prevents antibiotic resistance development and is effective in treating acne vulgaris associated with antibiotic resistant *P. acnes*. Any combinations containing succinic acid prevent *P. acnes* biofilm development in follicles.

[0010] In various other embodiments, the present invention includes a method of treating acne vulgaris. The method includes administering to a subject in need thereof, a topical composition comprising a combination of succinic acid and another API, such as

retinoid, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, or antibiotic, in a pharmaceutically acceptable preparation.

[0011] In various additional embodiments, the present invention includes a method of preparing a topical formulation for treatment of acne vulgaris. The method includes combining succinic acid with another API in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and another API produces additional effect in treating acne vulgaris, including synergistic or additive effect against *P. acnes*, prevention of antibiotic resistance development, prevention of *P. acnes* biofilm formation, or faster acting pharmaceutical composition.

[0012] In various embodiments, the present invention includes a topical composition for treating acne vulgaris. The composition may include succinic acid and an API that may be topical retinoid, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, antibiotics or combinations thereof, in a pharmaceutically acceptable preparation.

[0013] In various other embodiments, the present invention includes a method of treating acne vulgaris. The method includes administer to a subject in need thereof, a topical composition that may include succinic acid and an API selected from the group consisting of topical retinoids, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, clindamycin and combinations thereof in a pharmaceutically acceptable preparation.

[0014] In various other embodiments, the present invention includes a method of treating inflammatory acne vulgaris that contains a topical composition that includes succinic acid and another component API selected from the group consisting of topical retinoids, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, clindamycin and combinations thereof in a pharmaceutically acceptable preparation.

[0015] In various additional embodiments, the present invention includes a method of preparing a topical formulation for treatment of acne vulgaris. The method includes combining succinic acid and an API selected from the group consisting of retinoids, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, antibiotics, and combinations thereof in a pharmaceutically acceptable preparation.

[0016] In various embodiments, the compositions and preparations above can be provided to a subject in a pad, tube, and any container suitable for the delivery of the compositions and preparations so a subject.

[0017] These and other features, aspects and advantages of the present teachings will become better understood with reference to the following description, examples and appended claims.

DRAWINGS

[0018] Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0019] Figure 1 illustrates checkerboard assay using *P. acnes* for concentrations (mM) of succinic acid and salicylic acid used in each well in which the boxes highlighted in yellow (in the applications from which this application depends; C8 and H3 here) are the individual MIC values for each API; the green box (in the applications from which this application depends; C3) indicates where the concentrations of the individual MIC values intersect; the orange box (in the applications from which this application depends; E5) indicates the new MIC value obtained by the combination of succinic acid and salicylic acid; the boxes shaded in grey indicate bacterial growth; and the white boxes indicate no growth.

[0020] Figure 2 illustrates the ratios of salicylic acid to succinic acid determined by checkerboard assay using *P. acnes* in which the boxes highlighted in yellow (in the applications from which this application depends; C8 and H3 here) are the individual MIC values for each API; the green box (in the applications from which this application depends; C3) indicates where the concentrations of the individual MIC values for each API intersect; the orange box (in the applications from which this application depends; E5) indicates the new MIC value obtained by the combination of succinic acid and salicylic acid; the boxes shaded in grey indicate bacterial growth; and the white boxes indicate no growth.

[0021] Figure 3 illustrates a time-kill assay comparing succinic acid individually to succinic acid and salicylic acid combination in which each plate shows four dilutions for each time point starting with 1:10 (right) and ending with $1:10^4$ (left).

[0022] Figure 4 illustrates checkerboard assay using *P. acnes* for concentrations (mM) of succinic acid and azelaic acid used in each well in which the boxes highlighted in yellow (in the applications from which this application depends; H3 and C8 here) are the individual MIC values for each API; the green box (in the applications from which this application depends; C3) indicates where the concentrations of the individual MIC values intersect; the orange box (in the applications from which this application depends;

D4) indicates the new MIC value obtained by the combination of succinic acid and azelaic acid; the boxes shaded in grey indicate bacterial growth; and the white boxes indicate no growth.

[0023] Figure 5 illustrates the ratios of azelaic acid to succinic acid determined by checkerboard assay using *P. acnes* in which the boxes highlighted in yellow (in the applications from which this application depends; H3 and C8 here) are the individual MIC values for each API; the green box (in the applications from which this application depends; C3) indicates where the concentrations of the individual MIC values intersect; the orange box (in the applications from which this application depends; D4) indicates the new MIC value obtained by the combination of succinic acid and azelaic acid; the boxes shaded in grey indicate bacterial growth; and the white boxes indicate no growth.

[0024] Figure 6 illustrates checkerboard assay using *P. acnes* for concentrations (mM) of succinic acid and picolinic acid used in each well in which the boxes highlighted in yellow (in the applications from which this application depends; H3 and C8 here) are the individual MIC values for each API; the green box (in the applications from which this application depends; C3) indicates where the concentrations of the individual MIC values intersect and the orange box (in the applications from which this application depends; D4) indicates the new MIC value obtained by the combination of succinic acid and picolinic acid. The boxes shaded in grey indicate bacterial growth and white boxes indicate no growth.

[0025] Figure 7 illustrates the ratios of picolinic acid to succinic acid determined by checkerboard assay in which the boxes highlighted in yellow (in the applications from which this application depends; H3 and C8 here) are the individual MIC values for each drug; the green box (in the applications from which this application depends; C3) indicates where the concentrations of the individual MIC values intersect; the orange box (in the applications from which this application depends; D4) indicates the new MIC value obtained by the combination of succinic acid and picolinic acid; the boxes shaded in grey indicate bacterial growth; and the white boxes indicate no growth.

[0026] Figure 8 illustrates checkerboard assay using *P. acnes* for concentrations (mM) of succinic acid and benzoyl peroxide used in each well in which the boxes highlighted in yellow (in the applications from which this application depends; H2 and C8 here) are the individual MIC values for each API; the green box (in the applications from which this application depends; C2) indicates where the concentrations of the individual MIC values intersect; the orange box (in the applications from which this

application depends; D3) indicates the new MIC value obtained by the combination of succinic acid and benzoyl peroxide; the boxes shaded in grey indicate bacterial growth; and the white boxes indicate no growth.

[0027] Figure 9 illustrates the ratios of benzoyl peroxide to succinic acid determined by checkerboard assay in which the boxes highlighted in yellow (in the applications from which this application dependsH2 and C8 here) are the individual MIC values for each API; the green box (in the applications from which this application depends; C2) indicates where the concentrations of the individual MIC values intersect; the orange box (in the applications from which this application depends; D3) indicates the new MIC value obtained by the combination of succinic acid and benzoyl peroxide; the boxes shaded in grey indicate bacterial growth; and the white boxes indicate no growth.

[0028] Figure 10. illustrates checkerboard assay using *P. acnes* for concentrations (mM) of succinic acid and clindamycin used in each well in which the boxes highlighted in yellow (in the applications from which this application depends; H3 and C8 here) are the individual MIC values for each API; the green box (in the applications from which this application depends; C3) indicates where the concentrations of the individual MIC values intersect and the orange box indicates the new MIC value obtained by the combination of succinic acid and clindamycin; the boxes shaded in grey indicate bacterial growth; and the white boxes indicate no growth.

[0029] Figure 11 illustrates the ratios of clindamycin to succinic acid determined by checkerboard assay in which the boxes highlighted in yellow (in the applications from which this application depends; H3 and C8 here) are the individual MIC values for each API; the green box (in the applications from which this application depends; C3) indicates where the concentrations of the individual MIC values intersect; the boxes shaded in grey indicate bacterial growth; and the white boxes indicate no growth.

[0030] Figure 12 illustrates that succinic acid kills antibiotic resistant *P. acnes* strains in assay in which succinic acid was tested against *P. acnes* strains RMA# 20660 (black) and RMA# 20661 (grey) with resistance to erythromycin, clindamycin, minocycline and doxycycline: wherein the DRM1 (succinic acid) MIC value (determined at 15 mM for each strain) was defined as $\geq$ 90% decrease in bacterial growth as monitored at an optical density of 600 nm and the results reported as the mean of three individual experiments where ND means not detected and clindamycin showed no ability to inhibit RMA# 20660 or RMA# 20661 strains.

[0031] Figure 13 illustrates that *P. acnes* strains RMA# 20660 and RMA# 20661 but not *P. acnes* strain 6919 were resistant to clindamycin.

[0032] Figure 14 shows no resistance development in the presence of DRM1 (succinic acid).

[0033] Figure 15 shows that Formulation 1 and Formulation 2 inhibit *P. acnes* growth.

[0034] Figure 16 shows that Formulation 1 and Formulation 2 are bactericidal against *P. acnes*.

[0035] Figure 17 shows production of SE Microbiome Complex by S. epidermidis fermentation with selected prebiotic compounds.

[0036] Figure 18 shows healing of acne in a human subject after application of Formulation 1.

DETAILED DESCRIPTION

[0037] The present invention includes compositions and methods directed to the treatment of skin diseases, and in particular, inflammatory acne vulgaris. The compositions as well as treatment and formulation methods are based upon combinations of agents with antimicrobial, anti-hyperkeratinization, anti-comedogenic, and anti-inflammatory effects that are effective in treating acne vulgaris.

[0038] Abbreviations and Definitions

[0039] To facilitate understanding of the invention, a number of terms and abbreviations as used herein are defined below as follows:

[0040] As used herein, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a formulation" includes a plurality of such formulations and reference to "the method" includes reference to one or more methods and equivalents thereof known to those skilled in the art, and so forth.

[0041] As used herein, the term “about” is intended to refer to a range of values above and below a stated value such as for example, values encompassing 10% below up to 10% above a stated value.

[0042] The term “and/or” is intended to mean either or both of two recited elements.

[0043] "Active pharmaceutical ingredient" (“API”) refers to a substance, in particular an antimicrobial agent, in a pharmaceutical composition that is delivered for a desired effect.

[0044] As used herein, the term "combination" with respect to active agents refers to a composition of two or more active agents, in particular, agent with antimicrobial and/or anti-inflammatory and/or anti-hyperkeratinization and anti-commedogenic effects. In the present invention, a combination of active agents may include succinic acid and an API that may include retinoid, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, antibiotic, and combinations of two or more API's.

[0045] Reference herein to an API including, but not limited to succinic acid, retinoids, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide and antibiotics is intended to include pharmaceutically acceptable solvates, salts, hydrates or hydrated salts, their optical isomers, racemates, diastereomers, enantiomers or the polymorphic crystalline structures of the compounds.

[0046] In the Examples below, DRM1 refers to succinic acid.

[0047] The term "pharmaceutical composition" or 'pharmaceutical preparation" refers to a composition that combines one or more API's with a pharmaceutically acceptable carrier such that the composition is suitable for therapeutic use in vitro, in vivo or ex vivo.

[0048] As used herein, the term "pharmaceutically acceptable carrier" encompasses any suitable pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, various types of wetting agents and the like. The compositions also can include stabilizers and preservatives. Examples of carriers, stabilizers and adjuvants, can be found in Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins, Twenty-First edition (May 19, 2005).

[0049] A number of carrier systems have been developed, in particular for topical application, including by way of non-limiting examples, vesicular systems such as liposomes, niosomes, ethosomes and transfersomes. (Arora, S. et al., Dermal delivery of drugs using different, vesicular carriers: A comparative review, J Pharm. 2012;6:237-44).

[0050] One such vesicular carrier system is the niosome. As used herein, the term noisome refers to unilamellar or multilamellar vesicles in which an aqueous phase is encapsulated in highly ordered bilayer made up of nonionic surfactant (Vyas, J. et al., "Development of topical niosomal gel of benzoyl peroxide," International Journal of Nanotechnology, vol. 2011, Article ID 503158, 6 pages, 2011.).They are nonionic surfactant vesicles by which skin penetration and accumulation are increased in the superficial skin strata (Manconi, M. et al., Niosomes as carriers for tretinoin: III. A study

into the in vitro cutaneous delivery of vesicle-incorporated tretinoin, International Journal of Pharmaceutics, 2006 311(1-2):11–19.); (Vyas, A. et al., Carrier-Based Drug Delivery System for Treatment of Acne, The Scientific World Journal, 2014 Feb 9;2014:276260. doi: 10.1155/2014/276260. eCollection 2014.)

[0051] The term "synergy" as used herein is intended to refer the interaction of discrete antimicrobial agents in which the total antimicrobial effect is greater than the sum of the effects of the antimicrobial agent alone. Conversely, the term "antagonism" is intended to refer to an interaction of antimicrobial agents in which the total antimicrobial effect is less than the sum of the effects of the antimicrobial agents alone.

[0052] The term "checkerboard experimental design", is intended to refer to an in vitro two-dimensional array of serial concentrations of test compounds evaluated in paired or triple combinations for their affect in inhibiting the growth of a target microorganism, measured as a "minimal inhibitory concentration" (MIC), i.e. the lowest concentration that prevents of inhibits microbial growth. It is used as the basis for calculation of a "fractional inhibitory concentration index".

[0053] The term "fractional inhibitory concentration index" (FICI) refers to a value that compares the ratio of inhibitory concentration of a combination with that of individual agents separately. The formula used to calculate FICI is as follows:

$$FICI = MICA/B \,/\, MICA \text{ alone} + MICB/A \,/\, MICB \text{ alone}$$

[0054] The FIC Index (FICI) value is used to determine whether paired combinations of antimicrobial agents can exert inhibitory effects against tested organism that are more or less than the sum of their effects alone. In theory, FICI < 1.0 indicates synergy and FICI > 1.0) indicates antagonism. However, a more conservative calculation has been recommended such that FICI data may be interpreted as "synergy" for an FICI ≤ 0.5, "antagonism" for an FICI > 4.0 and "no interaction" interaction for an FICI > 0.5–4.0. (Odds, F.C., Synergy, antagonism, and what the chequerboard puts between them, Journal of Antimicrobial Chemotherapy (2003) 52, 1. As used herein, the FICI is interpreted as follows:

FICI $\leq$0.5 = synergy

FICI >0.5 – $\leq$1 = additive effect

FICI >1 – $\leq$4 = indifference

FICI > 4 = antagonism

[0055] Unless otherwise indicated, concentrations are given as mass weight percentages, i.e. w/w%. Mass weight percentages (w/w%) for combination formulations are calculated as follows:

mass % a = mass(a) ÷ (mass(a) + mass(b) + mass(c) + ...) x 100 (w/w%).

[0056] **Compositions**

[0057] The present invention includes compositions and treatment methods based upon compositions that are combinations of agents having antimicrobial, anti-inflammatory, anti-hyperkeratinization, and anti-commedogenic activities. Such compositions include succinic acid and an API selected from the group consisting of topical retinoids, salicylic acid, azelaic acid, picolinic acid, benzoyl peroxide, antibiotics and combinations thereof.

[0058] In various embodiments, the amount of succinic acid in the composition may be from about 0.1 to about 10 w/w% and in particular, from about 0.1, about 0.2, about 0.5 or about 0.75, about 1 w/w% to about 2, about 3, about 4, about 5, about 7.5 or about 10 w/w%. In various embodiments, the amount of succinic acid in the composition may be about 0.1, about 0.2, about 0.5, about 0.75, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9 or about 10 w/w%. The succinic acid is combined with one or more API's.

[0059] In various embodiments, salicylic acid may be included in the composition. Salicylic may be present in the composition in an amount of from about 0.05 to about 5 w/w% and in particular, from about 0.05, about 0.1, about 0.2, about 0.5 or about 0.75 w/w% to about 1, about 2, about 3, about 4 or about 5 w/w%. In various embodiments, the amount of salicylic acid in the composition may be about 0.05, about 0.1, about 0.2, about 0.5, about 0.75, about 1, about 2, about 3, about 4 or about 5 w/w%.

[0060] In various embodiments, azelaic acid may be included in the composition. Azelaic acid may be present in the composition in an amount of from about 0.05 to about 10 w/w% and in particular, from about 0.05, about 0.1, about 0.2, about 0.5 or about 0.75, about 1 w/w% up to about 2, about 3, about 4, about 5, about 7.5 or about 10 w/w%. In various embodiments, the amount of azelaic acid in the composition may be about 0.05, about 0.1, about 0.2, about 0.5, about 0.75, about 1, about 2, about 3, about 4 about 5, about 7.5 or about 10 w/w%

[0061] In various embodiments, picolinic acid may be included in the composition. Picolinic acid may be present in the composition in an amount of from about 0.01 to about 10 w/w% and in particular, from about 0.01, about 0,02, about 0.05, about 0.1, about 0.2 or about 0.5 w/w% to about 0.75, about 1, about 2, about 3, about 4, about 5, about 7.5 or about 10 w/w%. In various embodiments, the amount of picolinic acid in the composition may be about 0.01, about 0.02, about, about 0.05, about 0.1, about 0.2, about 0.5, about 0.75, about 1, about 2, about 3, about 4, about 5, about 7.5 or about 10 w/w%

[0062] In various embodiments, benzoyl peroxide may be included in the composition. Benzoyl peroxide may present in the composition in an amount of from about 0.01 to about 5 w/w% and in particular, from about 0.01, about 0,02, about 0.05, about 0.1, about 0.2 or about 0.5 w/w% to about 0.75, about 1, about 2, about 3, about 4 or about 5 w/w%. In various embodiments, the amount of benzoyl peroxide in the composition may be about 0.01, about 0.02, about, about 0.05, about 0.1, about 0.2, about 0.5, about 0.75, about 1, about 2, about 3, about 4 or about 5 w/w%

[0063] In various embodiments, antibiotic may be included in the composition. The antibiotic includes erythromycin, clindamycin, minocycline and doxycycline, or any other antibiotic with activity against *P. acnes*. Antibiotic may be present in the composition in an amount of from about 0.001 up to about 2 w/w% and in particular, from about 0.001, about 0.002, about 0.005 or about 0.0075, about 0.01, about 0.02, about 0.03, about 0.04, about 0.05 w/w% up to about 0.075, about 0.1, about 0.2, about 0.3, about 0.4, about 0.5 about 1 or about 2% w/w%. In various embodiments, the amount of antibiotic in the composition may be about 0.001, about 0.002, about 0.005, about 0.0075, about 0.01, about 0.02, about 0.03, about 0.04, about 0.05, about 0.06, about 0.07, about 0.08, about 0.09, about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 1% or about 2 w/w%.

[0064] The compositions of the present invention may be incorporated into a pharmaceutically acceptable carrier system which may include creams, gels, lotions or other types of suspensions that can stabilize the combination of agents and deliver them to the affected area of skin by topical applications. Pharmaceutically acceptable carrier systems may also contain ingredients that include, but are not limited to, saline, aqueous electrolyte solutions, ethanol, dimethyl sulfoxide, dimethyl isosorbide, isopropyl myristate, lauryl lactate, diisopropyl adipate, sodium lauryl sulfoacetate; ionic and nonionic osmotic agents such as sodium chloride, potassium chloride, glycerol, propylene glycol and dextrose; pH adjusters and buffers such as salts of hydroxide, phosphate, citrate, acetate, borate; and trolamine; antioxidants such as salts, acids and/or bases of bisulfite, sulfite, metabisulfite, thiosulfite, ascorbic acid, acetyl cysteine, cystein, glutathione, butylated hydroxyanisole, butylated hydroxytoluene, tocopherols, and ascorbyl palmitate; compounds such as lecithin, phospholipids; petroleum derivatives such as mineral oil and white petrolatum; fats such as lanolin, peanut oil, palm oil, soybean oil; mono-, di-, and triglycerides; polymers of acrylic acid such as carboxypolymethylene gel, and hydrophobically modified cross-linked acrylate copolymer; polysaccharides such as dextrans and glycosaminoglycans such as sodium hyaluronate. Such pharmaceutically acceptable carriers may be preserved against bacterial contamination using preservatives, including, but are not limited to, benzalkonium chloride, ethylene diamine tetra-acetic acid and its salts, benzethonium chloride, chlorhexidine, chlorobutanol, methylparaben, thimerosal, and phenylethyl alcohol, or may be formulated as a non-preserved formulation for either single or multiple use.

[0065] Methods of Treatment

[0066] The methods of treatment of the present invention are useful for the treatment of skin diseases including acne vulgaris and inflammatory acne vulgaris. Treatment of acne vulgaris may be by topically administering to a subject a composition that includes a combination of active agents.

[0067] The compositions of the present invention can be administered at a variety of intervals. In some instances, administration may be once a day. In other instances, administration can be less or more frequently, such as 1, 2, 3, or 4 times a day, 1 time every 2 days, or once a week.

[0068] The treatment methods may be monitored by following any of the pathogenic aspects of acne vulgaris including number of acne lesions, sebum excretion,

follicular keratinization, comedone formation, bacterial colonization of the follicle, *P. acnes* proliferation and/or inflammation.

EXAMPLES

[0069] Aspects of the present teachings may be further understood in light of the following examples, which should not be construed as limiting the scope of the present teachings in any way. In the examples below, DRM1 refers to succinic acid.

[0070] Example 1 - This example illustrates the antimicrobial effect of combinations of DRM1 (succinic acid) and salicylic acid using a checkerboard assay design.

[0071] Bacterial Cultivation

[0072] *P. acnes* (ATCC 6919) was cultured on Brucella plates supplemented with 0.1 g/L hemin, 0.01 g/L vitamin K, and 5% (v/v) defibrinated sheep's blood at 37°C under anaerobic conditions using a Gas-Pak (BD). For each assay, an inoculum of *P. acnes* was prepared by suspending a colony of *P. acnes* in 5 mL Reinforced Clostridium medium (RCM) and growing at 37°C and 250 RPM under anaerobic conditions for 30 hours. Cultures were harvested, washed with PBS and resuspended in fresh, sterile RCM broth to a concentration of 2 x 106 CFU/ml.

[0073] Minimum Inhibitory Concentration (MIC) Determination

[0074] Individual MIC values were determined for each drug according to the microbroth dilution method. Briefly, 2-fold serial dilutions of each drug (50 µl) were added to wells in a 96-well plate followed by 50 µl of the prepared *P. acnes* inoculum as described in Example 1. Media without *P. acnes* and medium with *P. acnes* only were used for negative and positive controls, respectively, and contained the highest concentration of solvent used in each drug analysis. Plates were incubated under anaerobic conditions using a Gas-Pak (BD) for 48 hours at 37°C. Following incubation, each well was resuspended by pipetting and the optical density at 600 nm (OD600) was determined on a plate reader. The MIC value was defined as the first well showing ≥ 90% reduction in growth compared to controls.

[0075] In vitro Checkerboard Assay

[0076] A checkerboard assay was used to determine if combining DRM1 (succinic acid) with commonly used topical treatments for acne showed synergy, indifference or antagonism between the drugs. Checkerboard assays were performed in 96 well plates as an 8 x 8 grid. Two-fold serial dilutions of API A (25 µl) were added at 4x the desired final concentration across 8 rows of the microplate. Two-fold serial dilutions

of API B (25 µl) were added at 4x the desired final concentration to 8 columns on the microplate so that the highest concentration of API A and the highest concentration of API B were in the same well at one corner of the 8 x 8 grid diagonal from the corner containing the lowest concentration of each drug. For each drug, a set of concentrations greater than and less than the MIC as calculated above was used. An inoculum of *P. acnes* (50 µl) as described in example 1 was added to each well containing API and the plate was incubated at 37°C for 48 hours under anaerobic conditions and the individual and combined MIC values were determined as stated in example 2 for the MIC assay. The FICI for each combination was determined using the following equation:

$$FICI = MICA/B \text{ / } MICA \text{ alone } + \text{ } MICB/A \text{ / } MICB \text{ alone}$$

[0077] Synergy was defined as an FICI ≤ 0.5, additivity was defined as an FICI $> 0.5 \leq 1.0$, indifference was defined as an FICI $> 1 \leq 4$ and antagonism was defined as an FICI > 4.0.

[0078] **Checkerboard Combination of DRM1 (succinic acid) and Salicylic acid**

[0079] Table 1, below, shows the MIC values for Salicylic Acid.

[0080] As shown in Figures 1 and 2 and Table 1, the combined MIC of the two drugs was found to be 3.75 mM DRM1 (succinic acid) and 1.9 mM salicylic acid as seen in Figure 1. This corresponded to an FICI of 0.5 suggesting synergy was occurring between DRM1 (succinic acid) and salicylic acid. Growth inhibition was seen when salicylic acid and DRM1 (succinic acid) were used at a ratio of 1:32 when the concentration of DRM1 (succinic acid) was $\geq$ 15 mM and the concentration of salicylic acid was $\geq$ 0.47 mM, when salicylic acid and DRM1 (succinic acid) were used in a ratio of 1:8 when the concentration of DRM1 (succinic acid) was $\geq$ 7.5 mM and the concentration of salicylic acid was $\geq$ 0.94 mM, when salicylic acid and DRM1 (succinic acid) were used in a ratio of 1:2 when the concentration of DRM1 (succinic acid) was $\geq$ 3.75 mM and the concentration of salicylic acid was $\geq$ 1.9 mM, when salicylic acid and DRM1 (succinic acid) were used in a ratio of 2:1 when the concentration of DRM1 (succinic acid) was $\geq$ 1.9 and the concentration of salicylic acid was $\geq$ 3.75 mM, and when salicylic acid and DRM1 (succinic acid) were used in a ratio of 4:1 when the concentration of DRM1 (succinic acid) was $\geq$ 0.94 mM and the concentration of salicylic acid was $\geq$ 3.75 mM as seen in Figure 2. Using the control *P. acnes* strain, ATCC 6919,

DRM1 (succinic acid) in combination with SA resulted in an FICI of 0.5 suggesting the two active pharmaceutical ingredients (APIs) are working in synergy. The most synergistic concentration of the two APIs occurred at a ratio of 2:1 (DRM1:SA) when DRM1 (succinic acid) (3.75 mM) was combined with SA (1.9 mM), at a quarter of the MIC concentration required for each component alone. (See Table 1 below)

[0081] Table 1. MIC's for Salicylic Acid in combination with Succinic Acid

Salicylic Acid (mM)	**DRM1 (Succinic Acid mM)**	**Ratio (Salicylic/DRM1)**	**FICI**	**Synergistic/ Additive/ Indifferent/ Antagonist**
0	15	--	--	--
7.5	0	--	--	--
1.9	3.75	1:2	1.9/7.5+3.75/15=0.5	Synergistic
≥0.47	≥ 15	1:32	≥0.47/7.5+≥15/15=≥1.06	Additive
≥0.94	≥7.5	1:8	≥0.94/7.5+≥7.5/15=≥0.625	Additive
≥1.9	≥3.75	1:2	≥1.9/7.5+≥3.75/15=≥0.5	Synergistic
≥3.75	≥1.9	2:1	≥3.75/7.5+≥1.9/15=≥0.626	Additive
≥3.75	≥0.94	4:1	≥3.75/7.5+≥0.94/15=≥0.563	Additive

[0082] Example 2 -- This example illustrates the time-kill assay of DRM1 (succinic acid) and Salicylic acid in Combination.

[0083] DRM1 (succinic acid) and salicylic acid were used individually at concentrations 2X their MIC values (30 mM DRM1 (succinic acid) and 15 mM salicylic acid) and in combination at 0.5X MIC for DRM1 (succinic acid, 7.5 mM) and 1X MIC for salicylic acid (7.5 mM) Assays were performed in RCM broth with 12% propylene glycol in 96-well plates. A final concentration of 5x105 CFU/ml *P. acnes* 6919 was used for each reaction and each assay was performed under anaerobic conditions using a Gas-Pak at 37°C. At various time points (0, 15, 30, 60 and 120 minutes) 10 µl of each sample was removed and diluted 1:10 to 1:104 in sterile PBS and 20 µl of each dilution was spread on a Brucella broth agar plate supplemented with 0.1 µg/ml hemin and 0.01 µg/ml vitamin K using the track plate method. Figure 3 shows a consistent growth between all three samples and the control at 0 minutes. After 15 minutes, growth was evident on the

individual DRM1 (succinic acid) and salicylic acid plates; however, no growth was detected on the DRM1(succinic acid)-salicylic acid combination plate. After 30 minutes of incubation, no growth was detected on any of the plates. The increased speed at which the DRM1 (succinic acid)-salicylic acid combination inhibited *P. acnes* growth suggests the two APIs performed in a synergistic or additive way.

[0084] Example 3 -- This example illustrates the antimicrobial effect of combinations of DRM1 (succinic acid) and azelaic acid using a checkerboard assay design.

[0085] Bacterial cultivation, MIC calculation and Checkerboard Assay design were as described in Example 1. Table 2, below, shows MIC values for Azelaic Acid.

[0086] As shown in Figures 4 and 5 and Table 2, the combined MIC of the two drugs was at 7.5 mM DRM1 (succinic acid) and 3.75 mM azelaic acid as seen in Figure 4. This corresponded to an FICI of 1.0 suggesting the interaction between DRM1 (succinic acid) and azelaic acid was additive. Growth inhibition was seen when azelaic acid and DRM1 (succinic acid) were used at a ratio 1:32 when the concentration of DRM1 (succinic acid) was $\geq$ 15 mM and the concentration of azelaic acid was $\geq$ 0.47 mM, when azelaic acid and DRM1 (succinic acid) were used in a ratio of 1:8 when the concentration of DRM1 (succinic acid) was $\geq$ 7.5 mM and the concentration of azelaic acid was $\geq$ 0.94 mM, when azelaic acid and DRM1 (succinic acid) were used in a ratio of 1:4 when the concentration of DRM1 (succinic acid) was $\geq$ 7.5 mM and the concentration of azelaic acid was $\geq$ 1.9 mM, when azelaic acid and DRM1 (succinic acid) were used in a ratio of 1:2 when the concentration of DRM1 (succinic acid) was $\geq$ 7.5 mM and the concentration of azelaic acid was $\geq$ 3.75 mM, when azelaic acid and DRM1 (succinic acid) were used in a ratio of 1:1 when the concentration of DRM1 (succinic acid) was $\geq$ 3.75 and the concentration of azelaic acid was $\geq$ 3.75 mM, when azelaic acid and DRM1 (succinic acid) were used in a ratio of 2:1 when the concentration of DRM1 (succinic acid) was $\geq$ 1.9 mM and the concentration of azelaic acid was $\geq$ 3.75 mM, and when azelaic acid and DRM1 (succinic acid) were used in a ratio of 8:1 when the concentration of DRM1 (succinic acid) was $\geq$ 0.94 mM and the concentration of azelaic acid was $\geq$ 7.5 mM as seen in Figure 5. DRM1 (succinic acid) in combination with AZA had additive effect and killed *P. acnes* at half the concentration each when used in combination. The concentration of AZA can be lowered which results in less side effect while maintaining efficacy of the combination.

[0087] Table 2. MIC's for Azelaic Acid in combination with Succinic Acid

Azelaic acid (mM)	DRM1 (Succinic Acid mM)	Ratio (Azelaic /DRM1)	FICI	Synergistic/ Additive/ Indifferent/ Antagonist
0	15	--	--	--
7.5	0	--	--	--
3.75	7.5	1:2	3.75/7.5+7.5/15=1.0	Additive
≥0.47	≥ 15	1:32	≥0.47/7.5+≥15/15=≥1.06	Indifferent
≥0.94	≥7.5	1:8	≥0.94/7.5+≥7.5/15=≥0.625	Additive
≥1.9	≥7.5	1:4	≥1.9/7.5 + ≥7.5/15=≥0.75	Additive
≥3.75	≥7.5	1:2	≥3.75/7.5+≥7.5/15=≥1.0	Additive
≥3.75	≥3.75	1:1	≥3.75/7.5+≥3.75/15=≥0.75	Additive
≥3.75	≥1.9	2:1	≥3.75/7.5+≥1.9/15=≥0.63	Additive
≥7.5	≥0.94	8:1	≥7.5/7.5+≥0.94/15=≥1.06	Indifferent

[0088] Example 4 -- This example illustrates the antimicrobial effect of combinations of DRM1 (succinic acid) and picolinic acid using a checkerboard assay design.

[0089] Bacterial cultivation, MIC calculation and Checkerboard Assay design were as described in Example 1. Table 3, below, shows MIC values for picolinic acid.

[0090] As shown in Figures 6 and 7 and Table 3, DRM1 (succinic acid, 0-60 mM) and picolinic acid (0-7.5 mM) were added to a 96-well plate in 2-fold serial dilutions and combined with *P. acnes* 6919 as described in example 1. The combined MIC of the two drugs was at 7.5 mM DRM1 (succinic acid) and 0.94 mM picolinic acid as seen in Figure 6. This corresponded to an FICI of 1.0 suggesting the interaction between DRM1 (succinic acid) and picolinic acid was additive. Growth inhibition was seen when picolinic acid and DRM1 (succinic acid) were used at a ratio 1:128 when the concentration of DRM1 (succinic acid) was ≥ 15 mM and the concentration of picolinic acid was ≥ 0.12 mM, when picolinic acid and DRM1 (succinic acid) were used in a ratio of 1:64 when the concentration of DRM1 (succinic acid) was ≥ 15 mM and the concentration of picolinic acid was ≥ 0.23 mM, when picolinic acid and DRM1 (succinic acid) were used in a ratio of 1:16 when the concentration of DRM1 (succinic acid) was ≥

7.5 mM and the concentration of picolinic acid was ≥ 0.47 mM, when picolinic acid and DRM1 (succinic acid) were used in a ratio of 1:8 when the of DRM1 (succinic acid) was ≥ 7.5 mM and the concentration of picolinic acid was ≥ 0.94 mM, when picolinic acid and DRM1 (succinic acid) were used in a ratio of 1:4 when the concentration of DRM1 (succinic acid) was ≥ 3.75 mM and the concentration of picolinic acid was ≥ 0.94 mM, when picolinic acid and DRM1 (succinic acid) were used in a ratio of 1:2 when the concentration of DRM1 (succinic acid) was ≥ 3.75 mM and the concentration of picolinic acid was ≥ 1.88 mM, when picolinic acid and DRM1 (succinic acid) were used in a ratio of 1:1 when the concentration of DRM1 (succinic acid) was ≥ 1.9 mM and the concentration of picolinic acid was ≥ 1.9 mM and when picolinic acid and DRM1 (succinic acid) were used in a ratio of 2:1 when the concentration of DRM1 (succinic acid) was ≥ 0.9 mM and the concentration of picolinic acid was ≥ 1.88 mM as seen in Figure 7. DRM1 (succinic acid) in combination with picolinic acid had additive effect and killed *P. acnes* at half the concentration each when used in combination. The concentration of picolinic acid can be lowered which results in less side effect while maintaining efficacy of the combination.

[0091] Table 3. MIC's for Picolinic Acid in combination with Succinic Acid

Picolinic acid (mM)	**DRM1 (Succinic Acid mM)**	**Ratio (Picolinic /DRM1)**	**FICI**	**Synergistic/ Additive/ Indifferent/ Antagonist**
0	15	--	--	--
1.88	0	--	--	
0.94	7.5	1:8	0.94/1.88+7.5/15=1.0	Additive
≥0.12	≥ 15	1:128	≥0.12/1.88+≥15/15=≥1.06	Indifferent
≥0.23	≥15	1:64	≥0.23/1.88+≥15/15=≥1.12	Indifferent
≥0.47	≥7.5	1:16	≥0.47/1.88 + ≥7.5/15=≥0.75	Additive
≥0.94	≥7.5	1:8	≥0.94/1.88+≥7.5/15=≥1.0	Additive
≥0.94	≥3.75	1:4	≥0.94/1.88+≥3.75/15=≥0.75	Additive
≥1.88	≥3.75	1:2	≥1.88/1.88+≥3.75/15=1.25	Indifferent
≥1.88	≥1.9	1:1	≥1.88/1.88+≥1.9/15=≥1.13	Indifferent
≥1.88	≥0.9	2:1	≥1.88/1.88+≥0.9/15=≥1.06	Indifferent

[0092] Example 5 -- This example illustrates the antimicrobial effect of combinations of DRM1 (succinic acid) and benzoyl peroxide (BPO) using a checkerboard assay design.

[0093] Bacterial cultivation, MIC calculation and Checkerboard Assay design were as described in Example 1. Table 4, below shows MIC values for BPO.

[0094] As shown in Figures 8 and 9 and Table 4, DRM1 (succinic acid, 0-7085 μg/ml) and benzoyl peroxide (0-250 μg/ml) were added to a 96-well plate in 2-fold serial dilutions and combined with *P. acnes* 6919 as described in example 3. DRM1 (succinic acid) had an individual MIC value of 1771 μg/ml and BPO had an individual MIC(succinic acid) value of 125 μg/ml. The combined MIC of the two drugs was at 885.7 μg/ml DRM1(succinic acid) and 62.5 μg/ml BPO as seen in Figure 8. This corresponded to an FICI of 1.0 suggesting the interaction between DRM1 (succinic acid) and BPO was additive. Growth inhibition was seen when BPO and DRM1 (succinic acid) were used at ratios of 1:113, 1:226, and 1:453 when DRM1 (succinic acid) was used at a concentration ≥ 1771 μg/ml and BPO was used at a concentration ≥ 15.6 μg/ml, ≥ 7.8 μg/ml, and ≥ 3.9 μg/ml, respectively, as seen in Figure 9. Growth inhibition was also seen at a ratio of BPO to DRM1 (succinic acid) of about 1:14 and 1:28 when the concentration of DRM1 (succinic acid) was ≥ 885.7 μg/ml and BPO was used at ≥ 62.5 μg/ml, and ≥ 31.25 μg/ml, respectively, or when the ratio of BPO to DRM1 (succinic acid) was about 1:7, 1:3.5, 1:1.8, or 1:0.88 at concentrations of BPO ≥ 125 μg/ml and concentrations of DRM1 (succinic acid) at ≥ 885.7 μg/ml, ≥ 442 μg/ml, ≥ 221 μg/ml, and ≥ 110 μg/ml, respectively. DRM1 (succinic acid) in combination with BPO had additive effect and killed *P. acnes* at half the concentration each when used in combination. The concentration of BPO can be lowered which results in less side effect while maintaining efficacy of the combination.

[0095] Table 4: MIC's for Benzoyl Peroxide (BPO) in combination with Succinic Acid

BPO (µg/ml)	DRM1 (Succinic Acid µg/ml)	Ratio (BPO /DRM1)	FICI	Synergistic/ Additive/ Indifferent/ Antagonist
0	1771	--	--	--
125	0	--	--	--
62.5	885.7	1:14	62.5/125+885.7/1771=1.0	Additive
≥3.9	≥1771	1:453	≥3.9/125+≥1771/1771=≥1.03	Indifferent
≥7.8	≥1771	1:226	≥7.8/125+≥1771/1771=≥1.06	Indifferent
≥15.6	≥1771	1:113,	≥15.6/125+≥1771/1771=≥1.12	Indifferent
≥31.25	≥885.7	1:28	≥31.25/125+≥885.7/1771=≥0.75	Additive
≥62.5	≥885.7	1:14	≥62.5/125 + ≥885.7/1771=≥1.0	-Additive-
≥ 125	≥ 885.7	1:7	≥ 125/125+≥885.7/1771=≥1.5	Indifferent
≥ 125	≥ 442	1:3.5	≥ 125/125+≥ 442/1771=1.25	Indifferent
≥ 125	≥ 221	1:1.8	≥ 125/125+≥ 221/1771=≥1.12	Indifferent
≥ 125	≥ 110	1:0.88	≥ 125/125+≥≥ 110/1771=≥1.06	Indifferent

[0096] Discussion of Examples: 1-5

[0097] Examples 1-5 illustrate DRM1 (succinic acid) combinations that maximize efficacy and minimize side effects:

[0098] DRM1 (succinic acid) increases efficacy of other acne treatments including salicylic acid, benzoyl peroxide, azelaic acid, picolinic acid, retinoids, etc. while reducing side effects of the second agents on the skin. This means that combinations can be used in which the amount of a side effect causing agent (such as benzoyl peroxide (BPO), salicylic acid, azelaic acid, retinoids, etc.) can be reduced while maximizing efficacy. We have identified DRM1 (succinic acid) containing combinations and the ratios between DRM1 (succinic acid) and the second agent that are optimal for acne treatment. All combinations improve the performance of the second agent without increasing side effects – they reduce side effects.). The most efficacious combination seems to be DRM1 (succinic acid) + salicylic acid at ratio 2:1. This combination shows

the highest synergistic effects against *P. acnes*, reduces inflammation (provided by DRM1), and has anti-hyperkeratinization (provided by salicylic acid) and anti-commedogenic (provided by salicylic acid) properties. This combination addresses three acne vulgaris diseases factors. In addition, the combinations result in faster killing of *P. acnes* (time kill assays in Example 2) and prevents biofilm formation in DRM1 concentration above 7.5mM (Example 8). The combinations of DRM1 (succinic acid) with a second agent can be optimized using specific molar ratios to achieve optimal efficacy while minimizing side effects. In addition to treating acne vulgaris in all patients including inflammatory acne vulgaris, DRM1 (succinic acid) containing combinations could be particularly useful in patients who are prone to side effects on the skin and would benefit from suing topical treatments that contain reduced amount of BPO, salicylic acid, azelaic acid, retinoids, etc.

[0099] Example 6 -- This example illustrates the antimicrobial effect of combinations of DRM1 (succinic acid) and clindamycin using a checkerboard assay design.

[0100] Bacterial cultivation, MIC calculation and Checkerboard Assay design were as described in Example 1. Table 5, below, shows MIC values for BPO.

[0101] As shown in Figures 10 and 11 and Table 5, DRM1 (succinic acid, 0-7085 μg/ml) and clindamycin (0-0.25 μg/ml) were added to a 96-well plate in 2-fold serial dilutions and combined with *P. acnes* 6919 as described in example 3. The combined MIC of the two drugs was at 1771 μg/ml DRM1 (succinic acid) and 0.06 μg/ml clindamycin, which were the same MIC values for each individual treatment as seen in Figure 10. This corresponded to an FICI of 2.0 suggesting the interaction between DRM1 (succinic acid) and clindamycin was indifferent with no indication of an antagonistic interaction. Growth inhibition was seen when clindamycin and DRM1 (succinic acid) were used at ratios of 1:28341, 1:56683, 1:113366, 1:226732, 1:453465 when DRM1 (succinic acid) was used at a concentration ≥ 1771 μg/ml and clindamycin was used at a concentration ≥ 0.063 μg/ml, ≥ 0.031 μg/ml, ≥ 0.016 μg/ml, ≥ 0.008 μg/ml, and ≥ 0.004 μg/ml, respectively, as seen in Figure 11.

[0102] Table 5. MIC's for Clindamycin in combination with Succinic Acid

Clindamycin (µg/ml)	DRM1 (Succinic Acid µg/ml)	Ratio (Clindamycin /DRM1)	FICI	Synergistic/ Additive/ Indifferent/ Antagonist
0	1771	--	--	--
0.063	0	--	--	--
0.063	1771	1:28341	0.063/0.063 + 885.7/1771=2.0	Indifferent
≥0.004	≥1771	1:453465	≥0.004/0.063+≥1771/1771=≥1.07	Indifferent
≥0.008	≥1771	1:226732,	≥0.008/0.063+≥1771/1771=≥1.13	Indifferent
≥0.016	≥1771	1:113366	≥0.016/0.063 + ≥1771/1771=≥1.27	Indifferent
≥0.031	≥1771	1:56683,	≥0.031/0.063+≥885.7/1771=≥1.52	Indifferent
≥0.063	≥1771	1:28341	≥0.063/0.063 + ≥885.7/1771=≥2.05	Indifferent
≥0.063	≥221.4	1:3514	≥0.063/0.063 + ≥221.4/1771=≥1.17	Indifferent
≥0.063	≥110.7	1:1757	≥0.063/0.063 + ≥110.7/1771=≥1.11	Indifferent

[0103] Table 6 summarizes the MIC values from Examples 1, 3, 4, 5 and 6.

[0104] Table 6. Summary of MIC values

API	API Conc.	DRM1 Conc. (Succinic Acid)	Ratio (API/ DRM1)	FICI	Synergistic/ Additive/ Indifferent/ Antagonist
Salicylic Acid	1.9 mM	3.75 mM	1:2	1.9/7.5+3.75/15=0.5	Synergistic
Azelaic acid	3.75 mM	7.5 mM	1:2	3.75/7.5+7.5/15=1.0	Additive
Picolinic Acid	0.94 mM	7.5 mM	1:8	0.94/1.88+7.5/15=1.0	Additive

API	API Conc.	DRM1 Conc. (Succinic Acid)	Ratio (API/ DRM1)	FICI	Synergistic/ Additive/ Indifferent/ Antagonist
Benzoyl Peroxide	62.5 µg/ml	885.7 µg/ml	1:14	62.5/125+885.7/1771=1.0	Additive
Clindamycin	0.063 µg/ml	1771 µg/ml	1:28341	0.063/0.063 + 885.7/1771=2.0	Indifferent

[0105] Example 7 -- This example shows that DRM1 (succinic acid) killed *P. acnes* strains that are resistant to antibiotics.

[0106] The MIC values for DRM1 (succinic acid) against multi-resistant *P. acnes* strains were determined as described in example 1. Briefly, *P. acnes* strains (RMA #20660 and RMA #20661) with low susceptibility to erythromycin, clindamycin, minocycline, and doxycycline were incubated with DRM1 (succinic acid, 0-120 mM) and the minimum inhibitory concentration was determined. As illustrated in Figure 12 and 13, DRM1 (succinic acid) was found to be bactericidal against antibiotic-resistant *P. acnes* strains RMA# 20660 and RMA# 20661. The MIC for DRM1 (succinic acid) against multi-resistant *P. acnes* strains was 15 mM. This was similar to the MIC value for non-resistant strains of *P. acnes* demonstrating that DRM1 (succinic acid) is able to kill *P. acnes* strains resistant to commonly used antibiotics.

[0107] Example 8 -- This example shows that DRM1 (succinic acid) prevents biofilm formation.

[0108] DRM1 (succinic acid) prevented biofilm formation in biofilm-forming strains at a concentration of 7.5 mM and above. Biofilm formation is an important factor in *P. acnes* pathogenicity and biofilm is detected in approximately half of hair follicles of acne patients.

[0109] Example 9 -- This example illustrates that DRM1 (succinic acid) prevents resistance development in *P. acnes* strains.

[0110] Resistance development may be measured by two methods: single step for the determination of spontaneous frequency of resistance development and serial passage for the determination of progressive resistance development. Single step resistance frequency was determined by spreading a large inoculum of *P. acnes* (10^9 to

10^{10} CFU) onto a blood agar plate containing 4x, 8x, 16x, or 32x the MIC for DMR1 (succinic acid) as described in Example 2. The spontaneous resistance frequency was calculated from the number of colonies that grow on plates containing DRM1 vs. the number of colonies that grow on DRM1-free agar. As shown in Figure 14, no colonies grew and therefore no resistance was detected. For progressive resistance selection, *P. acnes* (106 CFU/ml) will be exposed to a series of tubes containing serial 2-fold increasing concentrations of DRM1 (succinic acid) for a total of 10 cycles (72 hours each). The MIC of DMR1 (succinic acid) will be determined from the tubes containing the highest drug concentration. Tubes with the highest drug concentration that still shows turbidity will be used to inoculate (100 µl) a new series of tubes containing drug dilutions. To investigate whether DRM1 (succinic acid) could prevent the emergence of antibiotic resistance, experiments will be performed as described in the presence of 0.25 times the MIC of DRM1 (succinic acid). The resistance stability of each clone will be determined by sub-culturing onto drug-free agar for three cycles and determining the DRM1 (succinic acid) MIC via the microbroth dilution method as described in Example 1.

[0111] *P. acnes* strains did not develop spontaneous or progressive resistance to DMR1 (succinic acid) and including DRM1 (succinic acid) in combination with antibiotics for acne treatment prevents antibiotic resistance emergence and development.

[0112] Discussion of Examples: 6-9

[0113] Examples 6-9 illustrate that combinations with antibiotics prevents resistance development while reducing side effects.

[0114] DRM1 (succinic acid) combined with topical antibiotics can be used to replace side effect causing BPO in combinations containing antibiotics and BPO. DRM1 (succinic acid) is bactericidal. Many antibiotics are bacteriostatic which promotes resistance development. DRM1 (succinic acid) is effective against antibiotic resistant *P. acnes* strains and prevents emergence of antibiotic resistant *P. acnes* strains when used in combinations with antibiotics. In addition, DRM1 prevents biofilm formation.

[0115] Example 10: Synthetic SE Microbiome Complex

[0116] A synthetic SE Microbiome Complex (the "SE Microbiome Complex") containing a defined combination of *S. epidermis* metabolites and prebiotics was prepared by combining at least the following in a concentration of 0-10%:

1. At least one metabolite produced by *S. epidermidis* (*e.g.,* succinic acid, acetic acid, lactic acid, butyric acid, other short chain fatty acids, other metabolites including small molecules, peptides or proteins).
2. At least one prebiotic compound (*e.g.,* glycerol, sucrose, maltose, lactose, other carbohydrate or other compounds that supports fermentation and growth of *S. epidermidis*)

[0117] The components of the synthetic SE Microbiome Complex were produced by chemical synthesis or by microbial fermentation using wild-type or engineered microorganisms. The SE Microbiome Complex was optimized for bactericidal properties against *P. acnes*, skin tolerance, and cosmetic acceptance. The SE Microbiome Complex was formulated for use for the treatment of acne vulgaris.

[0118] Example 11: Formulation SE Microbiome Complex with salicylic acid for the treatment of acne vulgaris using medicated acne treatment pads

[0119] Acne treatment pads formulation (Formulation 1): The formulation was prepared that contained salicylic acid (1%), organic aloe vera (50%), propanediol (15%), organic grain alcohol (10%) and SE Microbiome Complex. The SE Microbiome complex consisted of 2% succinic acid, 1% Brij L4, 1% dimethyl isosorbide ether, and 1% glycerin (prebiotic compound). Optionally, organic lemon essential oil was added to a final concentration of 0-1% and grapefruit seed extract to a final concentration of 0-1%. Sodium hydroxide was used to adjust the solution pH to 3.9-4.5. Succinic acid was prepared by microbial fermentation or by chemical synthesis. Alternatively, succinic acid in the formulation was replaced by conditioned media prepared by fermenting S. epidermidis as follows: *S. epidermidis* was incubated in phenol red-free rich medium with glycerol (20 g/l) for one to six days. After that, microorganisms were discarded by centrifugation at 5,000 g for 30 min. Fermented media were then passed through 0.2-μm-pore-size filters. Conditioned media contained succinic acid and other S. epidermidis fermentation metabolites. Alternatively, conditioned media was used in addition to succinic acid. Alternatively, glycerin was replaced by other prebiotic compound (such as sucrose, maltose, lactose or other carbohydrates and other prebiotic compounds that support the fermentation of S. epidermidis). Alternatively, organic components in the formulation were replaced by non-organic equivalent components. The formulated solution was poured over pads (material - rayon, Lyocell, Polyester or similar) in a container. The pads were used for applying the topical treatment for acne vulgaris.

[0120] Example 12: Formulation of a salicylic acid and succinic acid combination for the acne spot treatment of acne vulgaris using a concentrated gel

[0121] Acne spot treatment formulation (Formulation 2) was prepared containing: Salicylic acid (2%), propanediol (15%), organic grain alcohol (10-20%) and SE Microbiome Complex – super strength (consisting of 4% succinic acid, 3% Brij L4, 5% dimethyl isosorbide). Sodium hydroxide was used to adjust the solution pH to 3.9-4.5. Hydroxyethyl cellulose or eco-gel was added to gel the solution at a concentration of 1-3%. Optionally, organic tee tree essential oil (0-10%) and lemon essential oil (0-1%) was added. Optionally, organic aloe vera (0-50%) was added. Optionally, glycerin or other prebiotic compounds (0-5%) were added (such as sucrose or other carbohydrates or prebiotic compounds that support the growth/fermentation of S. epidermidis). Alternatively, conditioned media of fermenting S. epidermidis (prepared as described in Example 1) was used instead of or in addition to succinic acid. Alternatively, organic components in the formulation were replaced by non-organic equivalent components. Gel was filled into tubes for application. Gel was used for topical treatment of acne vulgaris.

[0122] Example 13: Bacterial Cultivation

[0123] *P. acnes* (ATCC 6919) was cultured on reinforced Clostridial medium (RCM) agar plates at 37°C under anaerobic conditions using a Gas-Pak (BD). For each assay, an inoculum of *P. acnes* was prepared by suspending a colony of *P. acnes* in 5 mL RCM and growing at 37°C under anaerobic conditions for 72 hours. Cultures were harvested, washed with PBS and resuspended in fresh, sterile RCM broth to a concentration of 2 x 10^6 CFU/ml.

[0124] Example 14: Formulation 1 and Formulation 2 are inhibitory against *P. acnes*

[0125] Formulation 1 (Example 11) and Formulation 2 (Example 12) was incubated in 96-well plate with *P. acnes* prepared as described in Example 13. Control sample contained *P. acnes* in media only. Plates were incubated under anaerobic conditions using a Gas-Pak (BD) for 72 hours at 37°C. Following incubation, the plate was examined visually. Control samples exhibited growth as expected. No growth was observed in wells containing Formulation 1 and Formulation 2 (Figure 15). The MIC (determined by serial dilutions) was determined to be at 0.015% succinic acid for Formulation 1 and Formulation 2.

[0126] Example 15: Formulation 1 and Formulation 2 is bactericidal against *P. acnes*

[0127] The minimal bactericidal concentration for each formulated drug was determined according to the Clinical Laboratory Standards Institute (CLSI) document M26-A. Sample wells from the MIC assay in Example 14 were serially diluted 1:10 to $1:10^4$ in PBS and each dilution (5 µl) was plated on RCM agar plates.

[0128] The plates were incubated at 37°C in a Gas-Pak. Following incubation, Formulation 1 (from Example 11) and Formulation 2 (from Example 12) exhibited no growth (Figure 16). Control samples exhibited growth as expected. MBC concentration was determined as a concentration that shows a $\geq 3\log_{10}$ decrease in bacterial growth. The results showed that Formulation 1 and Formulation 2 are bactericidal against *P. acnes*. Using serial dilutions, the MBC was determined to be 0.015% succinic acid for Formulation 1 and 0.015% succinic acid for Formulation 2.

[0129] Example 16: Production of SE Microbiome Complex by S. epidermidis fermentation with prebiotic compounds and testing against P. acnes.

[0130] This example illustrates the growth inhibitory effects of *S. epidermidis* conditioned media prepared with various prebiotics against *P. acnes*.

[0131] Preparation of Prebiotics: Glycerol, sucrose, β-lactose, and D-(+)-maltose were purchased from Sigma-Aldrich. Stock solutions of each prebiotic (4% w/v) were prepared in water and filtered through a 0.22 µm filter for sterilization.

[0132] Preparation of *S. epidermidis* conditioned media: *S. epidermidis* (ATCC 12228) was cultured on trypic soy broth (TSB) agar plates at 37°C. For each assay, an inoculum of *S. epidermidis* was prepared by suspending one colony of *S. epidermidis* from a TSB agar plate into 7 ml of TSB broth and growing at 37°C, 215 RPM for 16 hours. The overnight culture was subcultured by adding 100 µl of overnight culture to 10 ml of fresh TSB. Once mid-log phase was reached, the cells were pelleted by centrifugation, washed with PBS and resuspended in fresh PBS. In 15 ml culture tubes, 4.9 ml of Rich Broth (20 g/L yeast extract, 6 g/L TSB) was combined with 40 µl 0.5% phenol red indicator, 5 ml of 4% prebiotic (glycerol, sucrose, β-lactose, or D-(+)-maltose) or PBS as a control, and *S. epidermidis* (10^5 CFU/ml) and placed in an anaerobic Gas-Pak (BD). The cultures were incubated at 37°C, 215 RPM for 6 days. Following fermentation, the cells were pelleted and the supernatants were filtered through a 0.22 µm filter for sterilization.

[0133] Minimum Inhibitory Concentration (MIC) Determination: Individual MIC values were determined for each conditioned medium according to the microbroth dilution method. *P. acnes* (ATCC 6919) was cultured in RCM broth and harvested during mid-log phase of growth. The bacterial pellet was washed and resuspended in PBS. A stock of *P. acnes* (10^7 CFU/ml) was prepared using the estimation that an OD600nm of 1.0 corresponds to about 10^7 CFU/ml. Two-fold serial dilutions of each conditioned medium (90 µl) in PBS were added to wells in a 96-well plate followed by 10 µl of the prepared *P. acnes* inoculum. Conditioned media without *P. acnes* and RCM medium with *P. acnes* only were used for negative and positive controls, respectively. Plates were incubated under anaerobic conditions using a Gas-Pak (BD) for 48 hours at 37°C. Following incubation, each well was resuspended by pipetting and the optical density at 600 nm (OD600) was determined on a plate reader. The MIC value was defined as the first well showing ≥ 90% reduction in growth compared to controls.

[0134] Fermentation of prebiotics by *S. epidermidis*: Figure 17 shows *S. epidermidis* cultures grown with (a) PBS, (b) glycerol, (c) β-lactose , (d) maltose, (e) sucrose under anaerobic conditions. After growing for six days under anaerobic conditions, the *S. epidermidis* cultures containing glycerol, sucrose, D-(+)-maltose and β-lactose showed a change of color from red to yellow indicating the acidification of the media when compared to the PBS control. No change in color occurred in PBS control (a). The acidification of the media was due to the formation of short chain fatty acid (*e.g.*, succinic acid, acetic acid, lactic acid, butyric acid) resulting from the fermentation of prebiotics by *S. epidermidis*. The glycerol sample was not as bright of a yellow compared to the other three prebiotics and still had a tint of pink. This indicates that sucrose, maltose and galactose prebiotics showed the most efficient fermentation and highest production of short chain fatty acids and therefore these are the preffered prebiotics to use in formulations for the treatment of acne.

[0135] *P. acnes* growth inhibition by *S. epidermidis* conditioned media: Conditioned media prepared by fermentation in the presence of prebiotics – glycerol, sucrose, D-(+)-maltose and β-lactose under anaerobic condition were able to inhibit P. acnes growth (undiluted media was the MIC). RCM control media prepared in the absence of prebiotics were not inhibitory.

[0136] **Example 17: Skin tolerability and cosmetic acceptance testing**

[0137] Formulation 1 and Formulation 2 with and without aloe vera was tested in human volunteers for skin tolerability and cosmetic acceptance. Evaluation was performed by using questionnaire and a scoring system. Score of 1-5 was used. Score 1 for skin tolerability corresponded to irritation, skin reddening. Score 5 for skin tolerability corresponded to no adverse reaction and excellent tolerability. Score 1 for cosmetic acceptability corresponded to unacceptable product due to smell and feel. Score 5 for cosmetic acceptability represented excellent cosmetic acceptability (smell and feel). Commercially available products were used as controls. Results are recorded in table 1. Results indicate that addition of aloe vera significantly improves skin tolerability and that Formulation 1 and Formulation 2 have significantly improved cosmetic acceptance (smell and feel) compared to commercially available products.

[0138] Table 7. Skin tolerability and cosmetic acceptance scores

Formulation	**Skin tolerability score**	**Cosmetic acceptance score**
Formulation 1 with aloe vera	5	5
Formulation 1 without aloe vera	4	4
Commercially available acne treatment pads with 1% salicylic acid	3	1
Formulation 2	5	5

[0139] Example 18: Testing of treatments containing combinations products in human subjects.

[0140] Formulation 1 (Example 11) and Formulation 2 (Example 12) were tested in human subjects. The Formulation 1 was applied using Acne Treatment Pads one or two times per day. Improvement in acne symptoms was observed (Figure 18, panel A vs. panels B and C). Panel A shows acne lesions prior to treatment, panels B and C show disappearance of acne lessions post treatment with Formulation 1. Formulation 2 (Acne Spot Treatment gel) was used as a spot treatment on individual acne lessions in human subjects. The subjects reported reduction in pain and inflammation in 5-10 minutes post application, significantly faster healing of acne lessions compared to benzoyl peroxide and lack of hyperpigmentation (dark spot side effect observed when benzoyl peroxide

was used). When Acne Spot Treatment was used in combination with hydrocolloid Acne Pimple Patches, the healing of acne lessions was visible in less than a day.

[0141] Example 19: Five step acne treatment system using combination of topical treatment containing SE Microbiome Complex, oral probiotics and oral supplements.

[0142] The five step treatment system combined the topical treatment containing SE Microbiome Complex with oral probiotics and oral supplements (such as zinc). Oral probiotics contained a diverse combination of probiotic strains of 500 million – 30 billion CFUs/capsule. The combination of probiotic strains included any combination of *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactococcus lactis*, or additional probiotic strains and optionally contained prebiotics (such as FOS or Arabinogalactan).

[0143] Five step treatment system consisted of:

[0144] Step 1: Washing face in the morning and evening.

[0145] Step 2: Applying Acne Treatment Pads (Formulation 1 from Example 11) to affected areas and Acne Spot Treatment (Formulation 2 from Example 12) to individual pimples.

[0146] Step 3: Covering whiteheads and inflamed pimples with Acne Pimple Patches (containing hydrocolloid) to treat blemishes overnight.

[0147] Step 4: Taking Probiotics (1-3 times daily) and 25-50 mg Zinc (1 time daily with a meal).

[0148] Step 5: Repeating for at least 2 months.

[0149] Compared to subjects using only topical products, the subjects who used topical products in combination with oral probiotics and zinc achieved improvement in acne symptoms.

[0150] Example 20: Combination products containing retinoids.

[0151] This example will be performed to screen combinations of DRM1 and retinoids (tretinoin, adapalene, tazarotene, isotretinoin, retinyl palmitate, retinol, or retinaldehyde) for antimicrobial and anti-inflammatory properties, and to determine if the compounds can decrease the amount of acne lesions in acne.

[0152] The minimal inhibitory concentration (MIC) and minimum bactericidal concentration of combinations of DRM1 and retinoids will be determined to monitor the

growth inhibitory effects of the combination. To determine the MIC values, DRM1 will be analyzed in concentrations ranging from 1-100 mM and the retinoids will vary in concentrations from 0.025%-0.1%. Two-fold serial dilutions of the DRM1-retinoid combination will be made in PBS (50µl). An inoculum of 2 x 10^6 CFU/ml *P. acnes* in RCM will be prepared and 50 µl will be added to the wells containing the DRM1-retinoid combinations.

[0153] Bactericidal properties of the DRM1 and retinoid combinations will be analyzed using the time-kill assay. Briefly, 0.4 ml of 10X the desired final concentration of DRM1 and retinoid will be added to 2.8 ml RCM broth along with 0.4 ml of 10^7 CFU/ml P. acnes. Media with *P. acnes* only will be used for a positive control. A separate tube will be prepared for each time point for all measured concentrations of the DRM1-retinoid combination. The assay will be performed under anaerobic conditions at 37°C. The reaction will be analyzed after 0, 0.5, 1, 2, 4, 8, and 24 hours. At each time point the corresponding tube will be removed and the sample will be diluted 1:10 to 1:10^5 in PBS. Each dilution will be spotted (10µl) in triplicate on RCM agar plates and incubated under anaerobic conditions using a Gas-Pak (BD) for 4-7 days at 37°C. Following incubation, the plate will be visually examined and the bacterial counts for each time point will be determined.

[0154] The best in vitro performing DRM1-retinoid combination, as determined by the MIC assay and time-kill assay, will be formulated into a topical cream, gel, or solution applied with pads. The topical formulation will be applied to acne infected areas twice a day (morning and night) following cleansing of the skin.

[0155] It is expected that the application of a DRM1-retinoid combination to acne infected skin will decrease the acne lesion counts when compared to controls – placebo, DRM1 alone or retinoid alone. It is expected that the count of both inflammatory and non-inflammatory lesions will decrease compared to controls.

[0156] Other Embodiments

[0157] The detailed description set-forth above is provided to aid those skilled in the art in practicing the present invention. However, the invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed because these embodiments are intended as illustration of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description

which do not depart from the spirit or scope of the present inventive discovery. Such modifications are also intended to fall within the scope of the appended claims.

[0158] References Cited

[0159] All publications, patents, patent applications and other references cited in this application are incorporated herein by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application or other reference was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. Citation of a reference herein shall not be construed as an admission that such is prior art to the present invention.

[0160] Other publications incorporated herein by reference in their entirety include:

[0161] Wang Y(1), Kuo S, Shu M, Yu J, Huang S, Dai A, Two A, Gallo RL, Huang CM. Staphylococcus epidermidis in the human skin microbiome mediates fermentation to inhibit the growth of *Propionibacterium acnes*: implications of probiotics in acne vulgaris. Appl Microbiol Biotechnol. 2014 Jan;98(1):411-24. doi: 10.1007/s00253-013-5394-8. Epub 2013 Nov 22.

CLAIMS

What is claimed is:

1. A topical composition for treating acne vulgaris, the composition comprising a combination of succinic acid and at least one other API suitable for the treatment of acne in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and API produces an improved effectiveness in treating acne vulgaris..

2. A topical composition for treating acne vulgaris, the composition comprising a combination of salicylic acid and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and salicylic acid produces a synergistic effect in treating acne vulgaris.

3. The composition of claims 1 or 2, wherein the salicylic acid and succinic acid are present in 1:2 ratio.

4. The composition of claims 1 or 2, wherein salicylic acid is present at concentration from 0.1 to 5%.

5. The composition of claims 1 or 2, wherein succinic acid is present at concentration from 0.1 to 10%.

6. A topical composition for treating acne vulgaris, the composition comprising a combination of azelaic acid and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and azelaic acid produces an improved effectiveness in treating acne vulgaris.

7. A topical composition for treating acne vulgaris, the composition comprising a combination of picolinic acid and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and picolinic acid produces an improved effect in treating acne vulgaris.

8. The composition of claim 7, wherein the picolinic acid is present in the composition, in an amount suitable for delivering from about 0.12 mM to about 1.88 mM to the skin and wherein the succinic acid is present in the composition, in an amount suitable for delivering from about 0.9 mM to about 15 mM to the skin.

9. The composition of claim 10, wherein the picolinic acid is present in the composition, in an amount suitable for delivering about 0.94 mM to the skin and the succinic acid is present in the composition, in an amount suitable for delivering about 7.5 mM.

10. A topical composition for treating acne vulgaris, the composition comprising a combination of benzoyl peroxide and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and benzoyl peroxide produces an improved effect in treating acne vulgaris.

11. A topical composition for treating acne vulgaris, the composition comprising a combination of antibiotic and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and antibiotic produces an improved effect in treating acne vulgaris in subjects with antibiotic resistant P. acnes strains.

12. A topical composition for treating acne vulgaris, the composition comprising a combination of retinoid and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and retinoid produces an improved effect in treating acne vulgaris

13. A method of treating acne vulgaris, the method comprising administering to a subject in need thereof, a topical composition comprising a combination of salicylic acid and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and salicylic acid produces a synergistic effect in treating acne vulgaris.

14. A method of treating acne vulgaris, the method comprising administering to a subject in need thereof, a topical composition comprising a combination of azelaic acid and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and azelaic acid produces an improved effect in treating acne vulgaris.

15. A method of treating acne vulgaris, the method comprising administering to a subject in need thereof, a topical composition comprising a combination of picolinic acid and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and picolinic acid produces an improved effect in treating acne vulgaris.

16. A method of treating acne vulgaris, the method comprising administering to a subject in need thereof, a topical composition comprising a combination of benzoyl peroxide and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and benzoyl peroxide produces an improved effect in treating acne vulgaris.

17. A method of treating acne vulgaris, the method comprising administering to a subject in need thereof, a topical composition comprising a combination of antibiotic and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and antibiotic produces an improved effect in treating acne vulgaris in subjects with antibiotic resistant P. acnes strains.

18. The method of claim 17, wherein the antibiotic is selected from the group consisting of erythromycin, clindamycin, minocycline, and doxycycline.

19. A method of treating acne vulgaris, the method comprising administering to a subject in need thereof, a topical composition comprising a combination of retinoid and succinic acid in a pharmaceutically acceptable preparation, wherein the combination of succinic acid and retinoid produces an improved effect in treating acne vulgaris.

20. A formulation for the treatment of acne vulgaris comprising salicylic acid (0.5-2w/w%), organic aloe vera (0-50w/w%), propanediol (15w/w%), organic grain alcohol (10-20w/w%), organic lemon essential oil (0-1w/w%), grapefruit seed extract (0-1w/w%), sodium hydroxide to adjust the solution pH to 3.9-4.5, and SE Microbiome Complex.

21. The formulation of claim 20, wherein the SE Microbiome complex comprises 0.5-4w/w% succinic acid, 1-3w/w% Brij L4, 1-5w/w% dimethyl isosorbide, 0-5w/w% carbohydrate-based prebiotic agent.

22. The formulation of claim 21, wherein the succinic acid is prepared by one of microbial fermentation or by chemical synthesis.

23. The formulation of claim 21, wherein sterile conditioned media prepared by fermenting *S. epidermidis* is used instead or in addition to succinic acid.

24. The formulation of claims 20 through 23, wherein organic components in the formulation are replaced by non-organic equivalent components.

25. The formulation of claims 20 through 24, wherein the formulated solution is poured over pads in a container.

26. A container comprising the pads of claim 25.

27. The formulation of claims 20 through 25, wherein gelling agent (hydroxyethyl cellulose, eco-gel or other gelling agent) is added at a concentration of 0.5-3w/w% to the solution to gel the solution.

28. The formulation of claims 20 through 27, wherein gel is filled into tubes for application.

29. A tube comprising the gel of claim 28.

30. A method of treating acne vulgaris of a patient, the method comprising the steps of: Washing patient areas affected by acne in the morning and evening with mild detergent; Applying solution containing SE Microbiome Complex to acne affected areas of patient and gel containing SE Microbiome Complex to individual acne lesions (pimples); Optionally Covering whiteheads and inflamed acne lesions (pimples) on patient with hydrocolloid-containing patches to treat blemishes overnight; Patient taking probiotics containing Lactobacus and Bifidobacterium (1-3 times daily) and 25-50 mg Zinc (1 time daily with a meal); and Repeating for at least 2 months.

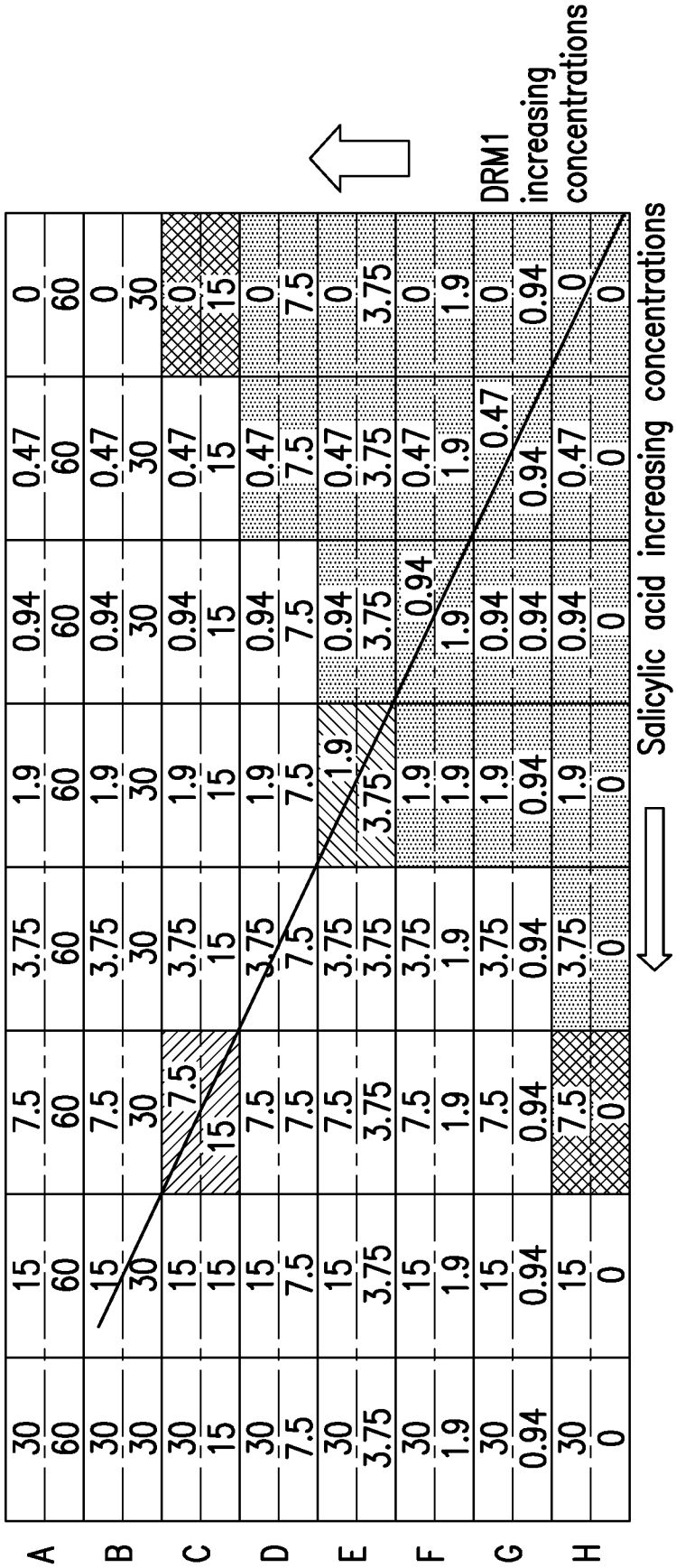

1 2 3 4 5 6 7 8
A 30 60 15 60 7.5 60 3.75 60 1.9 60 0.94 60 0.47 60 0 60
B 30 30 15 30 7.5 30 3.75 30 1.9 30 0.94 30 0.47 30 0 30
C 30 15 15 15 7.5 15 3.75 15 1.9 15 0.94 15 0.47 15 0 15
D 30 7.5 15 7.5 7.5 7.5 3.75 7.5 1.9 7.5 0.94 7.5 0.47 7.5 0 7.5
E 30 3.75 15 3.75 7.5 3.75 3.75 3.75 1.9 3.75 0.94 3.75 0.47 3.75 0 3.75
F 30 1.9 15 1.9 7.5 1.9 3.75 1.9 1.9 1.9 0.94 1.9 0.47 1.9 0 1.9
G 30 0.94 15 0.94 7.5 0.94 3.75 0.94 1.9 0.94 0.94 0.94 0.47 0.94 0 0.94
H 30 0 15 0 7.5 0 3.75 0 1.9 0 0.94 0 0.47 0 0 0
Salicylic acid increasing concentrations
DRM1 increasing concentrations

FIG. 1

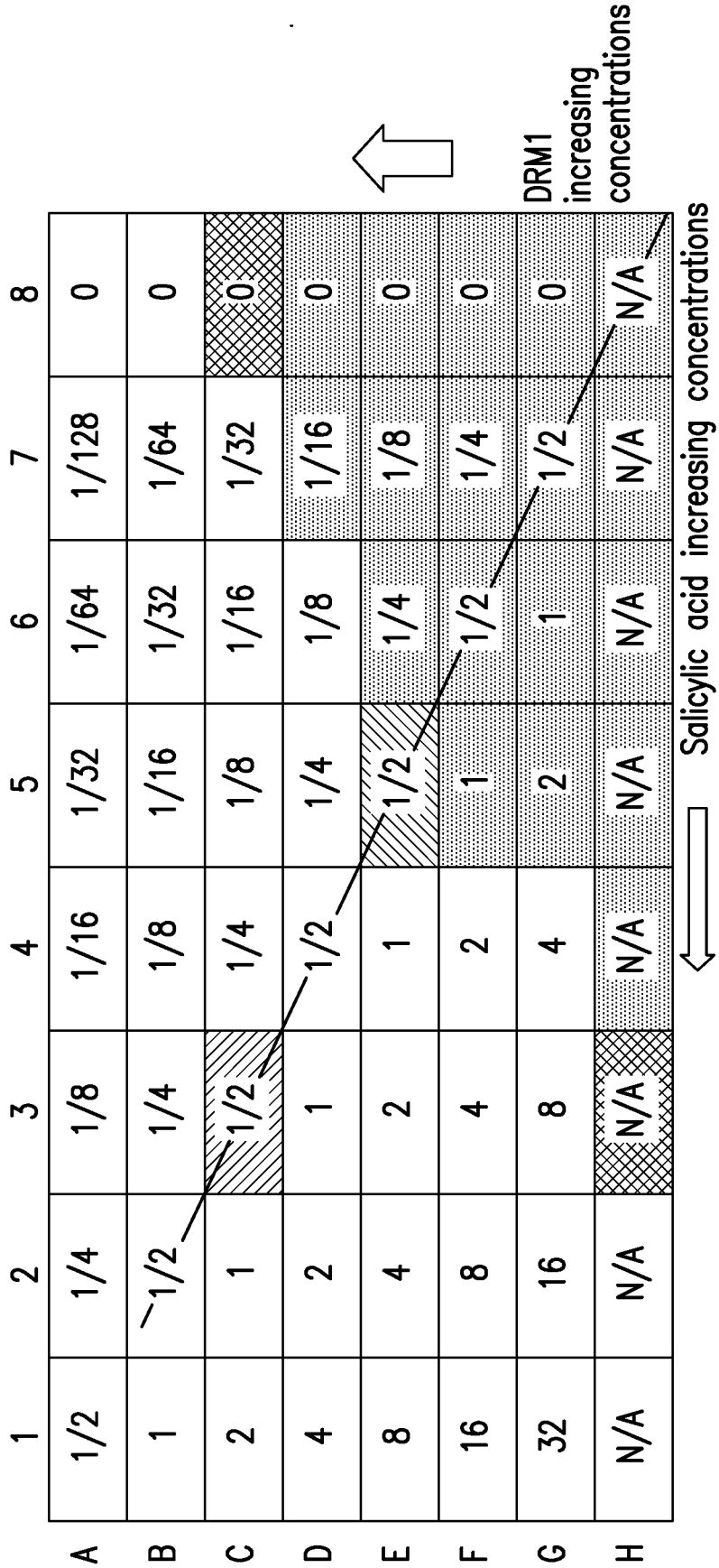
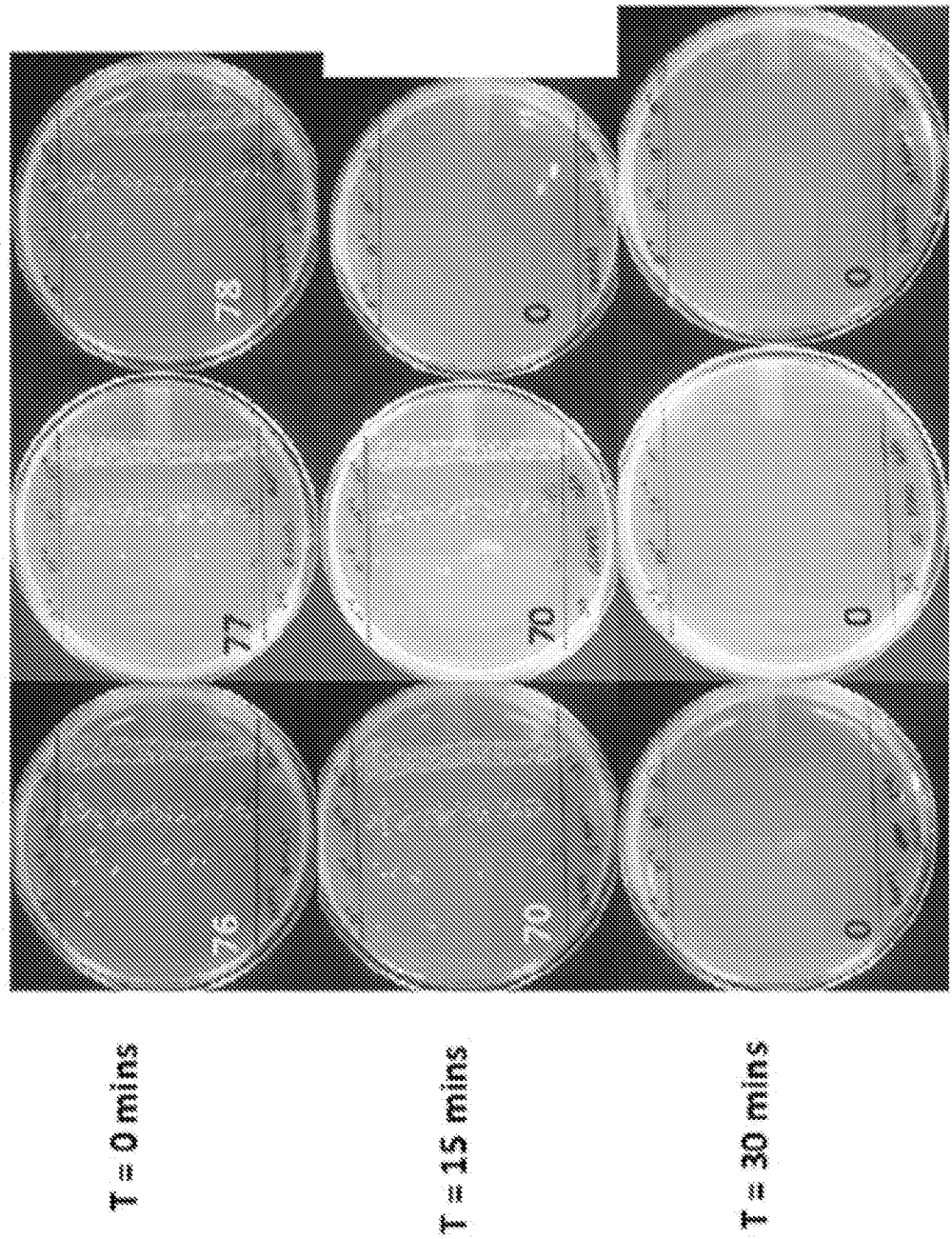

DRM1 increasing concentrations
Salicylic acid increasing concentrations
1 2 3 4 5 6 7 8
A 1/2 1/4 1/8 1/16 1/32 1/64 1/128 0
B 1 1/2 1/4 1/8 1/16 1/32 1/64 0
C 2 1 1/2 1/4 1/8 1/16 1/32 0
D 4 2 1 1/2 1/4 1/8 1/16 0
E 8 4 2 1 1/2 1/4 1/8 0
F 16 8 4 2 1 1/2 1/4 0
G 32 16 8 4 2 1 1/2 0
H N/A N/A N/A N/A N/A N/A N/A N/A

FIG.2

FIG. 3


2x MIC DRM1
2x MIC SA
DRM1/SA combo
T = 0 mins
T = 15 mins
T = 30 mins
76
77
78
70
70
0
0
0
0

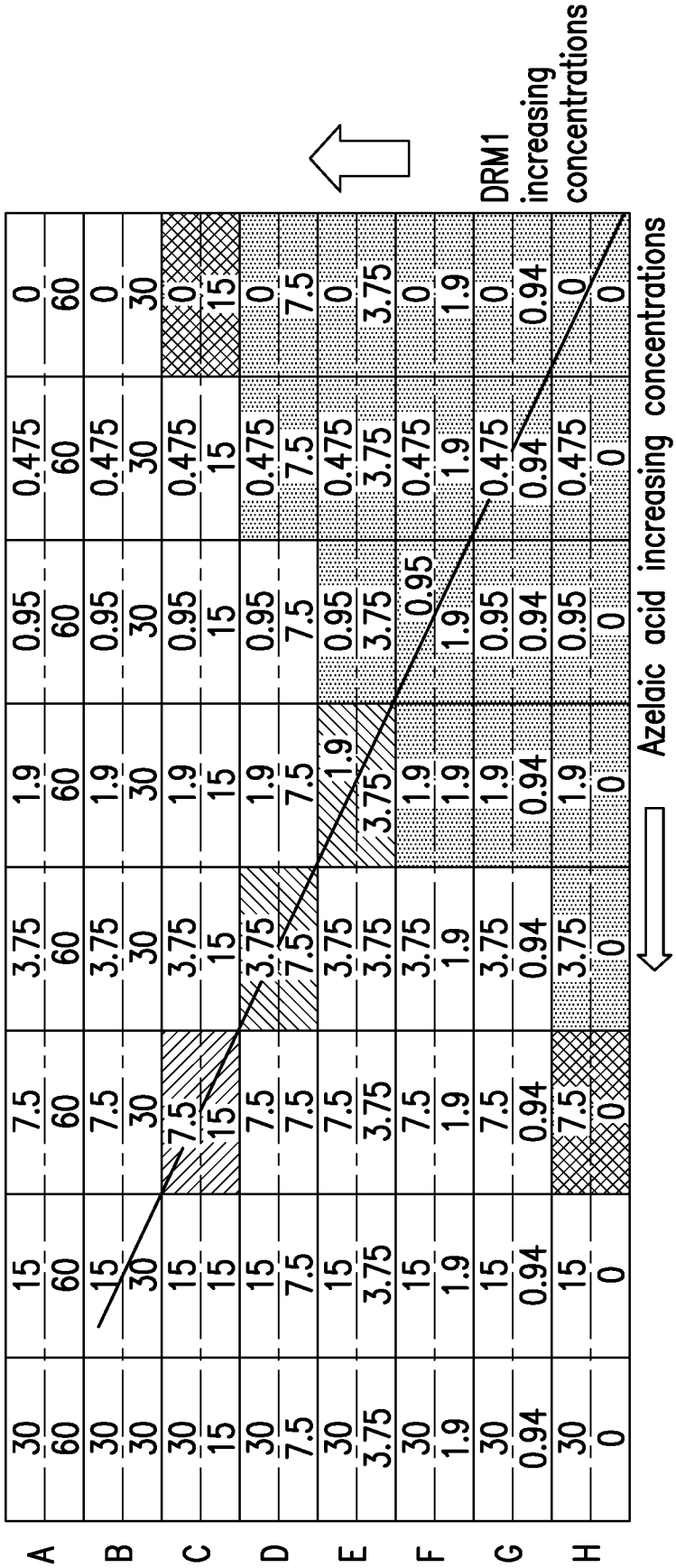

1 2 3 4 5 6 7 8
A 30 60 15 60 7.5 60 3.75 60 1.9 60 0.95 60 0.475 60 0 60
B 30 30 15 30 7.5 30 3.75 30 1.9 30 0.95 30 0.475 30 0 30
C 30 15 15 15 7.5 15 3.75 15 1.9 15 0.95 15 0.475 15 0 15
D 30 7.5 15 7.5 7.5 7.5 3.75 7.5 1.9 7.5 0.95 7.5 0.475 7.5 0 7.5
E 30 3.75 15 3.75 7.5 3.75 3.75 3.75 1.9 3.75 0.95 3.75 0.475 3.75 0 3.75
F 30 1.9 15 1.9 7.5 1.9 3.75 1.9 1.9 1.9 0.95 1.9 0.475 1.9 0 1.9
G 30 0.94 15 0.94 7.5 0.94 3.75 0.94 1.9 0.94 0.95 0.94 0.475 0.94 0 0.94
H 30 0 15 0 7.5 0 3.75 0 1.9 0 0.95 0 0.475 0 0 0
DRM1 increasing concentrations
Azelaic acid increasing concentrations

FIG.4

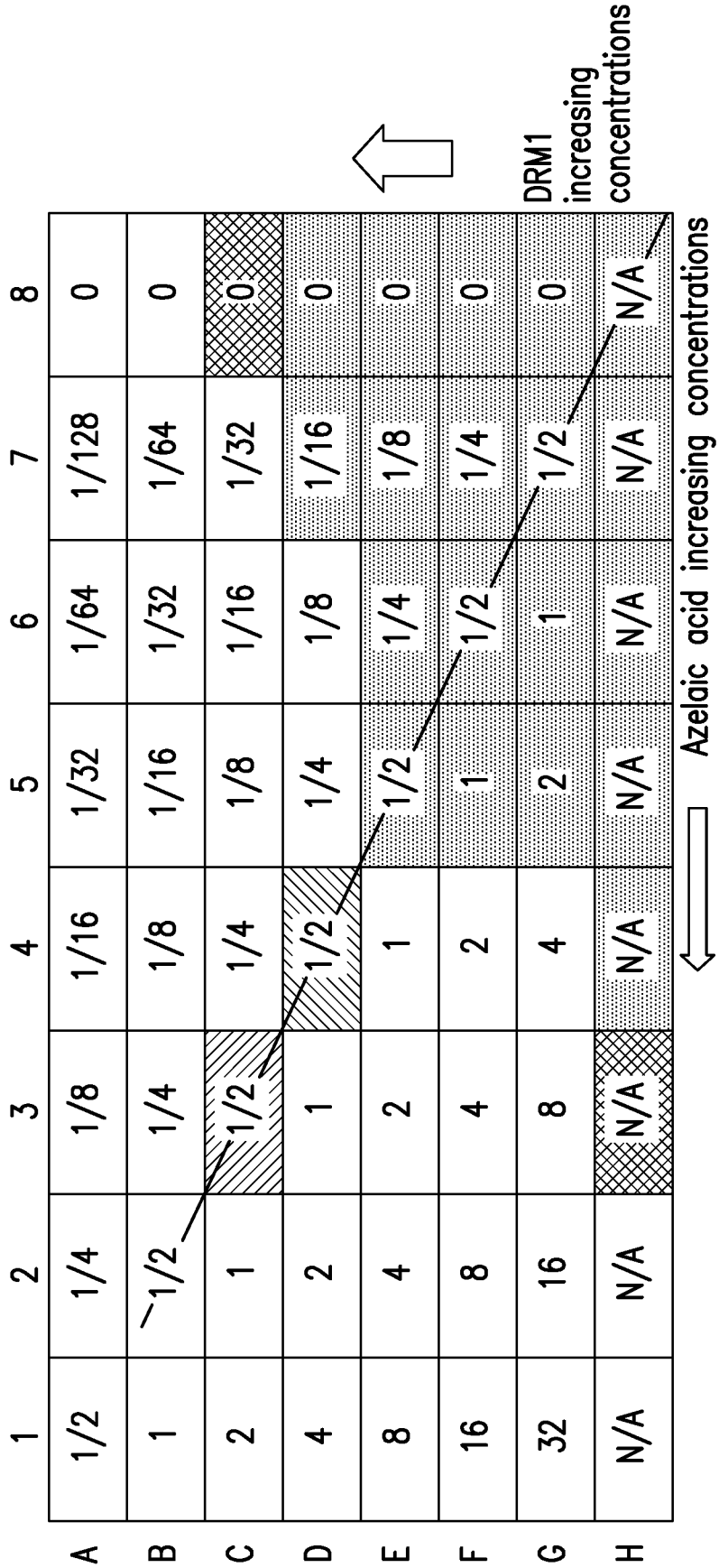

DRM1 increasing concentrations
Azelaic acid increasing concentrations
1 2 3 4 5 6 7 8
A 1/2 1/4 1/8 1/16 1/32 1/64 1/128 0
B 1 1/2 1/4 1/8 1/16 1/32 1/64 0
C 2 1 1/2 1/4 1/8 1/16 1/32 0
D 4 2 1 1/2 1/4 1/8 1/16 0
E 8 4 2 1 1/2 1/4 1/8 0
F 16 8 4 2 1 1/2 1/4 0
G 32 16 8 4 2 1 1/2 0
H N/A N/A N/A N/A N/A N/A N/A N/A

FIG.5

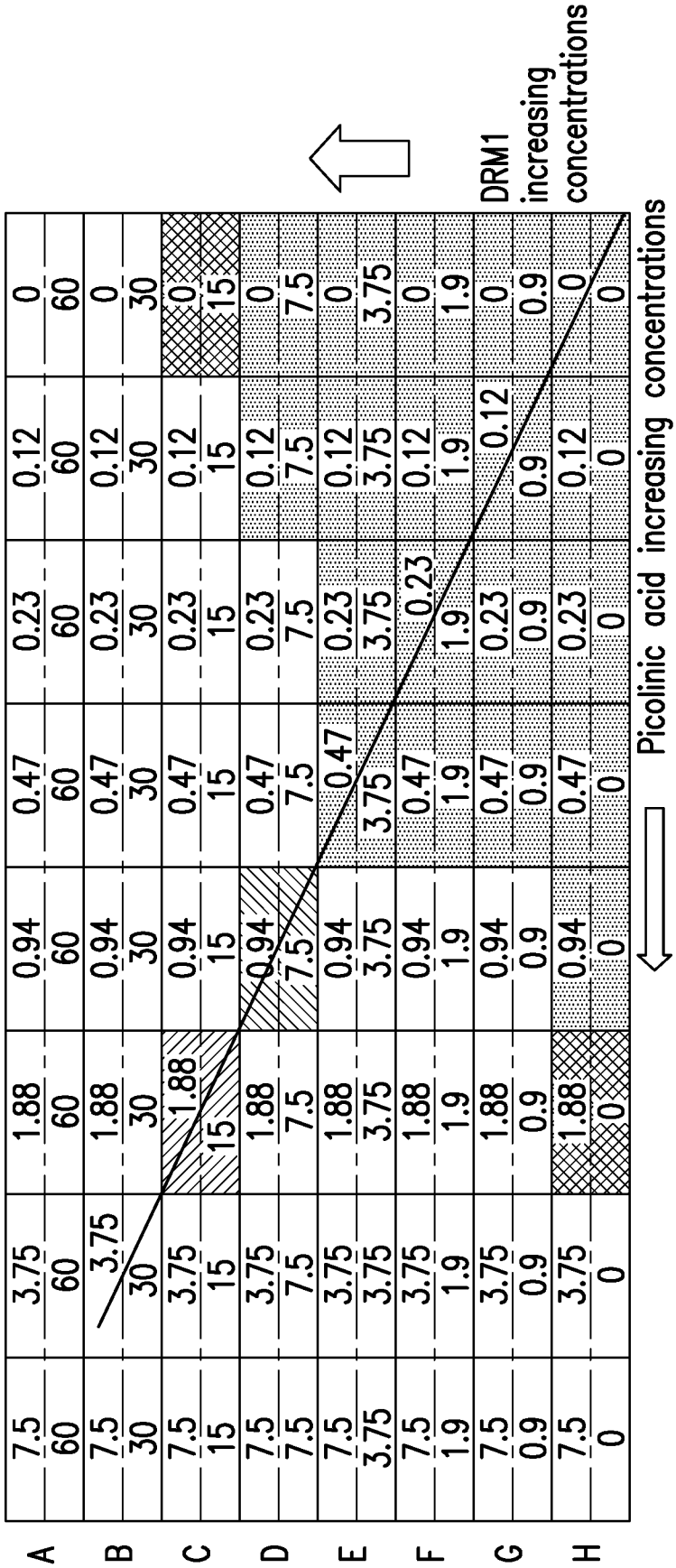

1
2
3
4
5
6
7
8
A
B
C
D
E
F
G
H
DRM1 increasing concentrations
Picolinic acid increasing concentrations

FIG.6

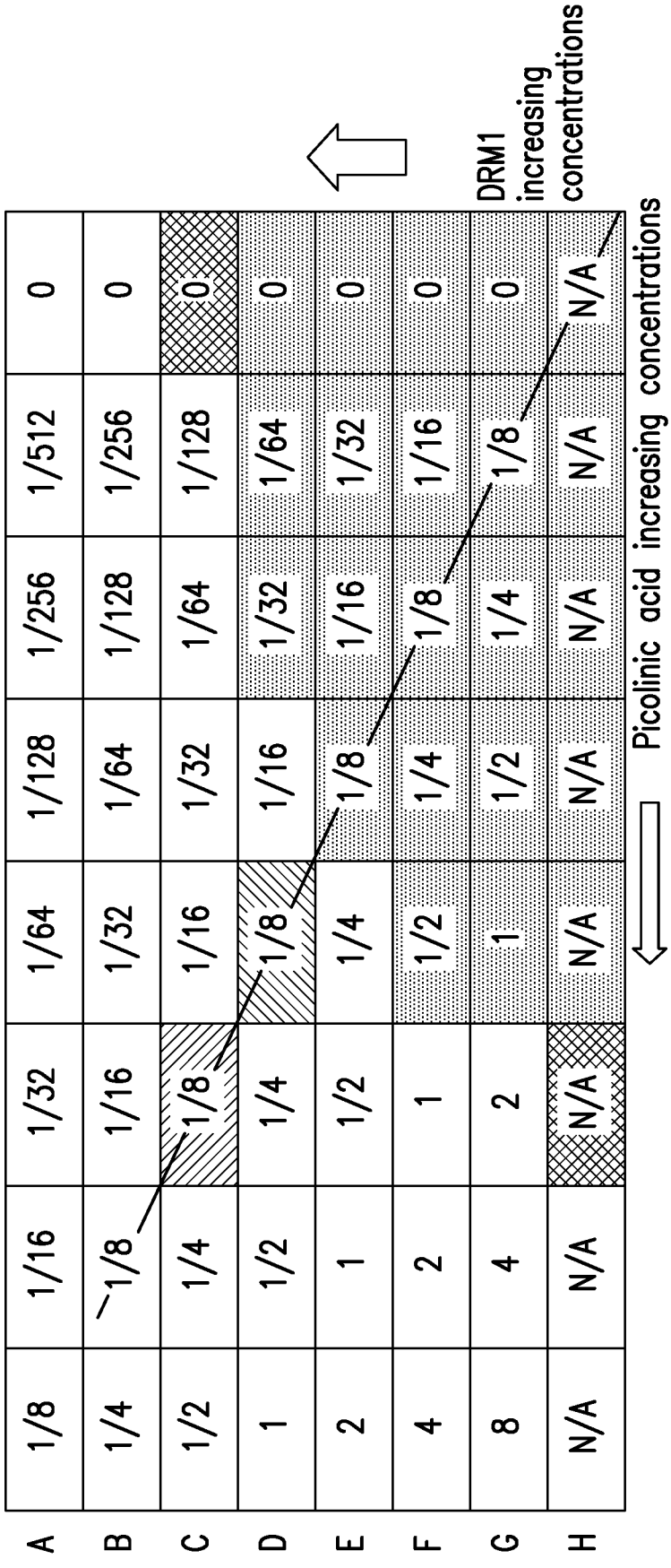

DRM1 increasing concentrations
Picolinic acid increasing concentrations
1 2 3 4 5 6 7 8
A 1/8 1/16 1/32 1/64 1/128 1/256 1/512 0
B 1/4 1/8 1/16 1/32 1/64 1/128 1/256 0
C 1/2 1/4 1/8 1/16 1/32 1/64 1/128 0
D 1 1/2 1/4 1/8 1/16 1/32 1/64 0
E 2 1 1/2 1/4 1/8 1/16 1/32 0
F 4 2 1 1/2 1/4 1/8 1/16 0
G 8 4 2 1 1/2 1/4 1/8 0
H N/A N/A N/A N/A N/A N/A N/A N/A

FIG. 7

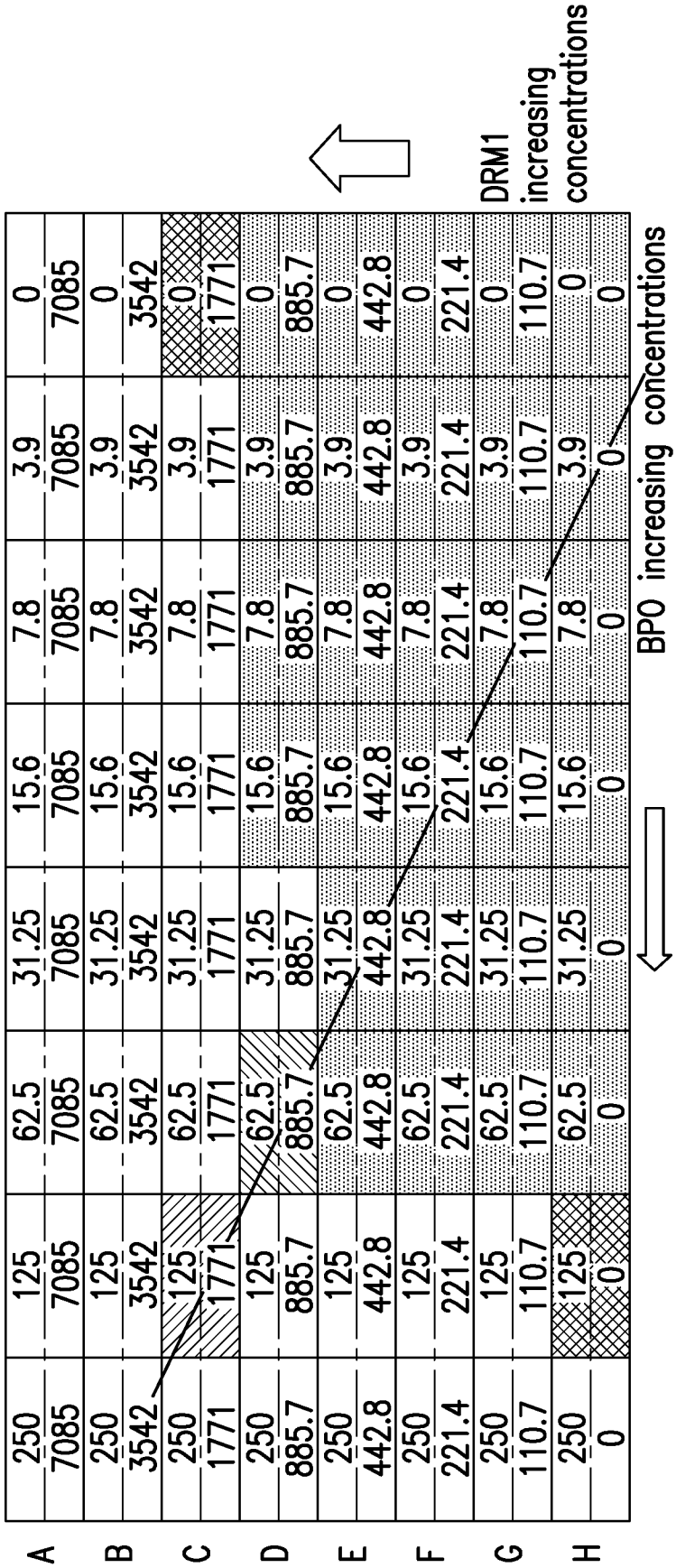

DRM1 increasing concentrations
BP0 increasing concentrations
A
B
C
D
E
F
G
H
1
2
3
4
5
6
7
8

FIG.8

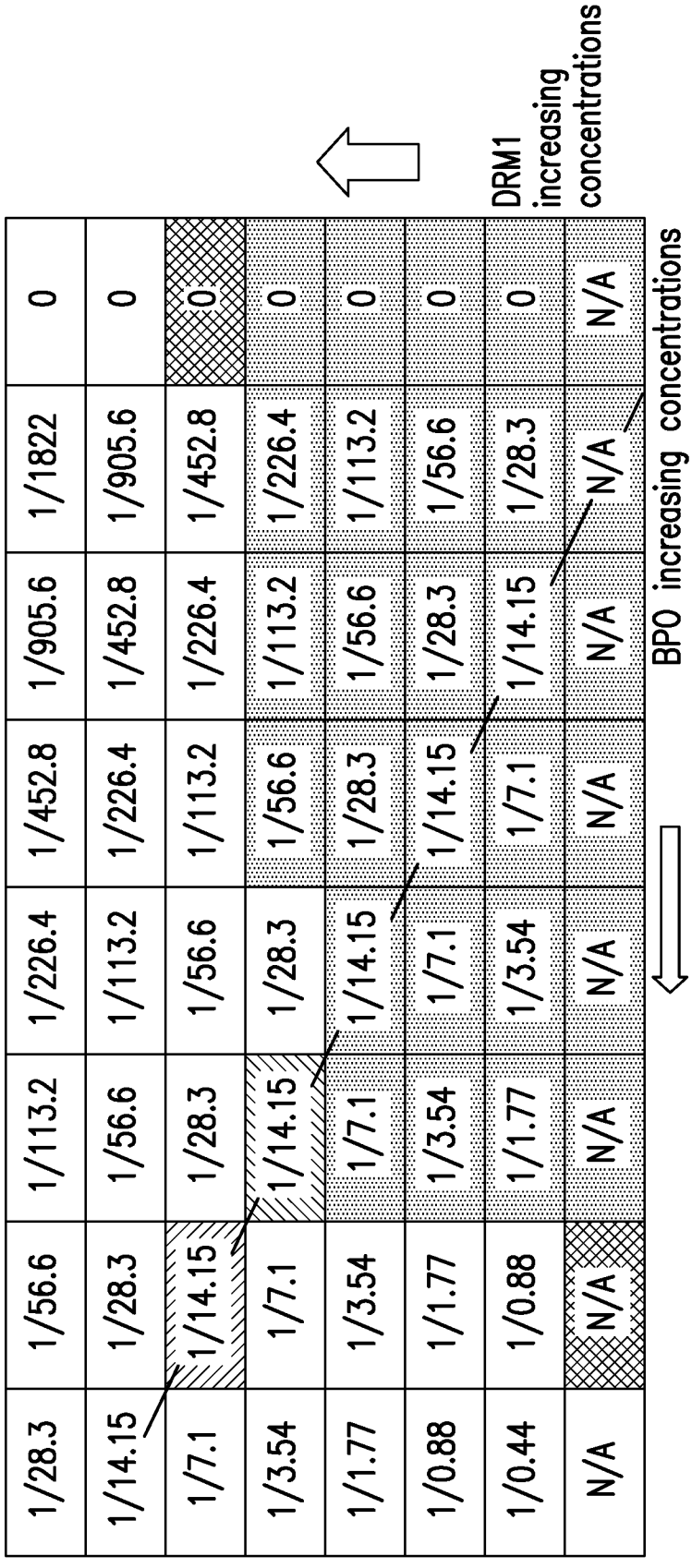

DRM1 increasing concentrations
BPO increasing concentrations
1 2 3 4 5 6 7 8
A 1/28.3 1/56.6 1/113.2 1/226.4 1/452.8 1/905.6 1/1822 0
B 1/14.15 1/28.3 1/56.6 1/113.2 1/226.4 1/452.8 1/905.6 0
C 1/7.1 1/14.15 1/28.3 1/56.6 1/113.2 1/226.4 1/452.8 0
D 1/3.54 1/7.1 1/14.15 1/28.3 1/56.6 1/113.2 1/226.4 0
E 1/1.77 1/3.54 1/7.1 1/14.15 1/28.3 1/56.6 1/113.2 0
F 1/0.88 1/1.77 1/3.54 1/7.1 1/14.15 1/28.3 1/56.6 0
G 1/0.44 1/0.88 1/1.77 1/3.54 1/7.1 1/14.15 1/28.3 0
H N/A N/A N/A N/A N/A N/A N/A N/A

FIG.9

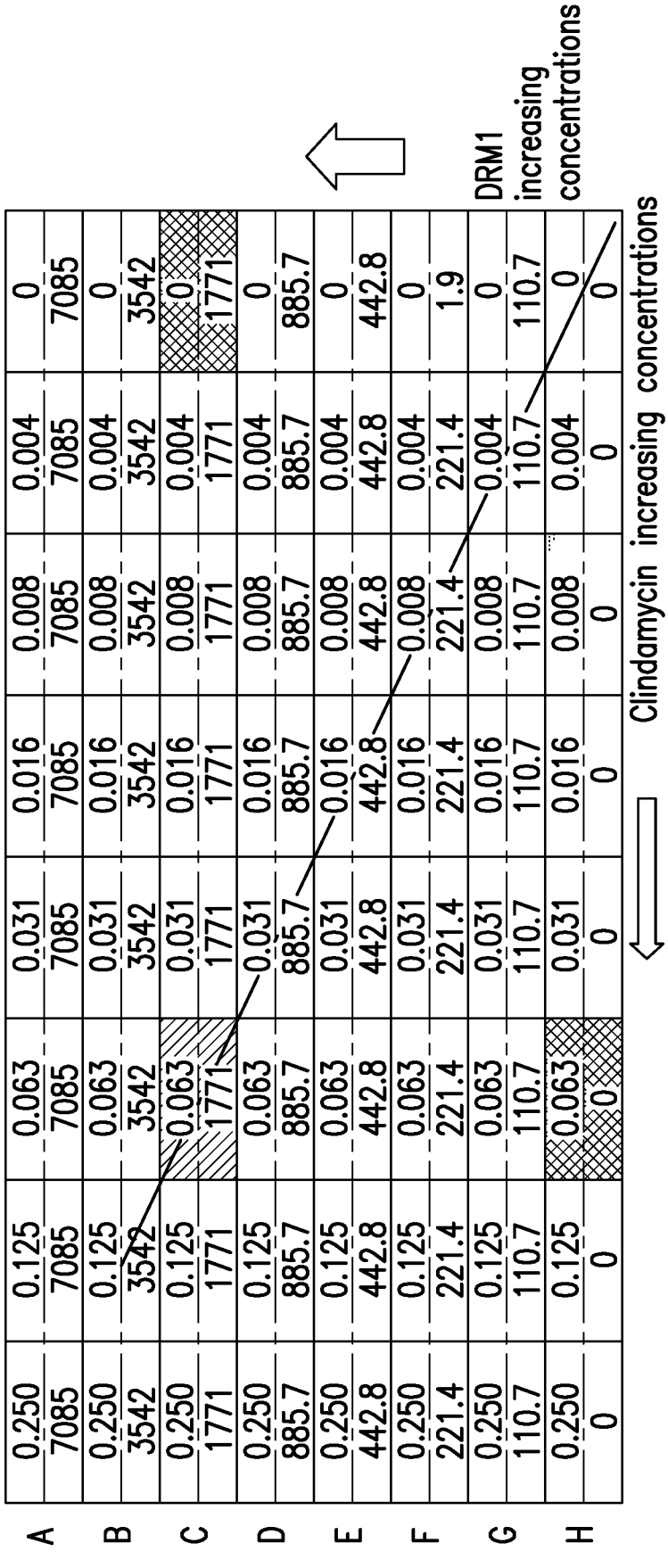

DRM1 increasing concentrations
Clindamycin increasing concentrations
1 2 3 4 5 6 7 8
A 0.250 0.125 0.063 0.031 0.016 0.008 0.004 0
B 0.250 0.125 0.063 0.031 0.016 0.008 0.004 0
C 0.250 0.125 0.063 0.031 0.016 0.008 0.004 0
D 0.250 0.125 0.063 0.031 0.016 0.008 0.004 0
E 0.250 0.125 0.063 0.031 0.016 0.008 0.004 0
F 0.250 0.125 0.063 0.031 0.016 0.008 0.004 0
G 0.250 0.125 0.063 0.031 0.016 0.008 0.004 0
H 0.250 0.125 0.063 0.031 0.016 0.008 0.004 0
7085 7085 7085 7085 7085 7085 7085 7085
3542 3542 3542 3542 3542 3542 3542 3542
1771 1771 1771 1771 1771 1771 1771 1771
885.7 885.7 885.7 885.7 885.7 885.7 885.7 885.7
442.8 442.8 442.8 442.8 442.8 442.8 442.8 442.8
221.4 221.4 221.4 221.4 221.4 221.4 221.4 1.9
110.7 110.7 110.7 110.7 110.7 110.7 110.7 110.7
0 0 0 0 0 0 0 0

FIG.10

FIG. 11

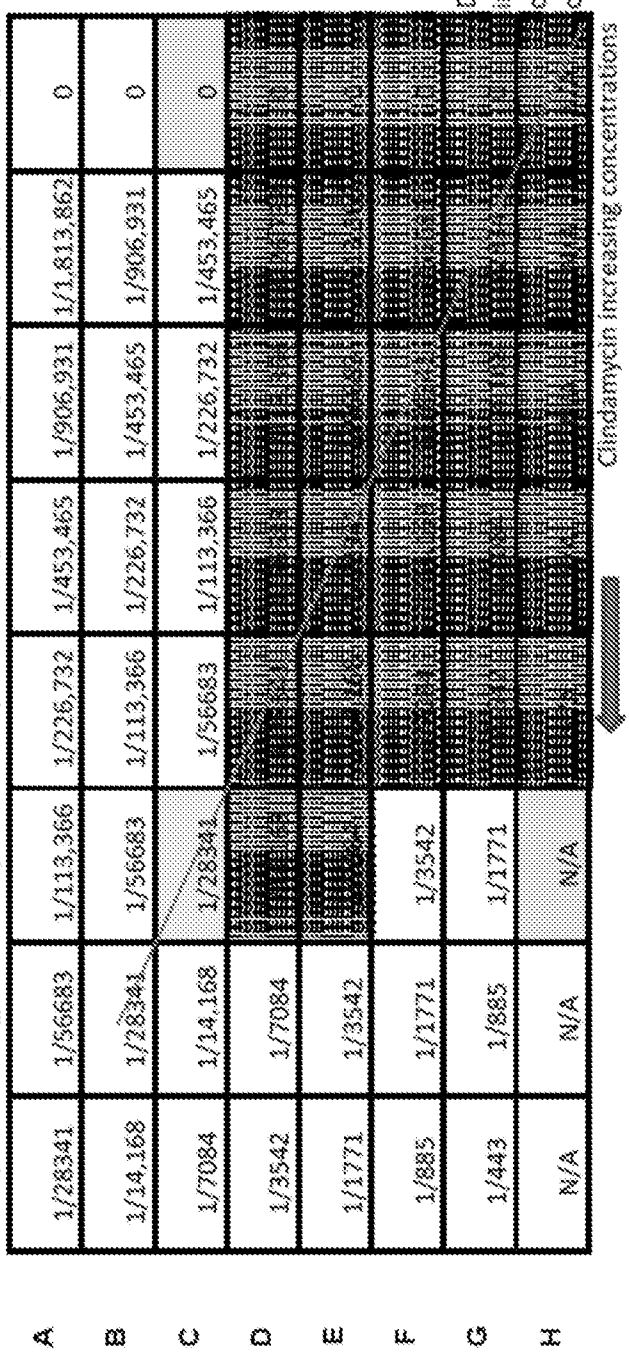

1 2 3 4 5 6 7 8
A 1/28341 1/56683 1/113,366 1/226,732 1/453,465 1/906,931 1/1,813,862 0
B 1/14,168 1/28341 1/56683 1/113,366 1/226,732 1/453,465 1/906,931 0
C 1/7084 1/14,168 1/28341 1/56683 1/113,366 1/226,732 1/453,465 0
D 1/3542 1/7084
E 1/1771 1/3542
F 1/885 1/1771 1/3542
G 1/443 1/885 1/1771
H N/A N/A N/A
Cindamycin increasing concentrations
ORM1 increasing concentrations

FIG. 12

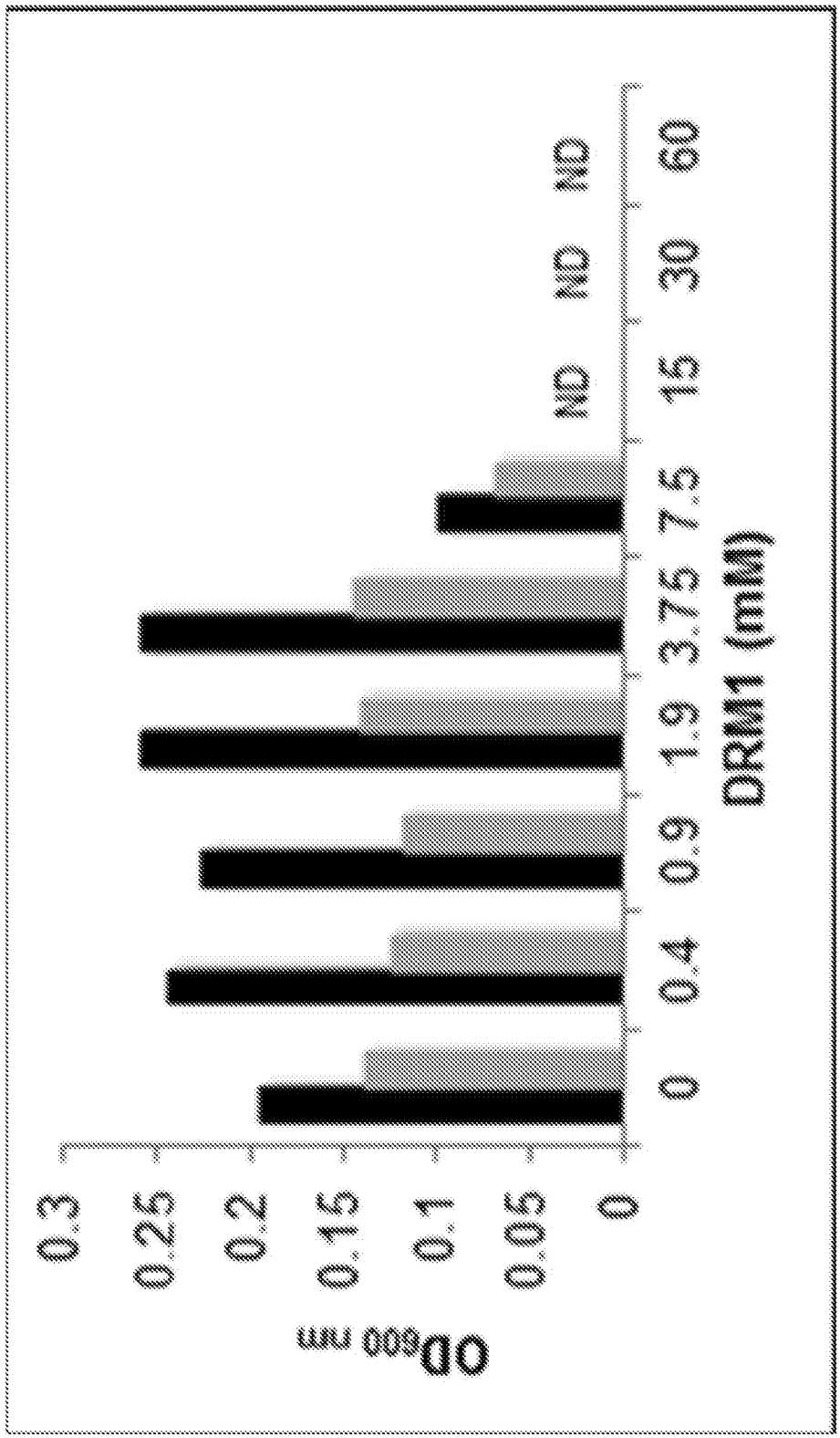
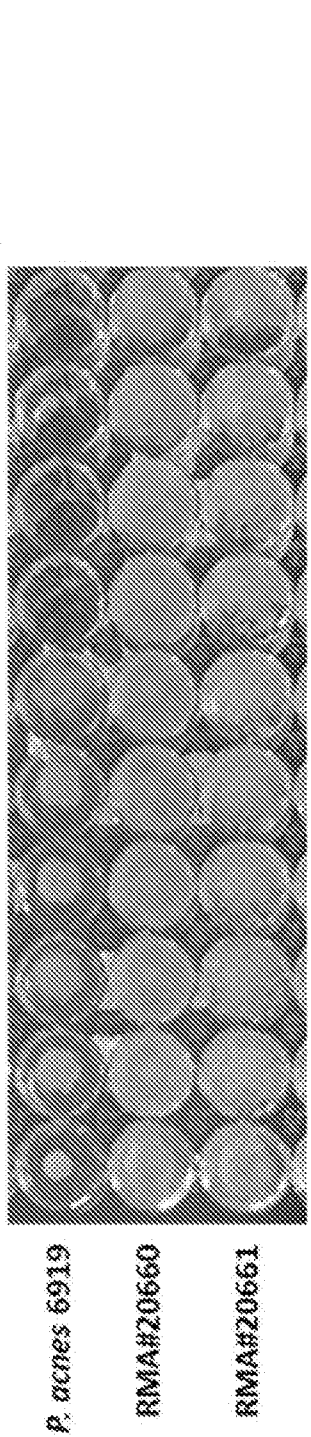

OD600 nm
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.4
0.9
1.9
3.75
7.5
15
30
60
ND
ND
ND
DRM1 (mM)

FIG. 13


0
.004
.008
.015
.03
.06
.125
.25
.5
1
ug/ml Clindamycin
P. acnes 6919
RMA#20660
RMA#20661

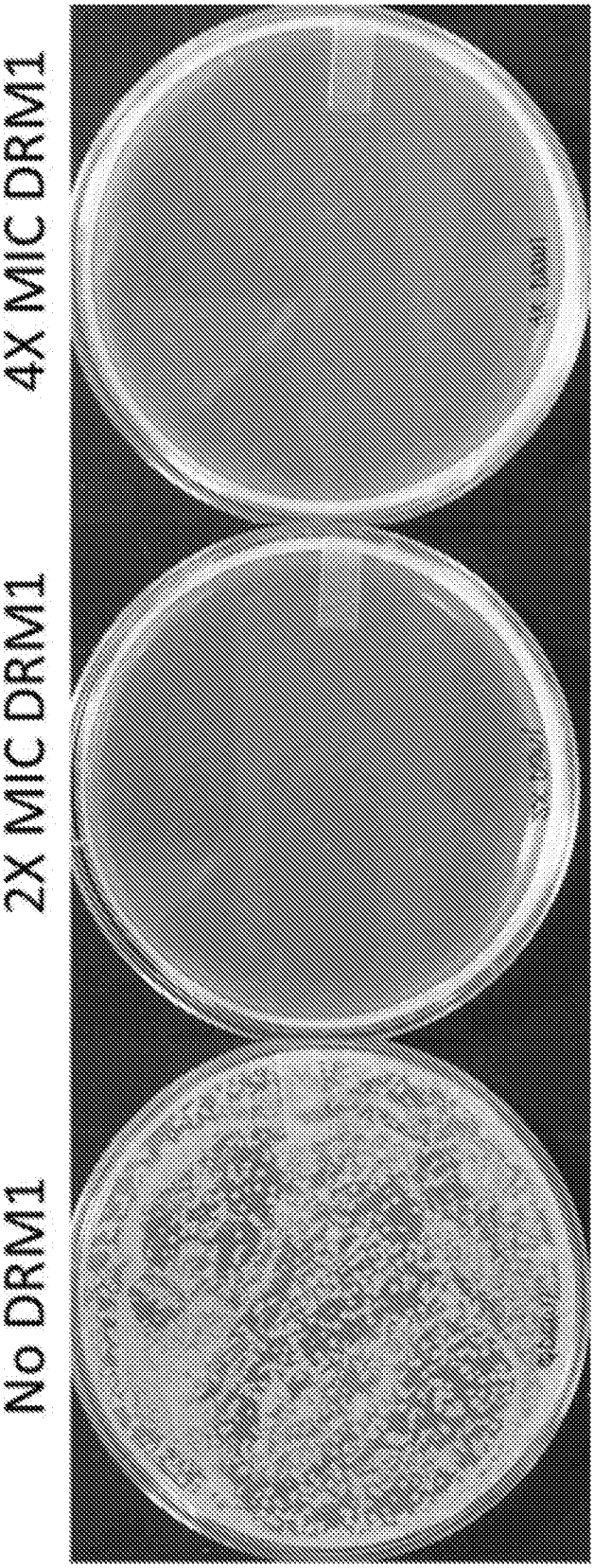


FIG. 14

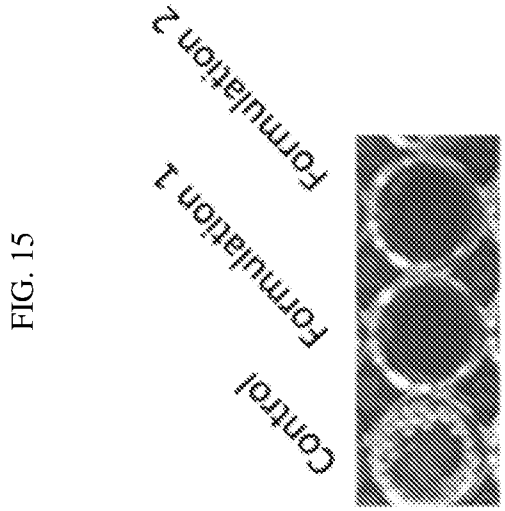
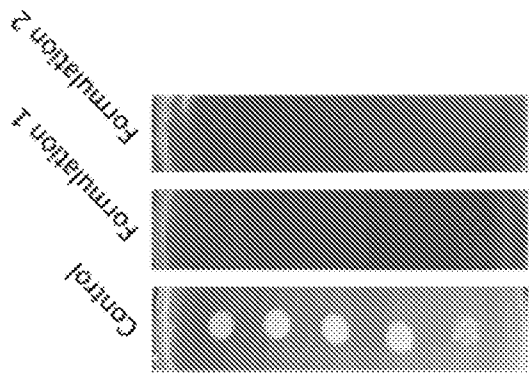


FIG. 15

FIG. 16



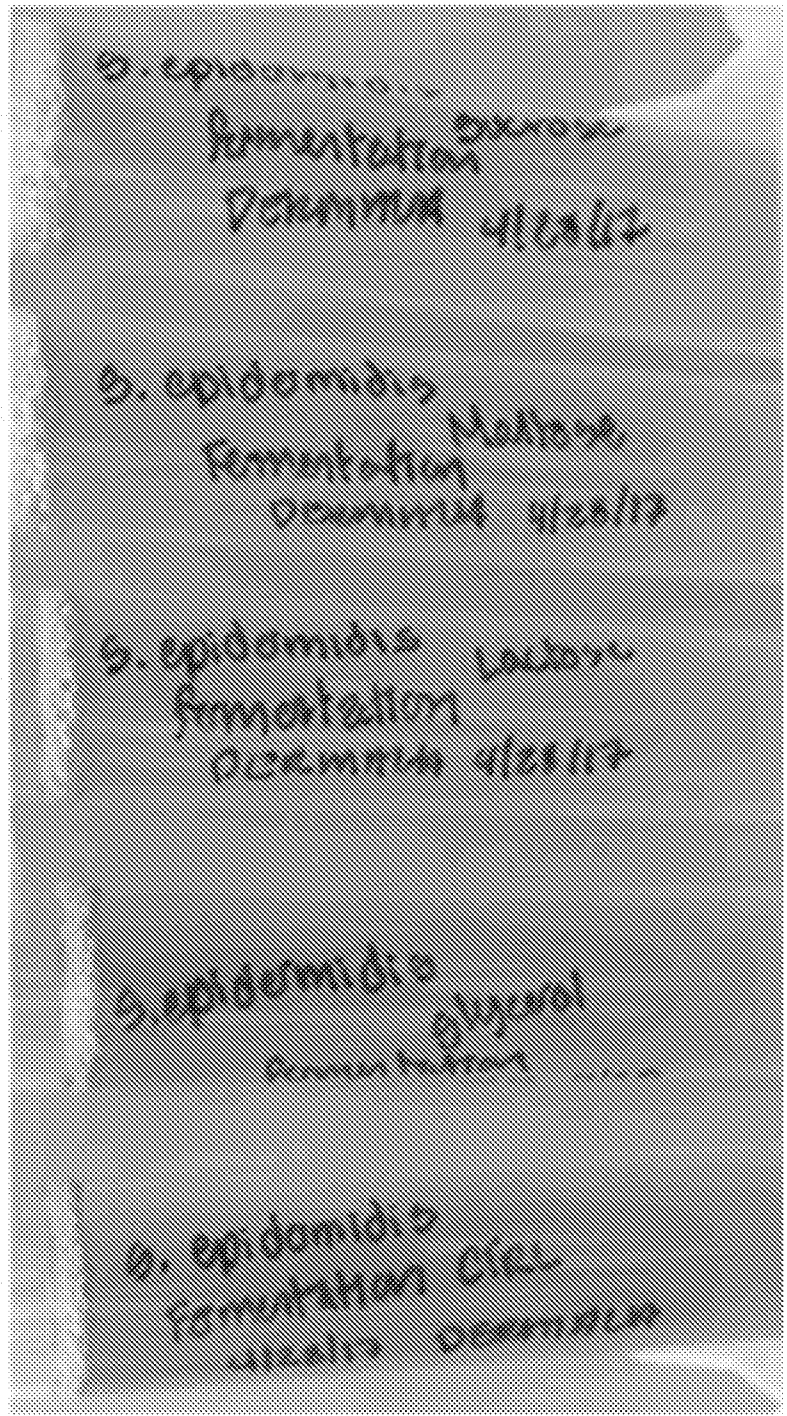


FIG. 17

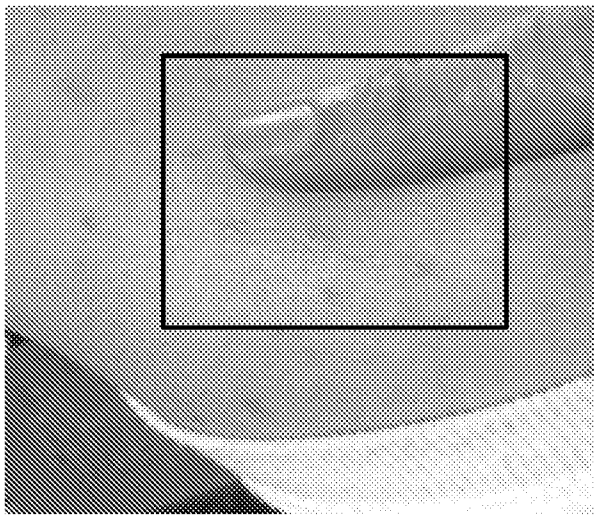


FIG.18C

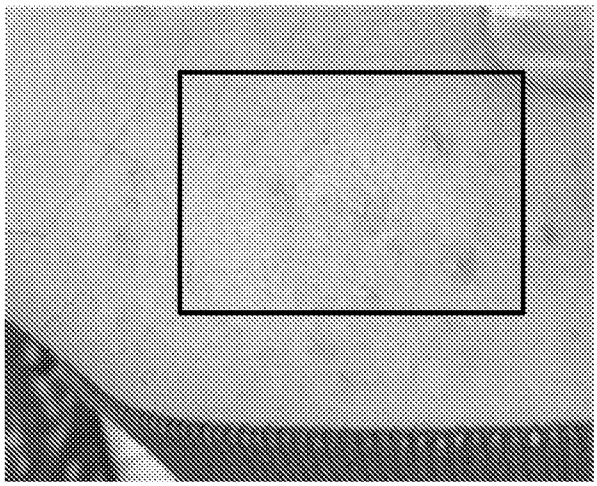


FIG.18B

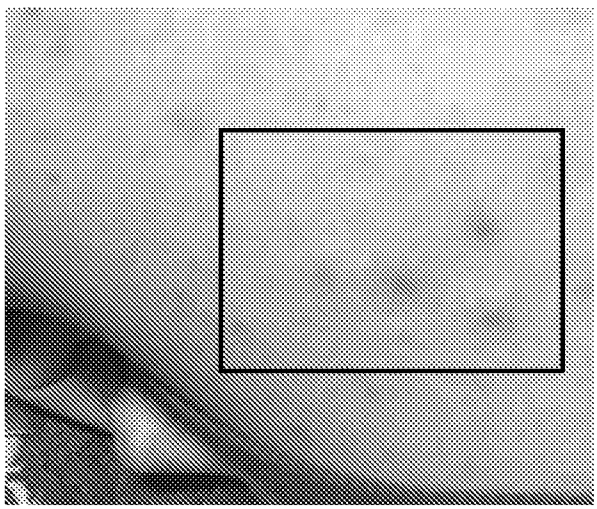


FIG.18A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/33346

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.: 24-29
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-19, directed a topical composition for treating acne vulgaris, the composition comprising a combination of succinic acid and at least one other API suitable for the treatment of acne

Group II: Claims 20-23, directed to a formulation for the treatment of acne vulgaris comprising salicylic acid (0.5-2w/w%), organic aloe vera (0-50w/w%), propanediol (15w/w%), organic grain alcohol (10-20w/w%), organic lemon essential oil (0-1w/w%), grapefruit seed extract (0-1w/w%), sodium hydroxide

*****Continued in Supplemental Box*****

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 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.:
1-19

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/US 17/33346

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 47/14, A61K 47/44, A61K 8/38 (2017.01)
CPC - A61K 31/327, A61K 9/0014, A61K 2800/59, A61K 8/368

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)
See Search History Document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 9,265,725 B2 (Tamarkin et al.) 23 February 2016 (23.02.2016); col 3, ln 40; col 12, ln 48-63; col 14, ln 47-48; col 35, ln 40-44	1, (3-5)/1
X --- Y	US 2007/0292355 A1 (Tamarkin et al.) 20 December 2007 (20.12.2007); abstract; para [0026], [0065], [0068], [0189], [0365]; claim 12	2, (3-5)/2, 6, 13-14 --- 18
X	US 2005/0239723 A1 (Amin et al.) 27 October 2005 (27.10.2005); para [0009], [0012], [0027], [0038]	7-9, 15
X	US 8,957,112 B2 (Mallard et al.) 17 February 2015 (17.02.2015); col 4, ln 8-21; col 9, ln 17-24; col 10, ln 65-67	10, 12, 16, 19
X --- Y	US 9,301,935 B2 (Uddin) 5 April 2016 (05.04.2016); col 2, ln 30-39, ln 57-64; col 5, ln 63-64; col 10, ln 22-23	11, 17 --- 18
A	US 2007/0048234 A1 (Waugh et al.) 1 March 2007 (01.03.2007); para [0036]	7, 15

☐ 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	Date of mailing of the international search report
14 July 2017	27 SEP 2017

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/33346

Continuation of Box III (Observations where unity of invention is lacking):

Group III: Claims 30, directed to a method of treating acne vulgaris of a patient, the method comprising the steps of: Washing patient areas affected by acne in the morning and evening with mild detergent; Applying solution containing SE Microbiome Complex to acne affected areas of patient and gel containing SE Microbiome Complex to individual acne lesions (pimples); Optionally Covering whiteheads and inflamed acne lesions (pimples) on patient with hydrocolloid-containing patches to treat blemishes overnight; Patient taking probiotics containing Lactobacus and Bifidobacterium (l-3 times daily) and 25-50 mg Zinc (1 time daily with a meal); and Repeating for at least 2 months.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I requires topical composition for treating acne vulgaris, the composition comprising a combination of succinic acid and at least one other API suitable for the treatment of acne, not required by groups II-III.

Group II requires a formulation for the treatment of acne vulgaris comprising salicylic acid, organic aloe vera, propanediol, organic grain alcohol, organic lemon essential oil, grapefruit seed extract, sodium hydroxide, not required by groups I and III

Common Technical Features:

Groups I-III share the technical feature formulation for the treatment of acne vulgaris comprising at least API. However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is being anticipated by US 9,265,725 B2 to Tamarkin et al. (hereinafter 'Tamarkin725'). Tamarkin725 discloses a topical composition (col 12, ln 48-49, a foamable therapeutic composition to a target site) for treating acne vulgaris (col 12, ln 59-63, wherein the disorder is selected from the group consisting of dermatological pain, dermatological inflammation, acne, acne vulgaris), the composition comprising a combination of succinic acid (col 12, ln 50-53, and a beneficially or therapeutically effective concentration of at least one benefit agent, selected from the group consisting of i. a dicarboxylic acid; col 14, ln 47-48, Non limiting examples of some elementary dicarboxylic acids (DCA's) are succinic acid) and at least one other API suitable for the treatment of acne (col 12, ln 49-50, the composition comprising a therapeutically effective amount of an active agent) in a pharmaceutically acceptable preparation (col 3, ln 40, d. a solvent selected from the group consisting of water; water is a pharmaceutically acceptable carrier, see instant specification para [0048]), wherein the combination of succinic acid and API produces an improved effectiveness in treating acne vulgaris (col 35, ln 40-44, The dicarboxylic acid or dicarboxylic ester may benefit by improving the solubility of the active agent or increasing the penetration of the active agent. The dicarboxylic acid or dicarboxylic ester may also provide a synergistic therapeutic effect in combination with the active agent).

As the shared technical features were known in the art at the time of the invention, they cannot be considered common technical features that would otherwise unify the groups. Therefore, Groups I-III lack unity under PCT Rule 13.

Note: claims 24-29 are unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).