An anti-bacterial formulation comprising (a) a pyridine thiol; (b) a bis-quinolinium salt having a cation of formula (I) wherein \( n \) is an integer from 3 to 18 and (c) a salicylic acid or derivative thereof provided that (b) and (c) are not dequalinium salicylate. An anti-bacterial formulation comprising (a) a bis-quinolinium salt having a cation of formula (I) wherein \( n \) is an integer from 3 to 18 and (b) a salicylic acid or derivative thereof provided that (b) and (c) are not dequalinium salicylate is also provided. Examples include topical anti-acne formulations comprising (1) zinc pyrithione, dequalinium chloride and salicylic acid; (2) dequalinium chloride and salicylic acid; (3) zinc pyrithione and salicylic acid and (4) zinc pyrithione and dequalinium chloride. The compositions are useful in the treatment of a condition which is caused, transmitted and/or exacerbated by propionibacteria activity, in particular acne.
Figure 1

FIC Isobologram of dequalinium chloride (DC) and zinc pyrithione (ZP) vs. P. acnes NCTC 737
Formulations

Field of the invention

This invention relates to anti-acne formulations, and to the use of certain combinations of compounds as anti-acne agents.

5 Background to the invention

Pyrithione, also known as 1-hydroxy-2(1H)-pyridinethione, 2-pyridinethiol-1-oxide or 2-mercaptopyridine N-oxide, is a type of pyridine thiol and is known for use as a bactericide and fungicide. It is typically used in the form of a metal salt (strictly, a chelated complex) such as zinc pyrithione. In particular the zinc salt is known as an antiseborrheic, an antifungal and an antibacterial agent, as well as for the treatment of scalp conditions such as dandruff and as an anti-acne agent. It has also been used as a cosmetic preservative, for inhibiting mould growth on fabrics in commercial laundries and as a preservative (persistence agent) for topical antiseptic formulations. (See Guthery, E et al, Am J Infect Control 33(1), 2005: 15-22.)

10 It is also known to use quaternary ammonium compounds, for example benzalkonium chloride, benzethonium chloride, cetalkonium chloride or dequalinium chloride, as antimicrobial agents. For example:

- US-3,147,182 describes a synergistic mixture of a dequalinium salt and a cetyl pyridinium salt in a topical antibacterial composition.

- US-4,006,218 refers to the use of dequalinium chloride as an antimicrobial agent in a topical antimicrobial composition, in combination with an alcohol, alkanol or phenol “potentiator”.

- US-4,176,197 discloses a topical anti-acne composition containing a quaternary ammonium halide together with a thio-amino acid or related compound.
• US-4,294,852 describes a topical composition for treating certain skin conditions such as psoriasis and eczema, which can contain a quaternary ammonium active such as dequalinium chloride or benzalkonium chloride.

• US-4,474,748 discloses a topical antimicrobial composition containing a synergistic mixture of a quaternary ammonium compound, such as dequalinium chloride or benzalkonium chloride, with a phenyl alkanol potentiator. The composition can be used to treat wounds or as a surgical scrub.

• US-6,001,864 discloses a topical antifungal composition containing a quaternary ammonium salt with an imidazole.

• US-6,015,816 describes antimicrobial compositions containing quaternary ammonium compounds attached to colloidal particles such as clays.

• US-6,423,750 describes the use of quaternary ammonium compounds such as benzalkonium chloride as topical anti-infective agents for the treatment of skin conditions.

• US-2006/0019987 describes the use of quaternary ammonium compounds as topical antiviral agents, referring to dequalinium chloride and benzalkonium chloride as candidate actives.

• JP-2005-350357 discloses the use of dequalinium chloride as an anti-perspirant and of benzalkonium chloride as a bactericide, in a deodorant formulation.

Salicylic acid (2-hydroxybenzoic acid) is well known for use as an anti-acne agent. It is a keratolytic which is widely used to prevent pores becoming blocked (Waller JM, Dreher F, Behnam S, Ford C, Lee C, Tiet T, Weinstein GD, Maibach HI, “Keratolytic properties of benzoyl peroxide and retinoic acid resemble salicylic acid in man”, Skin Pharmacol Physiol, 2006;19: 283-9). It thereby prevents comedogenesis (formation of blackheads and whiteheads) and trapping of the bacterium Propionibacterium acnes within obstructed follicles, which could otherwise result in visible inflammation.
The keratolytic action of salicylic acid is concentration dependent and it is often necessary to combine it with other actives to increase efficacy, especially against inflammatory lesions (see for example Touitou E, Godin B, Shumilov M, Bishouty N, Ainbinder D, Shouval R, Ingber A, Leibovici V, “Efficacy and tolerability of clindamycin phosphate and salicylic acid gel in the treatment of mild to moderate acne vulgaris”, *J Eur Acad Dermatol Venereol*, 2008;22: 629-31). There is therefore a need to find alternative anti-acne formulations in which the activity of salicylic acid can be boosted by the presence of a second active ingredient, ideally one which preferentially targets *P. acnes*. Salicylic acid alone has very weak antibacterial activity that is almost certainly insufficient to manifest against *P. acnes* on skin as typically formulated in concentrations of 2% w/v or less.

Moreover since acne is an extremely common condition and also a cause of significant distress to those suffering from it, there is always a need to find new and preferably more effective anti-acne treatments.

It has surprisingly been found that combinations of pyridine thiols such as zinc pyrithione with certain types of quaternary ammonium compound can be effective as antibacterial agents against propionibacteria, the bacteria implicated in inflammatory acne. It has also been found that the addition of salicylic acid to such combinations can provide improved anti-acne formulations.

**Statements of the invention**

According to a first aspect of the present invention there is provided an anti-acne formulation containing (a) a pyridine thiol (in particular a pyrithione or pyrithione derivative); (b) a bis-quinolinium salt which contains a cation of formula (I) below:

![Formula Image](image-url)
wherein \( n \) is an integer from 3 to 18; and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate.

This formulation is preferably suitable for topical application to, and/or contact with, the skin, in particular human skin. The pyridine thiol, the bis-quinolinium salt and the salicylic acid or derivative are therefore preferably contained in a pharmaceutically acceptable vehicle which can safely be applied to, and/or contacted with, the skin. A formulation which is “suitable for” topical application may also be adapted for topical application.

Suitable vehicles will be well known to those skilled in the art of preparing topical skin care or pharmaceutical preparations. The vehicle will typically be a fluid, which term includes a cream, paste, gel, lotion, foam, ointment or other viscous or semi-viscous fluid. The three actives (a) to (c) may each independently be present in the form of a solution or suspension, the term “suspension” including emulsions, micellar systems and other multi-phase dispersions as well as suspensions of particles of the relevant substances.

Any or all of the three actives (a) to (c) may, whether separately or together, be carried in or on a delivery vehicle which is suitable for targeting or controlling its release at the intended site of administration. Such vehicles include liposomes and other encapsulating entities, for example niosomes, aspasomes, cubosomes, ethosomes, microsponges, microemulsions, hydrogels and solid lipid nanoparticles.

In the context of the present invention, the pyridine thiol may for example be a 2-pyridine thiol, 3-pyridine thiol or 4-pyridine thiol, in particular a 2- or 4-pyridine thiol. It may be present in the form of a salt or other derivative, for instance a pyridine thiol oxide or hydroxide. It is preferably a pyrithione (i.e an N-oxide pyridine thiol) or tautomer or derivative thereof.

A pyrithione may be present in the form of a pyrithione derivative, e.g. a molecular and/or ionic complex containing the pyrithione group, such as for example a pyrithione salt or a dimer, oligomer or polymer containing a pyrithione or pyrithione salt.
monomer (for example, dipyrrithione, also known as di-2-pyridenedisulphide-1,1'-dioxide).

Suitable salts of pyridine thiols, in particular pyrithiones, include metal salts such as zinc, selenium, silver, copper and sodium salts, preferably zinc or copper, most preferably zinc (eg zinc-2-pyridinethiol-1-oxide).

The pyridine thiol may be present as a pyrithione salt, in particular a metal salt/complex such as are mentioned above. In an embodiment it is zinc pyrithione.

The pyridine thiol is preferably pharmaceutically acceptable, which term includes suitable for veterinary use.

A formulation according to the invention may contain a mixture of two or more different pyridine thiols.

The bis-quinolininium salt contains a cation of formula (I) above, in which n is an integer from 3 to 18, together with one or more appropriate counterions. n is suitably an integer from 4 to 15 or from 8 to 12, for example 10.

In a preferred embodiment of the invention, the bis-quinolinium salt is a dequalinium salt, in which n is 10. It may be selected from dequalinium chloride, dequalinium iodide, dequalinium acetate and mixtures thereof.

In the present context, the bis-quinolinimum salt will suitably be a pharmaceutically acceptable salt such as a halide (for example a chloride, bromide or iodide, in particular a chloride or iodide) or a carboxylate. Suitable carboxylates include the acetate, lactate, citrate, ascorbate, gluconate, laurate, myristate, palmitate, salicylate, undecenoate and aspirinate – of these, the acetate may be preferred.

In an embodiment of the invention, the bis-quinolinium salt is a halide. It may for example be a chloride. It may be an iodide.

The bis-quinolinium salt is preferably pharmaceutically acceptable, which term includes suitable for veterinary use.
A formulation according to the invention may contain a mixture of two or more different bis-quinolinium salts.

Either or both of the pyridine thiol and the bis-quinolinium salt may be replaced, in a formulation according to the invention, by a suitable derivative, in particular a pharmaceutically acceptable (which term includes acceptable for veterinary use, but suitably implies at least acceptability for human pharmaceutical use) derivative. It may be for example a salt, complex or solvate or a so-called “prodrug” form or protected form which reverts to an active form of the relevant compound at an appropriate time on or after administration. In an embodiment, however, the pyridine thiol is not used in the form of a prodrug or other protected form. In an embodiment, the bis-quinolinium salt is not used in the form of a prodrug or other protected form.

In a formulation according to the invention, the salicylic acid may be used in the form of a derivative such as in particular a salt. A salicylate salt may be for example a metal salt or ammonium salt. Suitable metal salts include the alkali metal salts (for example the sodium and potassium salts, in particular the former) and the alkaline earth metal salts (for example the calcium and magnesium salts). A metal salicylate may also be selected from bismuth salicylate, bismuth subsalicylate and transition metal salts such as zinc, copper or titanium salts.

Other salicylic acid derivatives include salicylic acid esters, in particular alkyl esters, more particularly C₁-C₂₀ or C₁-C₁₀ or C₁-C₆ alkyl esters such as methyl salicylate (“wintergreen”). Further derivatives include benzyl salicylate and betaine salicylate.

In an embodiment of the invention, the salicylic acid is present in the form of the free acid, although dependent on the pH of the formulation the acid may be present in dissociated form, typically as salicylate anions and protons.

The salicylic acid (or any derivative thereof used in a formulation according to the invention) may be either naturally or synthetically derived. It may for example be obtained from a natural source such as willow herb.
A “derivative” of salicylic acid is preferably a pharmaceutically acceptable derivative. It may be for example a salt, ester, complex or solvate or a so-called “prodrug” form or protected form which reverts to an active form of the relevant compound at an appropriate time on or after administration. In an embodiment, however, the salicylic acid is present in the form of the free acid (which may be present in dissociated form, as discussed above).

A formulation according to the invention may contain a mixture of two or more salicylic acid derivatives, or a mixture of salicylic acid with one or more derivatives thereof.

In a formulation according to the invention, the pyridine thiol and the bis-quinolinium salt are ideally present as active (i.e. antimicrobially, preferably antibacterially, active) agents. They may be present as anti-acne agents (i.e. agents which are active against a symptom and/or a cause of acne and/or against one or more micro-organisms associated with acne). Surprisingly, such agents have been found to act together synergistically to inhibit, and often to prevent, microbial (in particular bacterial, and more particularly propionibacterial) activity. In other words, they have been found to increase one another’s activity in a manner which can be synergistic compared to the sum of the activities of the two agents individually.

It is possible that the potentiation of one another’s antimicrobial activity by a pyridine thiol and a bis-quinolinium salt may be at least partly due to the formation of a reaction product having an antimicrobial activity greater than the sum of those of the individual reactants. The invention may thus embrace an anti-acne formulation containing, in addition to salicylic acid or a derivative thereof, a reaction product formed between a pyridine thiol and a bis-quinolinium salt (for example a dequalinium salt), in particular between zinc pyrithione and a dequalinium salt such as dequalinium chloride or dequalinium iodide; this reaction product may be formed in situ immediately prior to, or at the point of, use.

In a formulation according to the invention the pyridine thiol and the bis-quinolinium salt, and their relative proportions, are preferably such as to yield at least an additive level of antimicrobial or antibacterial activity compared to the activities of the
individual compounds alone (this is sometimes referred to as an “indifferent” interaction between the compounds). More preferably, the compounds and their relative proportions are such as to yield a synergistic effect on antimicrobial or antibacterial activity, by which is meant that the antimicrobial or antibacterial activity of the combination of the two compounds is greater than the sum of the individual activities of the same amounts of the two compounds used individually. An increased level of activity in these contexts may be manifested by a lower concentration of the compound(s) being needed to inhibit and/or to kill the relevant micro-organism, and/or by a larger zone of inhibition in a disc diffusion assay, and/or by a faster rate of microbial inhibition or killing.

In a formulation according to the invention, the salicylic acid or derivative is ideally present as an agent which is active against acne (which includes against a symptom and/or a cause of acne). The overall anti-acne activity of a formulation according to the invention can be enhanced by the inclusion of agents having two different but complementary modes of action, the pyridine thiol and the bis-quinolinium salt being active against propionibacteria and the salicylic acid or derivative being active as a keratolytic agent.

Antimicrobial activity may be growth inhibitory activity or more preferably biocidal (i.e. lethal to the relevant micro-organism). It preferably includes at least antibacterial activity, which can encompass activity against both Gram-positive and Gram-negative bacteria, in particular Gram-positive bacteria. It may comprise activity against sessile and/or planktonic bacteria.

A formulation according to the present invention preferably has antibacterial activity, at least against propionibacteria.

In the context of this invention, activity against a particular species of micro-organism may be taken to mean activity against at least one, preferably two or more, strains of that species.
Antibacterial activity may be or include the ability to disrupt and/or suppress biofilm formation by the relevant organism. The biofilm may in particular be formed by *Propionibacterium acnes*.

In the present context, the disruption of biofilm formation embraces any negative effect on the ability of a micro-organism to form, maintain or exist in a biofilm, and/or on a biofilm already formed by the organism. Thus, it may involve reducing the amount of a previously formed biofilm, and/or impairing such a biofilm. It may involve killing or inhibiting sessile bacteria within a biofilm.

Suppression of biofilm formation embraces any degree of impairment (including complete prevention) of the ability of a micro-organism to form, or more typically to co-aggregate with, a biofilm. It thus embraces total or partial impairment, including reducing the amount and/or strength of biofilm which the organism is able to form and/or the speed with which it is able to do so. It may involve preventing or reducing the growth or the rate of growth of an existing biofilm formed by the organism.

An anti-acne formulation according to the present invention is preferably active at least against Gram-positive bacteria, in particular against one or more bacteria selected from *Propionibacterium* spp.

A formulation according to the present invention is preferably active against bacteria associated with acne; it is thus preferably active against propionibacteria and/or other bacteria associated with acne and most preferably against one or more strains of *Propionibacterium acnes* and/or in some instances against one or more strains of *P. granulosum*.

The formulation is preferably active against micro-organisms, in particular bacteria and more particularly propionibacteria, which are wholly or partially resistant to one or more antibiotics, for instance those which are in common clinical use. It is ideally active against macrolide-lincosamide-streptogramin (MLS) resistant and/or macrolide-lincosamide-streptogramin-ketolide (MLSK) resistant strains of bacteria. In particular it may be active against erythromycin-resistant, clindamycin-resistant and/or tetracycline-resistant strains of bacteria, for example *P. acnes* strains, the term
tetracycline here referring to the class of antibiotics including for example minocycline and doxycycline as well as the specific antibiotic known as tetracycline.

Antimicrobial activity may be measured in conventional manner, for instance using the tests described in the examples below. Generally tests for activity involve treating a culture of the relevant micro-organism with the candidate antimicrobial compound or combination, incubating the treated culture under conditions which would ordinarily support growth of the organism, and assessing the level of growth, if any, which can occur in the presence of the candidate compound or combination.

In an embodiment, the pyridine thiol used in the present invention has a minimum inhibitory concentration (MIC), at least against propionibacteria, of 50 μg/ml or less, for example 10 μg/ml or less, or 5 or even 2 μg/ml or less. Its corresponding minimum biocidal concentration (MBC) may be 100 μg/ml or less, or 50 μg/ml or less, or 10 μg/ml or less. Suitably the ratio of its MIC to its MBC is from 0.01 to 1 or from 0.125 to 1, ideally from 0.5 to 1. The pyridine thiol may also exhibit such characteristics in the presence of at least one of, preferably both of, lipid (for example either Tween™80 or triolein at 1% v/v) and salt (sodium chloride) – these are species which can be present at the surface of the skin and hence performance in this context can be indicative of suitability for use in topical skin treatment formulations, in particular in the context of acne treatment.

In an embodiment, the bis-quinolinium salt used in the present invention has a minimum inhibitory concentration (MIC), at least against propionibacteria, of 125 μg/ml or less, for example 62.5 or 31.25 or 15.6 or in cases 10 μg/ml or less, such as from 31.25 to 3.9 μg/ml. Its corresponding minimum biocidal concentration (MBC) may be 125 μg/ml or less, or 62.5 or 31.25 or even 20 μg/ml or less. Suitably the ratio of its MIC to its MBC is from 0.125 to 1, ideally from 0.5 to 1. Again, the bis-quinolinium salt may also exhibit such characteristics in the presence of at least one of, preferably both of, lipid and salt, as described above in connection with the pyridine thiol activity.

MIC and MBC values may be measured using conventional assay techniques, for instance as described in the examples below.
The concentration of the pyridine thiol in the formulation might suitably be 0.01% w/v or greater, or 0.05% w/v or 0.1% w/v or greater. Its concentration might be up to 1% w/v, or up to 0.5 or 0.25% w/v.

The concentration of the bis-quinolinium salt in the formulation might suitably be 0.05% w/v or greater, or 0.1% w/v or greater, or 0.5% w/v or greater. Its concentration might be up to 10 or 5% w/v, or up to 2.5% w/v, or up to 2% w/v.

Due to the presence of the other compound, it may be possible for the concentration of either the pyridine thiol or the bis-quinolinium salt, at the site of action when the formulation is applied in vivo, to be less than the MBC, or even than the MIC, of that compound alone. For instance the concentration of at least one of the compounds at this point may be 0.8 or less times its MBC or MIC, such as 0.5 or less, 0.25 or less or 0.125 or less.

The weight ratio of the pyridine thiol in the formulation to that of the bis-quinolinium salt may for example be from 1:500 to 500:1, or from 1:50 to 50:1 or from 1:10 to 10:1 or from 1:50 to 1:1, or from 1:20 to 1:1, or from 1:10 to 1:1 or from 1:5 to 1:1. In an embodiment of the invention, the weight ratio of the pyridine thiol in the formulation to that of the bis-quinolinium salt is from 1:50 to 1:1, or from 1:20 to 1:1, or from 1:10 to 1:1, or from 1:50 or 1:20 or 1:10 to 1:2 or 1:5.

The concentration of the salicylic acid or derivative in a formulation according to the invention might suitably be 0.1 or 0.2 or 0.5% w/v or greater, for example 1 or 2% w/v or greater. Its concentration might be up to 5% w/v, or up to 3 or 2 or 1% w/v. In an embodiment of the invention, the concentration of the salicylic acid or derivative is from 0.5 to 5% w/v, or from 0.5 to 3% w/v, or from 0.5 to 2% w/v, such as about 2% w/v. In cases, for example in chemical peels for use against acne, its concentration might be up to 10 or 20 or even 30% w/v.

In an embodiment neither the pyridine thiol nor the bis-quinolinium salt is present, in a formulation according to the invention, either purely or even primarily as an antifungal agent.
The formulation of the invention is preferably suitable for, and more preferably adapted for, topical administration to human skin. It may take the form of a lotion, cream, ointment, varnish, foam, paste or gel or any other physical form known for topical administration. It may take the form of a solution or suspension. It may comprise a formulation which is, or may be, applied to a carrier such as a sponge, swab, brush, tissue, cloth, wipe, skin patch or dressing (which includes a bandage, plaster, skin adhesive or other material designed for application to a tissue surface) to facilitate its topical administration. It may be intended for pharmaceutical use, and/or for cosmetic or other non-medical care purposes (for example, for general hygiene or skin cleansing or for improving the appearance of the skin).

The vehicle in which the three actives (a) to (c) are contained may be any vehicle or mixture of vehicles which is suitable for topical application; the type chosen will depend on the intended mode and site of application. Many such vehicles are known to those skilled in the art and are readily available commercially. Examples may for instance be found in Williams’ “Transdermal and Topical Drug Delivery”, Pharmaceutical Press, 2003, and other similar reference books. See also Date, AA et al, Skin Pharmacol Physiol, 2006, 19(1): 2-16 for a review of topical drug delivery strategies, and also “Skin Delivery Systems”, 2006, John J Wille, Ed, Blackwell Publishing; “Textbook of Cosmetic Dermatology”, 2004, 3rd edition, Robert Baran, Howard I Maibach, Taylor & Francis; and “Skin Care Beyond the Basics”, 2001, Mark Lees, Milady. Any or all of the active agents may be present in the form of a suspension or other form of multi-phase dispersion, as described above.

Also as described above, the vehicle may be such as to target a desired site and/or time of delivery of the formulation. It may for instance target the formulation to the skin or hair follicles, most preferably to the hair follicles and/or pilosebaceous follicles. It may delay or otherwise control release of the formulation over a particular time period. Any or all of the actives (a) to (c) salt may be microencapsulated, for instance in liposomes – particularly suitable liposomes, for topical use, are those made from stratum corneum lipids, eg ceramides, fatty acids or cholesterol.

In some cases a polar vehicle may be preferred. In an embodiment, the vehicle may be primarily non-aqueous, and is suitably volatile. In another embodiment, the vehicle
may be aqueous. In cases the vehicle may be alcohol-based or silicon-based. The vehicle may include a solubilising agent to assist solubilisation of any or all of the components (a) to (c).

By way of example, a lotion or gel formulation may contain a mixture of water, an alcohol such as ethanol or phenoxyethanol and a glycol such as propylene glycol.

The formulation may contain standard excipients and other additives known for use in pharmaceutical formulations, in particular topical skin care formulations. Examples include perfumes, antioxidants, preservatives, stabilisers, gelling agents and surfactants; others may be found in Williams’ “Transdermal and Topical Drug Delivery”, supra.

Such a formulation may further contain additional active agents such as antimicrobial (in particular antibacterial) agents. For example, it may contain one or more agents selected from anti-acne agents, keratolytics, comedolytics, agents capable of normalising keratinocyte and/or sebocyte function, anti-inflammatories, anti-proliferatives, antibiotics, anti-androgens, sebum controlling agents, anti-pruritics, immunomodulators, agents which promote wound healing, additional antimicrobial agents and mixtures thereof; it may instead or in addition contain one or more agents selected from sunscreens, moisturisers and mixtures thereof. The formulation may in particular contain one or more agents selected from anti-acne agents, keratolytics, comedolytics, sebostatic/sebossuppressive agents, anti-inflammatories and additional antimicrobial (especially antibacterial) agents.

Generally speaking a formulation according to the invention may contain one or more agents which enhance the activity of another active agent present in the formulation, or reduce a side effect of such an active, or improve patient compliance on administration of the formulation. It may contain one or more agents which facilitate penetration of an active agent into microbial biofilms. It may contain one or more agents which control the site and/or rate of release of an active agent following administration.

An additional antimicrobial agent may for example be selected from the group consisting of biocides, disinfectants, antiseptics, antibiotics, bacteriophages, enzymes,
anti-adhesins, immunoglobulins, other antimicrobially active antioxidants and mixtures thereof. It is preferably active as a bactericide, in particular against propionibacteria.

It may however be preferred for the three components (a) to (c) to be the only active agents in the formulation, or at least to be the only antimicrobially active agents (or at least the only antibacterially active agents) and/or the only anti-acne active agents. It may be preferred for the components (a) and (b) to be the only antimicrobially (or at least the only antibacterially) active agents in the formulation.

In some cases it may be suitable for a formulation according to the present invention not to contain a cetyl pyridinium salt, for instance as disclosed in US-3,147,182.

In some cases it may be suitable for a formulation according to the present invention not to contain an imidazole (in particular miconazole nitrate), for instance as disclosed in US-6,001,864.

In some cases it may be suitable for a formulation according to the present invention not to contain an alkanol potentiator, in particular a phenyl alkanol, for instance as disclosed in US-4,474,748.

In some cases it may be suitable for a formulation according to the present invention not to contain both an alcohol and an organic acid as delivery vehicles, for instance as disclosed in US-4,294,852.

In some cases it may be suitable for a formulation according to the present invention not to contain an antibiotic, for instance as described in US-2006/0019987.

In some cases it may be suitable for a formulation according to the present invention not to contain a thio-amino acid or related compound, for instance as disclosed in US-4,176,197.

In some cases it may be suitable for a formulation according to the present invention not to contain a quaternary ammonium compound other than the bis-quinolinium salt, in particular a benzalkonium salt such as benzalkonium chloride.
In cases it may be suitable for a formulation according to the invention not to include an alcohol, alkanol or phenol (in particular cyclohexyl phenol) potentiator, for example of the kind referred to in US-4,006,218.

A formulation according to the invention may be incorporated into, and hence applied in the form of, another product such as a cosmetic; a skin or hair care preparation (for example a skin cleanser, toner or moisturiser; a cleansing preparation (for example a hand wash or facial wash); or a shampoo, conditioner, styling mousse or gel or hair spray); a deodorant or anti-perspirant; a cleansing preparation (for example a hand wash); a pharmaceutical (which includes veterinary) preparation; a cosmeceutical preparation; a toiletry product (for instance a bath or shower additive or a soap); or a laundry or other fabric treatment product. The formulation may be, or be incorporated into, a leave-on or a wash-off skin treatment product.

The invention therefore provides, according to a second aspect, a product which incorporates an anti-acne formulation according to the first aspect.

A formulation according to the invention may be marketed with an indication that it has antibacterial or anti-acne activity, or enhanced antibacterial or anti-acne activity. The marketing of such a formulation may for example include an activity selected from (a) enclosing the formulation in a container or package that comprises the relevant indication; (b) packaging the formulation with a package insert that comprises the indication; (c) providing the indication in a publication that describes the formulation; and (d) providing the indication in a commercial which is aired for instance on the radio, television or internet. The antibacterial or anti-acne activity of the formulation may be attributed, in such an indication, at least partly to the presence of the combination of the pyridine thiol and the bis-quinolinium salt, and/or to the salicylic acid or derivative.

The invention may involve assessing the antibacterial or anti-acne activity of the formulation during or after its preparation. It may involve assessing the antibacterial or anti-acne activity of the formulation both before and after incorporation of the pyridine thiol, the bis-quinolinium salt and/or the salicylic acid or derivative, for
example so as to confirm that one or more of them contribute to the relevant activity of the formulation.

The formulation of the invention may be prepared in situ, at or immediately before its point of use, for instance its application to the skin or another surface. Thus according to a third aspect, the present invention provides a kit for preparing an anti-acne formulation, for example a formulation according to the first aspect, the kit comprising sources of (a) a pyridine thiol, (b) a bis-quinolinium salt and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, together with instructions for combining the three compounds so as to make the formulation at or before the point of intended use, and/or for the co-administration of the three compounds to a surface such as the skin. Two or more of the actives (a) to (c) may be pre-combined in one or more single sources. Any or any combination of the three compounds may be present in suitable respective vehicles.

According to one embodiment, the formulation or kit of the invention may contain a pyridine thiol, a bis-quinolinium salt and salicylic acid or a derivative thereof, each encapsulated (for instance microencapsulated) in a separate delivery vehicle; this might for instance allow their release, and hence their contact with one another, only at the intended site of administration. Any two of the actives may be pre-combined in a suitable delivery vehicle.

A fourth aspect of the invention provides a method for preparing an anti-acne formulation, which method involves mixing together (a) a pyridine thiol, (b) a bis-quinolinium salt and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, preferably together with a pharmaceutically acceptable vehicle.

According to a fifth aspect of the invention there is provided a formulation (preferably a formulation according to the first aspect of the invention) containing (a) a pyridine thiol, (b) a bis-quinolinium salt and (c) salicylic acid or a derivative thereof other than dequalinium salicylate, for use in the treatment of acne.

In the context of the present invention, treatment of a condition encompasses both therapeutic and prophylactic treatment, of either an infectious or a non-infectious
condition, in either a human or animal but in particular a human, suitably on the skin. It may involve complete or partial eradication of the condition, removal or amelioration of associated symptoms, arresting subsequent development of the condition, and/or prevention of, or reduction of risk of, subsequent occurrence of the condition. It will typically involve use of the actives (a) to (c), and in particular of the pyridine thiol and the bis-quinolinium salt, as an antimicrobial combination, in particular as an antibacterial (preferably bactericidal) combination, more particularly against propionibacteria.

In the context of the present invention, treatment of a condition may in particular involve use of the formulation against one or more propionibacteria, including against propionibacterial biofilm formation. It may involve use against *P. acnes* and/or in some instances against *P. granulosum*.

The treatment is preferably administered topically.

The invented formulation is for use in the treatment of acne (ie as an anti-acne agent), which includes the treatment of acne lesions, for instance to reduce acne-related scarring.

Acne is a multifactorial disease of the pilosebaceous follicles of the face and upper trunk, characterised by a variety of inflamed and non-inflamed lesions such as papules, pustules, nodules and open and closed comedones. Its treatment can therefore encompass the treatment (which embraces prevention or reduction) of any of these symptoms, and references to use as an anti-acne agent may be construed accordingly.

In particular, the treatment of acne encompasses the treatment (including prevention) of lesions and/or scarring associated with acne. It also encompasses the treatment of a propionibacterial infection and/or the inhibition of propionibacterial activity which could cause or be otherwise associated with acne or its symptoms.

In general, the present invention will be used for the treatment of symptoms which are directly due to acne rather than for instance infections which may arise as a consequence of treating acne with other actives such as antibiotics, and/or secondary
infections caused by opportunistic pathogens, which can arise in skin already affected by acne.

Thus, in general terms the invention can provide a formulation containing (a) a pyridine thiol, (b) a bis-quinolinium salt and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, for use in the treatment of acne.

According to the fifth aspect of the invention, the formulation containing actives (a) to (c) may be prepared in situ, at or immediately before the point of administration. This aspect of the invention thus pertains to any use of a pyridine thiol, a bis-quinolinium salt and salicylic acid or a derivative thereof (other than dequalinium salicylate) in the treatment of acne, the three compounds being administered either simultaneously or sequentially.

According to a sixth aspect, the invention provides the use of (a) a pyridine thiol, (b) a bis-quinolinium salt and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, in the manufacture of a medicament (typically a formulation) for the treatment of acne. The pyridine thiol and the bis-quinolinium salt will typically be used as antimicrobial agents in the manufacture of the medicament. The salicylic acid or derivative thereof may be used as an anti-acne agent (in particular as a keratolytic agent) in the manufacture of the medicament.

The invention further provides, according to a seventh aspect, the use together of (a) a pyridine thiol, (b) a bis-quinolinium salt and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, as an anti-acne agent, or in the manufacture of an anti-acne formulation.

An eighth aspect provides a method for controlling the growth of a propionibacterium, the method comprising applying, to an area or surface which is infected or suspected to be infected or capable of becoming infected with the bacterium, a combination of (a) a pyridine thiol, (b) a bis-quinolinium salt and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate. Again the three compounds may be applied simultaneously or sequentially. They may be applied in a formulation according to the
first aspect of the invention. They may in particular be applied to an area or surface which is infected with the bacterium.

In this context, “controlling the growth” of a bacterium embraces inhibiting or preventing its growth, whether completely or partially, as well as killing either completely or partially a culture of the bacterium. It also embraces reducing the risk of subsequent growth of the bacterium in or on the area or surface being treated. It may embrace reducing the risk of transmission of the bacterium from the area or surface being treated to another area or surface and/or living body. The method of the invention may thus be used to treat an existing occurrence of the bacterium or to prevent or reduce the risk of a potential subsequent occurrence. Controlling the growth of a bacterium may also embrace the disruption and/or suppression of biofilm formation by the bacterium, as described above.

Again the area or surface to which the actives (a) to (c) are applied will typically be a surface such as human or animal tissue, in particular the skin, typically of a living human or animal. In this case the invented combination may be applied for therapeutic purposes or for non-therapeutic (eg purely cosmetic) purposes. Thus the method of the eighth aspect of the invention encompasses a method of treatment of a human or animal patient suffering from or at risk of suffering from acne, the method involving administering to the patient a therapeutically (which term includes prophylactically) effective amount of a formulation containing (a) a pyridine thiol, (b) a bis-quinolinium salt and (c) salicylic acid or a derivative thereof other than dequalinium salicylate. The formulation is suitably administered in an antimicrobially effective amount.

In accordance with the eighth aspect of the invention, the formulation is suitably administered to a human patient. The patient is suitably suffering from acne. The method of the eighth aspect of the invention preferably involves applying a formulation according to the first aspect.

A ninth aspect of the invention provides the use of a formulation containing (a) a pyridine thiol, (b) a bis-quinolinium salt and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, for non-therapeutic purposes. In an embodiment of this aspect, the formulation is used as an anti-acne or skin care agent for non-
therapeutic purposes, for example for cosmetic purposes such as to improve the appearance of the skin.

A tenth aspect of the invention provides the use of a pyridine thiol and a bis-quinolinium salt in an anti-acne formulation, in combination with salicylic acid or a derivative thereof, other than dequalinium salicylate, for the purpose of increasing the anti-acne activity of the formulation and/or of reducing the amount of the salicylic acid or derivative in the formulation without or without undue loss of anti-acne activity.

An increase in anti-acne activity may be as compared to that of the salicylic acid or derivative alone, at the same concentration as used when combined with the pyridine thiol and bis-quinolinium salt. Ideally the increase is as compared to the sum of the activities of the pyridine thiol/bis-quinolinium salt combination and the salicylic acid or derivative individually, again at the same respective concentrations as used when all are combined.

A reduction in the amount of the salicylic acid or derivative in the formulation may be as compared to the amount which would otherwise have been used in the formulation in order to achieve a desired level of activity, in particular in order to have acceptable efficacy in the context of its intended use. The reduction may be manifested by reduced side effects which would otherwise have been observed during use of the formulation, for example local irritation and/or undesirable systemic absorption of the salicylic acid or derivative. According to the invention, the pyridine thiol and bis-quinolinium salt may therefore be used for the dual purposes of reducing an undesired property of a formulation containing salicylic acid or a derivative thereof, without undue loss of anti-acne activity.

Preferably the pyridine thiol and bis-quinolinium salt are used without any reduction in anti-acne activity compared to the level exhibited by the formulation prior to their addition. More preferably they are used to give an increase in anti-acne activity. They may however be used to reduce the amount of the salicylic acid or derivative present, and/or its associated side effects, whilst maintaining the anti-acne activity of the resultant formulation at a level, albeit lower than that which it would otherwise have exhibited, which is still acceptable in the context of its intended use.
According to an eleventh aspect, the invention provides an anti-acne formulation containing (a) a bis-quinolinium salt which contains a cation of formula (I) below:

\[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{N}^+ \quad \text{(CH}_2\text{n}) \\
\text{N}^+ \\
\text{NH}_2 \\
\end{array}
\]

wherein \(n\) is an integer from 3 to 18, and (b) salicylic acid or a derivative thereof, provided that (a) and (b) are not both dequalinium salicylate.

A twelfth aspect provides a formulation according to the eleventh aspect, for use in the treatment of acne. Preferably the treatment involves topical application of the formulation.

A thirteenth aspect provides the use of a formulation according to the eleventh aspect, in the manufacture of a medicament (typically a formulation) for the treatment of acne. The bis-quinolinium salt and the salicylic acid or derivative will typically be used as an anti-acne combination in the manufacture of the medicament.

A fourteenth aspect provides a method for controlling the growth of a propionibacterium, the method comprising applying, to an area or surface which is infected or suspected to be infected or capable of becoming infected with the bacterium, a combination of (a) a bis-quinolinium salt as defined above and (c) salicylic acid or a derivative thereof, provided that (a) and (b) are not both dequalinium salicylate.

A fifteenth aspect provides a method of treatment of a human or animal patient suffering from or at risk of suffering from acne, the method involving administering to the patient a therapeutically (which term includes prophylactically) effective amount of a formulation according to the eleventh aspect of the invention. The formulation may be administered in an antibacterially effective amount. It is suitably administered topically.
According to a sixteenth aspect, the invention provides an anti-acne formulation containing (a) a pyridine thiol and (b) salicylic acid or a derivative thereof, provided that the salicylic acid derivative is not dequalinium salicylate.

A seventeenth aspect provides a formulation according to the sixteenth aspect, for use in the treatment of acne. Preferably the treatment involves topical application of the formulation.

An eighteenth aspect provides the use of a formulation according to the sixteenth aspect, in the manufacture of a medicament (typically a formulation) for the treatment of acne. The pyridine thiol and the salicylic acid or derivative will typically be used as an anti-acne combination in the manufacture of the medicament.

A nineteenth aspect provides a method for controlling the growth of a propionibacterium, the method comprising applying, to an area or surface which is infected or suspected to be infected or capable of becoming infected with the bacterium, a combination of a pyridine thiol and salicylic acid or a derivative thereof, provided that the salicylic acid derivative is not dequalinium salicylate.

A twentieth aspect provides a method of treatment of a human or animal patient suffering from or at risk of suffering from acne, the method involving administering to the patient a therapeutically (which term includes prophylactically) effective amount of a formulation according to the sixteenth aspect of the invention. The formulation may be administered in an antibacterially effective amount. It is suitably administered topically.

Throughout the description and claims of this specification, the words “comprise” and “contain” and variations of the words, for example “comprising” and “comprises”, mean “including but not limited to”, and do not exclude other moieties, additives, components, integers or steps.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite
article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Preferred features of each aspect of the invention may be as described in connection with any of the other aspects.

Other features of the present invention will become apparent from the following examples. Generally speaking the invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims and drawings). Thus features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

Moreover unless stated otherwise, any feature disclosed herein may be replaced by an alternative feature serving the same or a similar purpose.

The present invention will now be further described with reference to the following non-limiting examples and the accompanying Figure 1, which is an isobologram showing FIC (fractional inhibitory concentration) values for mixtures of zinc pyrithione and dequalinium chloride against a propionibacterial strain, as referred to in Example 5 below.

Detailed description

Experimental tests were conducted to determine the antibacterial and anti-acne activities of formulations according to the invention.

Test micro-organisms

The primary test micro-organism used was a propionibacterial strain, Propionibacterium acnes NCTC 737. This is the type strain of the genus; it is fully susceptible to antibiotics.
The propionibacteria are clinically significant due to their involvement in acne, which is a very common, complex and multi-factorial skin disease in which \textit{P. acnes} and other \textit{Propionibacterium} spp (for example \textit{P. granulosum}) play key roles. They are also opportunistic pathogens in compromised hosts. Thus, activity observed against these micro-organisms is expected to be a good predictor of activity against acne.

Other propionibacterial strains were also tested, as described in Example 7 below. These included certain antibiotic resistant propionibacteria, such as the two \textit{P. acnes} strains designated PRP-010 and PRP-039 which are resistant respectively to macrolides-lincosamides-streptogramins-ketolides (MLSK) and to macrolides-lincosamides-streptogramins (MLS) and tetracycline – in other words, PRP-010 is resistant to erythromycin and clindamycin, and PRP-039 to erythromycin, clindamycin and tetracycline.

In addition, certain strains of \textit{P. granulosum}, another bacterium involved in acne, were also tested in Example 7.

The propionibacteria were cultured and maintained on Wilkins-Chalgren Anaerobe Medium (agar and broth) at pH 6.0; all cultures were incubated anaerobically at 37°C for 72 hours.

The following tests were carried out to assess antimicrobial activity against the test organisms.

\textit{(a) Minimum inhibitory concentration (MIC) assay}

This is a standard international method for quantitatively assessing the antimicrobial activity of a compound in a liquid medium. The method used a sterile 96-well microtitre plate, capable of holding about 200 \( \mu \)l of liquid per well. The wells contained liquid culture medium and ranges of decreasing concentrations of the relevant test compound in doubling dilutions (eg 1000, 500, 250, 125…\( \mu \)g/ml, etc.) down to 0.49 \( \mu \)g/ml). The culture medium was as described above.

The wells were inoculated with a liquid suspension of freshly grown micro-organism and incubated under the conditions described above. After incubation, the microtitre
plate was examined visually (with the aid of a light box) for cloudiness in each well, which would indicate microbial growth. The MIC value was recorded as the lowest concentration of test compound required to inhibit microbial growth, i.e., the lowest concentration for which the liquid in the well remained clear.

The assays were conducted in duplicate and included both negative (culture medium with no micro-organisms) and positive (culture medium plus diluting solvent plus micro-organism) controls.

Since inhibition does not necessarily indicate killing of microbial cells, merely that growth as visible to the naked eye has been inhibited, it is desirable to conduct a further test (the MBC assay described below) to establish the concentration of the test compound needed to kill the test organism.

(b) Minimum bactericidal concentration (MBC) assay

This assay, normally carried out after an MIC assay, determines the minimum concentration of a compound that is lethal to the micro-organism being tested.

Following an MIC assay, a 5 μl sample was withdrawn from the first microtitre well that showed positive growth and from all the subsequent wells that showed no growth. These samples were then individually sub-cultured on antibiotic-free agar medium, under the incubation conditions described above. Following incubation they were examined visually for bacterial growth. The MBC was taken to be the lowest test compound concentration for which the incubated sample showed no growth.

The ratio of MIC to MBC should ideally be as close to 1 as possible. This facilitates selection of the lowest possible effective concentration of a test compound with a reduced risk of selecting a sub-lethal concentration which could promote resistance or allow the target bacterial population to recover.

(c) Disc diffusion assay (DDA)

This is an internationally recognised standard method for qualitatively assessing the antimicrobial activity of a compound.
A sterile paper disc was impregnated with a sample of the test compound in a suitable solvent and 30 minutes allowed for the solvents to evaporate (where possible). The disc was then placed on an agar plate onto which the test micro-organism had been inoculated. The plate was then incubated under the conditions described above, following which it was examined visually for signs of microbial growth. If the test compound had antimicrobial activity, a circular zone of no growth would be obtained around the disc. The diameter of this zone of “inhibition” was measured using a ProtoCOL™ automated zone sizer (Synbiosis, Cambridge, UK). In general, a greater diameter and/or area of the zone of inhibition indicates a greater antimicrobial activity in the relevant test compound, although other factors such as test compound mobility through the agar gel may also influence the result.

(d) *Synergy disc diffusion assay (SDDA)*

This is a variation on the DDA method, in which two or more compounds are tested together for their combined antimicrobial activity.

Where for example the assay was used to test a combination of two test compounds A and B, the two compounds were placed on a single paper disc and the above described DDA procedure repeated. An increase in diameter of the zone of inhibition, compared to the greater of the zone diameters for the two compounds individually, was taken to indicate potential antimicrobial synergy. In practical terms, an increase of greater than 5 mm could be treated as significant.

An analogous procedure was used to test combinations of three test compounds.

(e) *Fractional inhibitory concentration (FIC) assay*

This assay was used to determine the mode of interaction between two antimicrobial test compounds A and B. It was similar to the MIC assay, utilising a 96-well microtitre plate and liquid culture medium. The test compounds were added together to each well at a range of concentrations starting at their respective MIC values and descending in doubling dilutions as with the MIC assay. Typically an 8 x 8 array of wells could be used to combine 8 different concentrations of compound A (from its
MIC downwards, including zero) with 8 different concentrations of compound B (ditto).

The wells were inoculated with freshly grown micro-organism and incubated under the conditions described above.

As for the MIC assay, the results were read by the naked eye. A minimum inhibitory concentration was recorded for each combination of A and B. A fractional FIC index (FICI) was then calculated for each compound in that mixture, and these two indices were added together to give an overall FICI indicative of the mode of interaction.

Thus for each mixture tested, the FIC for compound A \((\text{FIC}_A) = \text{MIC for } (A + B) / \text{MIC for } A\) alone. Similarly the FIC for compound B \((\text{FIC}_B) = \text{MIC for } (A + B) / \text{MIC for } B\) alone. The overall FICI = \(\text{FIC}_A + \text{FIC}_B\).

An FICI of 0.5 or less was taken to indicate synergy, a value from 0.5 to 4.0 an indifferent effect and values greater than 4.0 antagonism (ie the two compounds counter one another’s activity, leading overall to a diminished antimicrobial effect) (see Odds FC, “Synergy, antagonism, and what the chequerboard puts between them”, *J. Antimicrob Chemother*, 2003; 52: 1). These results can be depicted visually on a plot (isobologram) of FIC\(_A\) against FIC\(_B\) for the mixtures tested.

*Example 1 – activity against P. acnes (pyridine thiol + bis-quinolinium salt + salicylic acid)*

This example used *P. acnes* NCTC 737 as the test organism.

DDA assays, as described above, were carried out using a combination of the test compounds (a) dequalinium chloride (DC), dissolved in ethanol; (b) zinc pyrithione (ZP), dissolved in DMSO; and (c) salicylic acid (SA), dissolved in ethanol. All three compounds were sourced from Sigma-Aldrich, UK.

For each experiment, 50 \(\mu\)g of the DC was loaded onto each disc, 200 \(\mu\)g of the ZP and 200 \(\mu\)g of the SA. All the DDA experiments were conducted in triplicate.
The results are shown in Table 1 below; all are collated from a number of experiments.

Table 1

<table>
<thead>
<tr>
<th>Test</th>
<th>(S)DDA (mm)</th>
<th>SDDA increase (mm)</th>
<th>SDDA area increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>15.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZP</td>
<td>17.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC + ZP + SA</td>
<td>41.33</td>
<td>23.99</td>
<td>468.1</td>
</tr>
</tbody>
</table>

The Table 1 data show that the combination of pyridine thiol, bis-quinolinium salt and salicylic acid can be active against *P. acnes* NCTC 737. This in turn indicates the likely utility of such combinations as anti-acne agents.

It is also of note that the combination shows significantly enhanced antibacterial activity compared to the activities of the individual components DC, ZP and SA alone. The SA, which is unlikely to contribute to antibacterial activity but which is known to be active as a keratolytic agent, will enhance the overall anti-acne efficacy of the combination by complementing the antibacterial activity of the pyridine thiol and bis-quinolinium salt.

*Example 2 – activity against P. acnes (bis-quinolinium salt + salicylic acid)*

This example also used *P. acnes* NCTC 737 as the test organism.

DDA assays, as described above, were carried out using a combination of dequalinium chloride (DC) and salicylic acid (SA), both dissolved in ethanol. For each experiment, 50 μg of the DC was loaded onto each disc and 200 μg of the SA. All the DDA experiments were conducted in triplicate.
The zone diameter observed for the DC/SA combination was 13.81 (± 0.18) mm. This again shows that the combination of bis-quinolinium salt and salicylic acid can be active against *P. acnes* NCTC 737, and that such combinations are likely to be of use as anti-acne agents, particularly in view of the additional keratolytic action of the salicylic acid.

**Example 3 – activity against P. acnes (pyridine thiol + salicylic acid)**

Example 2 was repeated but using a combination of zinc pyrithione (ZP), dissolved in DMSO, and salicylic acid (SA) dissolved in ethanol. For each experiment, 200 μg of the ZP was loaded onto each disc and 200 μg of the SA. All the DDA experiments were conducted in triplicate.

The zone diameter observed for the ZP/SA combination was 20.35 (± 1.10) mm. This shows that the combination of pyridine thiol and salicylic acid can be active against *P. acnes* NCTC 737, and that such combinations are likely to be of use as anti-acne agents, particularly in view of the additional keratolytic action of the salicylic acid.

**Example 4 – activity against P. acnes (pyridine thiol + bis-quinolinium salt)**

This example used *P. acnes* NCTC 737 as the test organism.

MIC, MBC and DDA assays, as described above, were carried out using the test compounds (a) dequalinium chloride (DC), dissolved in ethanol and (b) zinc pyrithione (ZP), dissolved in DMSO.

Mixtures of the two test compounds were then subjected to SDDA assays as described above. Increases in zone diameter (mm) were measured with respect to the DC, which was the compound showing the larger zones of inhibition during the previous individual disc diffusion assays.

For the (S)DDA experiments, 50 μg of the DC was loaded onto each disc, and 200 μg of the ZP. All the (S)DDA experiments were conducted in triplicate.
The MIC and MBC results are shown in Table 2 below and the (S)DDA results in Table 3. All results are collated from a number of experiments.

### Table 2

<table>
<thead>
<tr>
<th>Test compound</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
<th>MIC/MBC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>7.8</td>
<td>15.6</td>
<td>0.5</td>
</tr>
<tr>
<td>ZP</td>
<td>0.98</td>
<td>7.8</td>
<td>0.125</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Test compound</th>
<th>DDA (mm)</th>
<th>SDDA with DC (mm)</th>
<th>SDDA increase (mm)</th>
<th>SDDA area increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>16.68 (± 0.47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZP</td>
<td>15.85 (± 1.42)</td>
<td>45.39 (± 5.17)</td>
<td>28.7</td>
<td>640.1</td>
</tr>
</tbody>
</table>

These data show that both zinc pyrithione and dequalinium chloride alone are active against *P. acnes* NCTC 737.

Surprisingly, however, when the two compounds are combined the data indicate a synergistic antibacterial interaction between them, with a significant increase in zone diameter and area over those exhibited by either compound alone.

*Example 5 – activity against P. acnes spp (pyridine thiol + bis-quinolinium salt in FIC assays)*

Mixtures of dequalinium chloride and zinc pyrithione, containing various relative proportions of the two actives, were then subjected to FIC assays against *P. acnes* NCTC 737, as described above. The results were used to prepare FIC isobolograms. All assays were conducted in triplicate.
The overall FICI obtained for the mixtures was 0.5, representing the mean of three replicates. This indicates a synergistic interaction. A representative isobologram is shown in Figure 1; the dashed line indicates where overall FICIs (ie FIC_{DC} + FIC_{ZP}) equal 1, which would indicate a purely indifferent interaction. Figure 1 clearly demonstrates the synergistic activity of the combination of the pyrithione and the bis-quinolinium salt against *P. acnes* NCTC 737.

*Example 6 – activity against P. acnes (another bis-quinolinium salt)*

The general method of Example 4 was repeated using another bis-quinolinium salt, dequalinium iodide (DI), which was also obtained from Sigma-Aldrich, UK. This compound was tested against *P. acnes* NCTC 737, both alone and in combination with ZP.

For the (S)DDA experiments, 50 µg of the DI was loaded onto each disc, and 200 µg of the ZP. The DI and ZP were both dissolved in DMSO. Increases in zone diameter (mm) were measured with respect to the ZP, which was the compound showing the larger zones of inhibition during the previous individual disc diffusion assays.

All the (S)DDA experiments were conducted in triplicate.

The MIC and MBC results are shown in Table 4 below and the (S)DDA results in Table 5. All results are collated from a number of experiments.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
<th>MIC/MBC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI</td>
<td>3.9</td>
<td>125</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test compound</th>
<th>DDA (mm)</th>
<th>SDDA with DI (mm)</th>
<th>SDDA increase (mm)</th>
<th>SDDA area increase (%)</th>
</tr>
</thead>
</table>

Table 5
<table>
<thead>
<tr>
<th>Test compound</th>
<th>DDA (mm)</th>
<th>SDDA with DI (mm)</th>
<th>SDDA increase (mm)</th>
<th>SDDA area increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI</td>
<td>21.92 (± 0.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZP</td>
<td>46.44 (± 4.59)</td>
<td>63.90 (± 5.19)</td>
<td>17.45</td>
<td>89.29</td>
</tr>
</tbody>
</table>

These data show that the DI alone is active against *P. acnes* NCTC 737. Surprisingly, however, when the DI is combined with ZP the data indicate a synergistic antibacterial interaction between them, with a significant increase in zone diameter and area over those exhibited by either compound alone.

*Example 7 – activity against other propionibacterium spp*

Using DC and ZP as the test compounds, the activity of each compound alone and in combination was determined, using (S)DDA tests, against a number of other *Propionibacterium* spp strains. Some of these strains have known antibiotic resistance.

50 μg of the DC and/or 200 μg of the ZP were loaded onto each disc. All the experiments were conducted in triplicate.

The results are shown in Table 6 below; the resistance phenotype for each of the test strains is indicated.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Resistance phenotype</th>
<th>DDA DC (mm)</th>
<th>DDA ZP (mm)</th>
<th>SDDA DC + ZP (mm)</th>
<th>SDDA increase (mm)</th>
<th>SDDA area increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. granulosum</em> NCTC 11865</td>
<td>None</td>
<td>14.08 (±1.08)</td>
<td>23.95 (±2.31)</td>
<td>39.36 (±11.05)</td>
<td>15.42</td>
<td>170.20</td>
</tr>
<tr>
<td><em>P. acnes</em> PRP-002</td>
<td>Tet/MLS</td>
<td>20.04 (±1.93)</td>
<td>25.79 (±1.52)</td>
<td>42.96 (±3.55)</td>
<td>17.16</td>
<td>177.34</td>
</tr>
</tbody>
</table>

32
<table>
<thead>
<tr>
<th>Test organism</th>
<th>Resistance phenotype</th>
<th>DDA DC (mm)</th>
<th>DDA ZP (mm)</th>
<th>SDDA DC + ZP (mm)</th>
<th>SDDA increase (mm)</th>
<th>SDDA area increase (%)</th>
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<td>P. acnes PRP-003</td>
<td>Tet</td>
<td>21.27 (±0.92)</td>
<td>26.82 (±8.21)</td>
<td>33.91 (±4.85)</td>
<td>7.09</td>
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<td>P. acnes PRP-004</td>
<td>Tet</td>
<td>20.45 (±1.81)</td>
<td>12.43 (±0.64)</td>
<td>31.34 (±1.25)</td>
<td>10.89</td>
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<td>P. granulosum PRP-005</td>
<td>MLSK</td>
<td>20.55 (±0.47)</td>
<td>20.04 (±0.82)</td>
<td>26.00 (±1.46)</td>
<td>5.45</td>
<td>60.02</td>
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<td>P. granulosum PRP-006</td>
<td>MLS</td>
<td>26.82 (±0.31)</td>
<td>32.58 (±3.44)</td>
<td>36.89 (±0.94)</td>
<td>4.32</td>
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<td>P. acnes PRP-007</td>
<td>Clin</td>
<td>18.91 (±0.78)</td>
<td>10.79 (±0.62)</td>
<td>23.43 (±0.82)</td>
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<td>P. acnes PRP-008</td>
<td>Clin</td>
<td>19.73 (±0.82)</td>
<td>14.90 (±1.08)</td>
<td>27.75 (±0.31)</td>
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<td>P. acnes PRP-010</td>
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<td>MLS</td>
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<td>29.70 (±3.21)</td>
<td>36.17 (±1.25)</td>
<td>6.47</td>
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<td>P. acnes PRP-023</td>
<td>MLSK</td>
<td>19.32 (±1.98)</td>
<td>17.37 (±1.55)</td>
<td>30.73 (±2.57)</td>
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<td>16.24 (±0.99)</td>
<td>28.26 (±2.76)</td>
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<td>P. acnes PRP-039</td>
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<td>23.02 (±2.62)</td>
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<td>44.81 (±2.62)</td>
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<td>P. granulosum PRP-044</td>
<td>MLS</td>
<td>17.37 (±0.18)</td>
<td>18.50 (±0.31)</td>
<td>31.86 (±0.94)</td>
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<td>P. acnes PRP-046</td>
<td>None</td>
<td>21.38 (±1.46)</td>
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<td>Tet/MLS</td>
<td>21.38 (±2.31)</td>
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<td>P. granulosum PRP-055</td>
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<td>29.08 (±1.70)</td>
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<td>19.42 (±1.60)</td>
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<td>27.13 (±0.31)</td>
<td>7.71</td>
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<td>P. acnes PRP-068</td>
<td>Ery</td>
<td>20.45 (±0.78)</td>
<td>14.90 (±1.52)</td>
<td>34.53 (±1.11)</td>
<td>14.08</td>
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<td>Resistance phenotype</td>
<td>DDA DC (mm)</td>
<td>DDA ZP (mm)</td>
<td>SDDA DC + ZP (mm)</td>
<td>SDDA increase (mm)</td>
<td>SDDA area increase (%)</td>
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<td><em>P. acnes</em> PRP-101</td>
<td>Tet/MLS</td>
<td>16.96 (±0.82)</td>
<td>14.39 (±0.64)</td>
<td>33.30 (±0.82)</td>
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<td><em>P. acnes</em> PRP-102</td>
<td>Tet/MLS</td>
<td>21.68 (±0.99)</td>
<td>16.85 (±0.47)</td>
<td>29.80 (±1.70)</td>
<td>8.12</td>
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[Abbreviations: American Type Culture Collection (ATCC), National Collection of Type Cultures (NCTC), Propionibacterium Panel Number (PRP), Tetracycline (Tet), Erythromycin (Ery), Clindamycin (Clin), Macrolide-Lincosamide-Streptogramin (MLS), Macrolide-Lincosamide-Streptogramin-Ketolide (MLSK).]

5 When the DC and ZP are combined, the synergistic interaction initially observed against *P. acnes* NCTC 737 was similarly observed, to a greater or lesser degree, against all of the propionibacteria tested. This indicates the utility of the combination either to treat or to prevent infections associated with such bacteria, in particular acne. These results are likely to be of particular clinical value for the antibiotic resistant test strains.

**Example 8 – topical anti-acne formulations**

The results from Examples 1 to 7 show that the combination of a pyridine thiol and a bis-quinolinium salt can be an effective antibacterial agent, in particular against the bacteria associated with acne, with a synergistic impact on the antibacterial activity of the combination compared to those of the individual compounds alone. They also show that the combination of a pyridine thiol, a bis-quinolinium salt and salicylic acid can be antibacterially active, as can the combination of a bis-quinolinium salt with salicylic acid and the combination of a pyridine thiol with salicylic acid.

20 These results can be of use in preparing antibacterial formulations, in particular for topical application to the skin, for either prophylactic or therapeutic use in any context where such bacteria are thought to be involved as possible sources of infection.
Even in cases where the invented combination of actives has an additive, as opposed to synergistic, antibacterial activity compared to those of the individual compounds, this can be of considerable benefit when preparing formulations for topical use. One of the compounds may be used to replace a proportion of the other, thus lowering any side effects and/or other undesirable properties of the combination without undue loss of antibacterial activity. The presence of the keratolytically active salicylic acid can contribute additional anti-acne activity, complementing the antibacterial activity of the other active(s) present in the formulation.


An alternative topical anti-acne formulation may be prepared by combining (a) a pyridine thiol such as zinc pyrithione, (b) a bis-quinolinium salt, in particular a dequalinium salt, and (c) salicylic acid or a pharmaceutically acceptable derivative thereof, other than dequalinium salicylate, again in a suitable fluid vehicle and optionally together with conventional additives. A further alternative topical anti-acne formulation may similarly be prepared by combining a bis-quinolinium salt, in particular a dequalinium salt, with salicylic acid or a pharmaceutically acceptable derivative thereof. A yet further alternative topical anti-acne formulation may be prepared by combining a pyridine thiol such as zinc pyrithione with salicylic acid or a pharmaceutically acceptable derivative thereof.

Such formulations may be prepared and administered using known techniques. They may for example take the form of creams, lotions or gels.
The concentrations of the active agents may be in the ranges described above, and will be determined based on the intended uses of the formulations, their intended modes of administration and the activities of the particular chosen active agents.
Claims

1. An anti-acne formulation containing (a) a pyridine thiol; (b) a bis-quinolinium salt which contains a cation of formula (I) below:

   ![Chemical Structure](attachment:image.png)

   (I)

   wherein n is an integer from 3 to 18; and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate.

2. A formulation according to claim 1, which is suitable for topical application to human skin.

3. A formulation according to claim 1 or claim 2, wherein the pyridine thiol is a pyrithione or tautomer or derivative thereof.

4. A formulation according to claim 3, wherein the pyrithione is present in the form of a metal pyrithione salt.

5. A formulation according to claim 4, wherein the pyrithione is selected from the group consisting of zinc, selenium, silver, copper and sodium pyrithiones and mixtures thereof.

6. A formulation according to claim 5, wherein the pyrithione is zinc pyrithione.

7. A formulation according to any one of the preceding claims, wherein in formula (I), n is an integer from 8 to 12.

8. A formulation according to claim 7, wherein the bis-quinolinium salt is a dequalinium salt.
9. A formulation according to any one of the preceding claims, wherein the bis-
quinolinium salt is a halide.

10. A formulation according to claim 9, wherein the bis-quinolinium salt is a
chloride.

11. A formulation according to any one of claims 1 to 8, wherein the bis-
quinolinium salt is a carboxylate.

12. A formulation according to any one of the preceding claims, wherein the
salicylic acid derivative is a salicylate.

13. A formulation according to claim 12, wherein the salicylate is a metal
salicylate.

14. A formulation according to any one of claims 1 to 11, wherein the salicylic acid
is present in the form of the free acid.

15. A formulation according to any one of the preceding claims, wherein the
concentration of the pyridine thiol is 0.05% w/v or greater.

16. A formulation according to any one of the preceding claims, wherein the
concentration of the pyridine thiol is up to 1% w/v.

17. A formulation according to any one of the preceding claims, wherein the
concentration of the bis-quinolinium salt is 0.1% w/v or greater.

18. A formulation according to any one of the preceding claims, wherein the
concentration of the bis-quinolinium salt is up to 5% w/v.

19. A formulation according to any one of the preceding claims, wherein the
weight ratio of the pyridine thiol to the bis-quinolinium salt is from 1:50 to
50:1.
20. A formulation according to claim 19, wherein the weight ratio of the pyridine thiol to the bis-quinolinium salt is from 1:20 to 1:1.

21. A formulation according to any one of the preceding claims, wherein the concentration of the salicylic acid or derivative is 0.1% w/v or greater.

22. A formulation according to any one of the preceding claims, wherein the concentration of the salicylic acid or derivative is up to 5% w/v.

23. A formulation according to any one of the preceding claims, which additionally contains one or more agents selected from anti-acne agents, keratolytics, comedolytics, agents capable of normalising keratinocyte and/or sebocyte function, anti-inflammatories, anti-proliferatives, antibiotics, anti-androgens, sebum controlling agents, anti-pruritics, immunomodulators, agents which promote wound healing, additional antimicrobial agents, and mixtures thereof.

24. A formulation according to any one of the preceding claims, which is in the form of a cream, paste, gel, lotion, foam, ointment, varnish or other viscous or semi-viscous fluid.

25. A formulation according to any one of the preceding claims, which is, or may be, applied to a carrier selected from a sponge, swab, brush, tissue, cloth, wipe, pad, skin patch, skin adhesive or other material designed for application to a tissue surface.

26. An anti-acne formulation which is substantially as herein described.

27. A product containing an anti-acne formulation according to any one of the preceding claims.

28. A product according to claim 27, which is a cosmetic, a skin or hair care preparation, a pharmaceutical or cosmeceutical preparation or a toiletry product.
29. A kit for preparing an anti-acne formulation, the kit comprising sources of (a) a pyridine thiol, (b) a bis-quinolinium salt which contains a cation of formula (I) as defined in claim 1, and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, together with instructions for combining the three compounds so as to make the formulation at or before the point of its intended application, and/or for the co-administration of the three compounds to a surface.

30. A method for preparing an anti-acne formulation, which method involves mixing together (a) a pyridine thiol, (b) a bis-quinolinium salt which contains a cation of formula (I) as defined in claim 1, and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate.

31. A formulation containing (a) a pyridine thiol, (b) a bis-quinolinium salt which contains a cation of formula (I) as defined in claim 1, and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, for use in the treatment of acne.

32. A formulation containing (a) a pyridine thiol, (b) a bis-quinolinium salt which contains a cation of formula (I) as defined in claim 1, and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, for use in the treatment of a condition affecting the human or animal body, which condition is caused by, transmitted by and/or exacerbated by propionibacterial activity.

33. Use of (a) a pyridine thiol, (b) a bis-quinolinium salt which contains a cation of formula (I) as defined in claim 1, and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, in the manufacture of a medicament for the treatment of acne.

34. A method for controlling the growth of a propionibacterium, the method comprising applying, to a non-living area or surface which is infected or suspected to be infected or capable of becoming infected with the bacterium, a formulation containing (a) a pyridine thiol, (b) a bis-quinolinium salt which
contains a cation of formula (I) as defined in claim 1, and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate.

35. Use of a formulation containing (a) a pyridine thiol, (b) a bis-quinolinium salt which contains a cation of formula (I) as defined in claim 1, and (c) salicylic acid or a derivative thereof, other than dequalinium salicylate, as an anti-acne or skin care agent for non-therapeutic purposes.

36. Use of a pyridine thiol and a bis-quinolinium salt which contains a cation of formula (I) as defined in claim 1, in an anti-acne formulation, in combination with salicylic acid or a derivative thereof, other than dequalinium salicylate, for the purpose of increasing the anti-acne activity of the formulation and/or of reducing the amount of the salicylic acid or derivative in the formulation without or without undue loss of anti-acne activity.

37. An anti-acne formulation containing (a) a bis-quinolinium salt which contains a cation of formula (I) below:

\[
\text{(I)}
\]

wherein \( n \) is an integer from 3 to 18, and (b) salicylic acid or a derivative, provided that (a) and (b) are not both dequalinium salicylate.

38. A formulation according to claim 37, for use in the treatment of acne.
**Application No:** GB0921378.6  
**Examiner:** Dr Natalie Coombs  
**Claims searched:** 1-38  
**Date of search:** 19 March 2010

**Patents Act 1977: Search Report under Section 17**

**Documents considered to be relevant:**

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<td>GB995492 A (JOHNSON &amp; JOHNSON) See whole document especially page 2 lines 22-36 and lines 71-82</td>
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<td>WO2008/035078 A1 (SYNTOPIX LIMITED) See whole document especially page 1 lines 7-14</td>
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**Field of Search:**

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC X:
Worldwide search of patent documents classified in the following areas of the IPC
A61K; A61P; A61Q
The following online and other databases have been used in the preparation of this search report
CAS ONLINE, EPODOC, WPI, BIOSIS, MEDLINE

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