(54) Title: USE OF HALOGENATED SALICYLANILIDES FOR THE TREATMENT OF ACNE

(57) Abstract: A halogenated salicylanilide (other than a brominated salicylanilide), or a pharmaceutically acceptable derivative thereof, for use in the treatment of a propionibacterial condition and/or of acne. The salicylanilide may in particular be a chlorinated salicylanilide such as niclosamide.
USE OF HALOGENATED SALICYLANILIDES FOR THE TREATMENT OF ACNE

Field of the invention

This invention relates to the use of certain compounds as anti-acne agents.

Background to the invention

Certain types of salicylanilide, including halogenated salicylanilides, are known for use as anthelmintics and are used in particular to destroy or expel tapeworms in domestic animals.

Certain brominated salicylanilides are also known to have antibacterial and antifungal activities, and are used for example as disinfectants in medicated soaps: these include 4',5-dibromosalicylanilide (also known as dibromosalan, CAS #: 87-12-7); 3,5-dibromosalicylanilide (also known as metabolosalan, CAS #: 2577-72-2); and 3,4',5-tribromosalicylanilide (also known as tribromosalan, CAS #: 87-10-5) which is used as a bacteriostat in detergents.

FR-1.568.910 for example describes the use of halogenated (in particular brominated) salicylanilides as topical disinfectants. EP-1 362 581 mentions the use of tribromosalan as an antimicrobial agent, in combination with an anti-perspirant, in a pharmaceutical or cosmetic composition for topical application to the skin. EP-0 934 742 also refers to tribromosalan as an antimicrobial agent, for use in topical antimicrobial cleansers. US-4,205,061 discloses an oral antimicrobial composition containing a synergistic combination of 3,5-dibromo-3'-trifluoromethyl salicylanilide and cetyl pyridinium chloride; the composition is intended to help prevent plaque and gingival diseases, without tooth staining.

Other halogenated salicylanilides are known for use as antimicrobial agents in certain contexts. For example, CH-506 292 discloses an oral health care composition,
particularly for use against dental caries, which contains a halogenated trifluoromethyl salicylanilide and an alkali metal trimetaphosphate.

WO-02/28819, meanwhile, describes the use of naphthyl-substituted salicylanilides as topical antibacterial and anti-inflammatory agents; the inclusion of the naphthyl group is said to confer activity against a range of bacteria.

Salicylanilides are also known as 2-hydroxy-N-phenylbenzamides or 2-hydroxybenzanilides. They have the following basic structure:

Specific known halogenated salicylanilides include:

- Closantel (CAS #: 57808-65-8), which is a halogenated salicylanilide also known as N-[5-chloro-4-[(4-chlorophenyl)cyanomethyl]-2-methylphenyl]-2-hydroxy-3,5-di-iodobenzamide, and is used as a veterinary anthelmintic. This is a broad spectrum antiparasitic agent used against several species and developmental stages of trematodes, nematodes and arthropods. The anti-trematode activity of closantel is mainly used against liver fluke. Its anti-nematode and anti-arthropod activity are especially used against species which feed on blood or plasma. The drug is widely used in sheep and cattle and can be used either parenterally (s.c. or i.m.) or orally for both prophylactic and therapeutic purposes. It is available as drench, bolus and injectable formulations. Closantel has also been combined with mebendazole and several other benzimidazoles in drench formulations for sheep and with levamisole in a bolus for cattle.

- Niclosamide, which is another halogenated salicylanilide and is also known as 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide. It is used as a
veterinary anthelmintic against cestodes, and its ethanolamine salt is also
known for use as a molluscicide.

- Rafinoxanide (3’-chloro-4’-(p-chlorophenoxy)-3,5-diiodosalicylanilide), which is
  known for veterinary use as a fasciolicide and anthelmintic.

- Oxyclozlanide (3,3’,5,5’,6-pentachloro-2’-hydroxsalicylanilide), which again is
  known for veterinary use as an anthelmintic, primarily against trematodes.

It has now surprisingly been found that salicylanilides such as these can be
antibacterially active, in particular against propionibacteria, and can therefore be used
as anti-acne agents.

10 Statements of the invention

According to a first aspect of the present invention there is provided a halogenated
salicylanilide (other than a brominated salicylanilide), or a pharmaceutically
acceptable derivative thereof, 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 (in particular caused or transmitted by) propionibacteria.

The propionibacteria are known to be implicated in acne. Thus, a second aspect of the
invention provides a halogenated salicylanilide (other than a brominated
salicylanilide), or a pharmaceutically acceptable derivative thereof, for use as an anti-
acne agent (ie, for use in the treatment of acne or acne lesions).

Instead or in addition, the salicylanilide may be for use against an opportunistic
infection which is caused, transmitted and/or exacerbated by (in particular caused by)
propionibacteria, for instance an infection associated with an indwelling surgical
device (a prosthetic joint, for example). It may be for use in treating an infected
wound, burn or ulcer. It may be for use against any other infection or condition which
involves or can involve propionibacteria, for example an eye infection such as
endophthalmitis. In cases it may be used in the treatment of body odour, in particular
in the axilla and/or feet, since this is a condition in which propionibacteria can sometimes be implicated.

A halogenated salicylanilide is substituted at either or both of its phenyl rings with one or more halo groups. According to the present invention, however, the salicylanilide is not substituted with any bromo groups. A halo substituent may be selected from fluoro, chloro and iodo, in particular chloro and iodo.

In an embodiment, the salicylanilide is substituted with two or more halo groups, for example three or more.

The salicylanilide may also be substituted, at either or both of its phenyl rings, with one or more substituents selected from hydroxyl, nitro, cyano, phenoxy, optionally substituted C$_1$-C$_4$ alkyl (in particular methyl) and ether groups –OR. In an ether group –OR, R may be selected for example from optionally substituted C$_1$-C$_4$ alkyl and optionally substituted phenyl.

Substituents for substituted C$_1$-C$_4$ alkyl groups may be selected for example from cyano (–CN), halo (other than bromo), hydroxyl, nitro, C$_1$-C$_4$ alkyl and optionally substituted phenyl, in particular cyano and optionally substituted phenyl.

Substituents for substituted phenyl groups may be selected for example from cyano, halo (other than bromo), hydroxyl, nitro, C$_1$-C$_4$ alkyl and optionally substituted phenyl, in particular halo and C$_1$-C$_4$ alkyl, more particularly halo.

The salicylanilide may for example be substituted with one or more chloro groups, for example two or more chloro groups (in other words, it may be a chlorinated salicylanilide). It may instead or in addition be substituted with one or more, for example two or more, iodine atoms.

Because the present invention does not extend to the use of brominated salicylanilides, the salicylanilide should not for example be dibromsalan, metabromsalan or tribromsalan.
In an embodiment of the invention, the salicylanilide is selected from closantel, niclosamide, rafoxanide, oxyclozanide and mixtures thereof.

In an embodiment, the salicylanilide is selected from closantel, niclosamide and mixtures thereof. In another embodiment, the salicylanilide is niclosamide.

A "pharmaceutically acceptable derivative" of a halogenated salicylanilide may be a derivative which is acceptable for veterinary use. A "derivative" may for example be selected from salts, esters, solvates and also so-called "prodrug" forms or protected forms which revert to an active form of the relevant compound at an appropriate time on or after administration.

In particular, the salicylanilide may be used in the form of a pharmaceutically acceptable salt, for example a metal salt or an ammonium salt (in particular the NH$_4^+$ salt). Suitable metal salts include the alkali metal salts (for example the sodium or potassium salts, in particular the former) and the alkaline earth metal salts (for example the calcium salt). Other potential salts include substituted ammonium (for example alkanolammonium, in particular ethanolammonium) salts, and piperazine salts.

In particular where the salicylanilide is closantel, it may be used in the form of its sodium salt.

In particular where the salicylanilide is niclosamide, it may be used in the form of the free base, or of a salt such as the ethanolammonium or piperazine salt, and/or in the form of a hydrate such as a monohydrate.

The salicylanilide or derivative may in particular be for use against one or more bacteria associated with acne, more particularly the propionibacteria. It may for example be for use against one or more strains of *Propionibacterium acnes* and/or in some instances *P. granulosum*.

A third aspect of the present invention provides the use of a halogenated salicylanilide (other than a brominated salicylanilide), or a pharmaceutically acceptable derivative thereof, in the preparation of a medicament (typically a formulation) 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 (in particular caused or transmitted by) propionibacteria.

A fourth aspect provides the use of a halogenated salicylanilide (other than a brominated salicylanilide), or a pharmaceutically acceptable derivative thereof, in the preparation of a medicament (typically a formulation) for use in the treatment of acne or acne lesions.

The salicylanilide or derivative is suitably used as an antibacterial, in particular an anti-propionibacterial, agent in the medicament. It is suitably used as an anti-acne agent (ie, as an agent which is active against acne (which includes against a symptom and/or a cause of acne) and/or against one or more micro-organisms associated with 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. 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 salicylanilide or derivative as a bactericide, in particular against propionibacteria.

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.

The treatment of acne also encompasses the treatment and/or prevention of lesions and/or scarring associated with acne.
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.

In accordance with all aspects of the invention, a mixture of two or more halogenated salicylanilides, or derivatives thereof, may be used as an antibacterial and/or anti-acne agent.

In embodiments of the invention, the salicylanilide or derivative is applied topically. It is preferably used in the form of a formulation which is suitable for topical application to, and/or contact with, the skin, in particular human skin. It is therefore preferably contained in a pharmaceutically acceptable vehicle which can safely be applied to, and/or contacted with, the skin and/or other epithelia.

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 salicylanilide or derivative may be present in the form of a solution or suspension, the term “suspension” including emulsions, micellar systems and other multi-phase dispersions.

The salicylanilide or derivative may in general, however, be delivered by any appropriate route, whether local or systemic. It may for example be delivered orally, for instance in the form of a tablet, capsule, powder, granules, solution or suspension. In this case the salicylanilide or derivative should be used in the form of a formulation which is suitable and/or adapted for oral ingestion. Again suitable vehicles for use in such formulations will be well known to those skilled in the art of preparing pharmaceutical preparations for oral delivery.
Alternatively the salicylanilide or derivative may be delivered transdermally, for instance via a skin patch.

The salicylanilide or derivative may 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, microsponges, microemulsions, hydrogels and solid lipid nanoparticles.

The antibacterial activity of the salicylanilide or derivative may be growth inhibitory activity or more preferably biocidal (i.e., lethal to the relevant organism). In the context of this invention, activity against a particular species of bacterium 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; thus, in accordance with the invention, the halogenated salicylanilide may be used to treat a condition which is caused, transmitted and/or exacerbated by microbial biofilm formation, in particular biofilm formation which is caused or exacerbated by, or which otherwise involves (in particular which is caused by), propionibacteria.

In the present context, the disruption of biofilm formation embraces any negative effect on the ability of a bacterium to form, maintain or exist in a biofilm, and/or on a biofilm already formed by the bacterium. 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 bacterium 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 bacterium 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 bacterium.
A formulation prepared or used according to the invention is preferably active against one or more propionibacteria which are wholly or partially resistant to one or more antibiotics, for instance those which are in common clinical use. For example, the formulation is ideally active against erythromycin-resistant, clindamycin-resistant and/or tetracycline-resistant *P. acnes* strains of bacteria, 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.

Antibacterial 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 bacterium with the candidate antibacterial compound, incubating the treated culture under conditions which would ordinarily support growth of the bacterium, and assessing the level of growth, if any, which can occur in the presence of the candidate compound.

Preferably the salicylanilide or derivative has a minimum inhibitory concentration (MIC), at least against propionibacteria, of 250 μg/ml or less, more preferably 100 or 50 μg/ml or less, most preferably 25 or 15 or 10 or 5 or in cases 2 or even 1 or 0.5 μg/ml or less. Its corresponding minimum biocidal concentration (MBC) is preferably 250 μg/ml or less, more preferably 100 or 50 μg/ml or less, most preferably 25 or 15 or 10 or 5 or in cases 2 or even 1 or 0.5 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. More preferably the salicylanilide or derivative also exhibits such characteristics in the presence of at least one of, preferably both of, lipid 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.

The concentration of the salicylanilide or derivative in a formulation prepared or used according to the invention, in particular a formulation for topical delivery, might suitably be 0.05 or 0.1 % w/v or greater, preferably 0.3 or 0.5 % w/v or greater. Its concentration might be up to 5 % w/v, for example up to 3 or 2 or 1.5 or 1 % w/v. Its concentration may for instance be from 0.05 to 2 % w/v or from 0.1 to 1.5 % w/v, for example about 1 % w/v.
For oral delivery, from about 50 mg to 2 g of the salicylanilide or derivative may be
administered daily, whether as a single dose or as two or more divided doses. Thus the
salicylanilide or derivative may be formulated in dosage forms – for example tablets or
capsules – containing up to about 2 g, for instance from about 50 mg to 2g, of the
active substance.

As discussed above, a formulation prepared or used according to the invention may be
suitable for, and more preferably adapted for, topical administration to human skin. It
may take the form of a lotion, cream, ointment, foam, paste, gel or any other physical
form known for topical administration, including for instance 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, in particular to a wound) to facilitate its
topical administration. It may be intended for pharmaceutical (which includes
veterinary but is preferably human) use, and/or for cosmetic or other non-medical care
purposes (for example, for general hygiene or skin cleansing).

In such cases the vehicle in which the salicylanilide or derivative is 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, A. A. et
strategies.

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. It may delay or otherwise control release of the formulation over a
particular time period. The salicylanilide or derivative 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. Where the formulation is intended for use on the skin, the vehicle may be primarily non-aqueous, although in the case of an anti-acne treatment an aqueous vehicle may be used. The vehicle is suitably volatile. In cases it may be alcohol-based or silicon-based.

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/or other additives known for use in pharmaceutical or veterinary formulations, in particular topical skin care formulations. Examples include emollients, perfumes, antioxidants, preservatives, stabilisers, gelling agents and surfactants; others may be found in Williams' "Transdermal and Topical Drug Delivery", supra. For the treatment of acne, however, it may be preferred for the formulation not to contain an emollient.

The formulation may contain additional active agents. It may for example contain one or more additional agents selected from anti-acne agents, keratolytics, comedolytics, agents capable of normalising keratinocyte and/or sebocyte function, anti-inflammatories, anti-proliferatives, antibiotics, anti-androgens, sebostatic/sebosuppressive agents, anti-pruritics, immunomodulators, agents which promote wound healing, additional antimicrobial (in particular antibacterial) agents and mixtures thereof. It may in particular contain one or more agents selected from anti-acne agents, keratolytics, comedolytics, sebostatic/sebosuppressive agents, anti-inflammatories and additional antibacterial agents. It may instead or in addition contain one or more agents selected from sunscreens, moisturisers and mixtures thereof.

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

Generally speaking a formulation prepared or used 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 however be preferred for the salicylanilide or derivative to be the only active agent in the formulation, or at least to be the only antimicrobially or bacteriologically active agent and/or the only anti-acne active agent.

In an embodiment of the invention, it may be preferred for the formulation not to contain a halogenated trifluoromethyl salicylanilide, for instance as described in CH-506 292.

In an embodiment, it may be preferred for the formulation not to contain an alkali metal trimetaphosphate, for instance as described in CH-506 292.

In an embodiment, it may be preferred for the formulation not to contain a naphthyl-substituted salicylanilide, for instance as described in WO-02/28819.

In an embodiment, it may be preferred for the formulation not to contain an anti-perspirant, for instance as described in EP-1 362 581.

In an embodiment, it may be preferred for the formulation not to contain an anchoring agent of the type referred to in EP-0 934 742, in particular an anchoring agent selected from C3 to C6 polyols, adloses and ketoses, more particularly glycerin.

In an embodiment, it may be preferred for the formulation not to contain 3,5-dibromo-3′-trifluoromethyl salicylanilide and/or cetyl pyridinium chloride, for instance as described in US-4,205,061.

In an embodiment, it may be preferred for the salicylanilide or derivative not to be for use against a bacterial infection within the oral cavity, in particular dental caries. It may be preferred for the salicylanilide or derivative not to be for use as a general skin disinfectant, such as in a hand or face wash or other general cleansing preparation. It may be preferred for the salicylanilide or derivative not to be for use as an anti-inflammatory agent. It may be preferred for the salicylanilide or derivative not to be for use against plaque and/or gingival diseases, and/or to reduce tooth staining.
In an embodiment, it may be preferred for the salicylanilide or derivative not to be applied topically within the oral cavity, and/or not to be adapted or intended for use in that way.

A formulation prepared or used according to the invention may be marketed with an indication that it has antibacterial and/or anti-acne activity, or enhanced antibacterial and/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 and/or anti-acne activity of the formulation may be attributed, in such an indication, at least partly to the presence of the halogenated salicylanilide or derivative.

The invention may involve assessing the antibacterial and/or anti-acne activity of the formulation during or after its preparation, for instance against one or more propionibacteria. It may involve assessing the antibacterial and/or anti-acne activity of the formulation both before and after incorporation of the salicylanilide or derivative, for example so as to confirm that it contributes to the antibacterial and/or anti-acne activity of the formulation.

A formulation prepared or used 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, or a shampoo, conditioner, styling mousse or gel or hair spray), a deodorant or anti-perspirant, a cleansing preparation (for example a facial wash), a pharmaceutical (which includes veterinary) preparation, a cosmeceutical preparation, or a toiletry product (for instance a bath or shower additive or a soap).

A fifth aspect of the present invention 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 halogenated salicylanilide (other than a brominated salicylanilide) or
derivative thereof. The salicylanilide or derivative is suitably applied in a formulation of the type described above, for instance topically. It may in particular be applied to an area or surface which is infected with the relevant bacterium.

"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 organism. It also embraces reducing the risk of subsequent growth of the bacterium in the area or on the surface being treated. The method of the invention may thus be used to treat an existing occurrence of the bacterium or to prevent a potential subsequent occurrence.

Again the area or surface to which the salicylanilide or derivative is applied will typically be a surface such as human or animal tissue, in particular the skin, typically of a living human being. In this case the salicylanilide or derivative may be applied for therapeutic purposes or for non-therapeutic (eg, purely cosmetic) purposes.

Thus according to a sixth aspect of the invention, there is provided a method of treatment of a human or animal patient suffering from or at risk of suffering from a condition which is caused by, transmitted by and/or exacerbated by (in particular caused or transmitted by) propionibacteria, the method involving administering to the patient a therapeutically (which term includes prophylactically) effective amount of a halogenated salicylanilide (other than a brominated salicylanilide) or a pharmaceutically acceptable derivative thereof. Again the salicylanilide or derivative may be administered by any appropriate route, for example orally or more preferably topically. It may be administered in a formulation of the type described above.

According to a seventh aspect there is provided a method of treatment of a patient suffering from or at risk of suffering from acne or acne lesions, the method involving administering to the patient a therapeutically (which term includes prophylactically) effective amount of a halogenated salicylanilide (other than a brominated salicylanilide) or a pharmaceutically acceptable derivative thereof. The salicylanilide or derivative may be administered by any appropriate route, for example orally or more preferably topically. It may be administered in an anti-acne formulation of the type described above.
In accordance with the sixth and seventh aspects of the invention, the salicylanilide or
derivative is suitably administered to a human patient. The patient is suitably suffering
from the relevant condition, in particular acne.

According to an eighth aspect, the present invention provides an antibacterial or anti-
acone formulation containing a halogenated salicylanilide (other than a brominated
salicylanilide) or pharmaceutically acceptable derivative thereof, together with a
pharmaceutically acceptable vehicle, the formulation being adapted for topical
application, in particular to human skin.

A ninth aspect provides an antibacterial or anti-acne formulation containing a
halogenated salicylanilide (other than a brominated salicylanilide) or pharmaceutically
acceptable derivative thereof, together with a pharmaceutically acceptable vehicle, the
formulation being adapted for oral delivery, in particular to a human patient.

The invention also provides, according to a tenth aspect, a product which incorporates
an antibacterial or anti-acne formulation according to the eighth or the ninth aspect.

An eleventh aspect provides the use of a halogenated salicylanilide (other than a
brominated salicylanilide), or a derivative thereof, as an anti-propionibacterial and/or
anti-acne agent.

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.

**Detailed description**

Experimental tests were conducted to determine the anti-propionibacterial activity of formulations prepared 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. This is a very common, complex and multi-factorial skin disease in which *P. acnes* and other *Propionibacterium* spp. (for example *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 2 below. These included certain antibiotic-resistant propionibacteria, such as the two *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 *P. granulosum*, another bacterium involved in acne, were
also tested in Example 2.

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 antibacterial activity against the test
organisms.

(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 μl 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…μg/ml, etc..
down to 0.49 μg/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, ie, 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 microbial 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 microbial population to recover.

(c) *Agar dilution MIC* assay

This is a standard international method for quantitatively assessing the antimicrobial activity of a compound in a solid medium. The test compound was prepared to 40× the highest concentration required (eg, 10 mg/ml for a final concentration of 250 μg/ml) and a series of doubling dilutions were performed in a suitable solvent. A set amount of these antimicrobial stock solutions was then added to molten agar medium (ca 55 °C), mixed thoroughly, poured into sterile Petri dishes and allowed to cool/set. The culture medium was as described above.

A Multipoint™ Inoculator (AQS Manufacturing Ltd, UK) was used to inoculate the plates by spotting the inocula onto the surface of the agar, delivering approximately 1 to 2 μl per spot (yielding $10^5$ CFU (colony forming units) per spot).
The plate(s) were then incubated under the conditions described above, following which they were examined visually for signs of bacterial growth. The MIC value was ascertained when there was a marked reduction in, or total loss of, growth on the test plate at the lowest concentration as compared to that of the growth on the control plate.

The assays were conducted in triplicate and included a positive control (culture medium, diluting solvent and inoculum).

(d) 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.

(e) Supplemented disc diffusion assays

The DDA test may be carried out using an agar gel supplemented with lipid and/or salt to simulate some of the major components present in human skin and to assess whether these substances might affect the antimicrobial activity observed for the test compound. Performance under these conditions can provide a more reliable indication of activity on topical application. The supplement used in Example 1 below was sodium chloride (100 mM).
Example 1 – activity against Propionibacterium spp

The following experiments all used P. acnes NCTC 737 as the test organism.

MIC, MBC and DDA assays, as described above, were carried out using as the test compounds closantel, niclosamide, rafoxanide and oxyclozanide (all ex Sigma Aldrich, UK). The solvents used were DMSO for the closantel and niclosamide, and ethanol for the rafoxanide and oxyclozanide. All four actives were used in the form of their free bases rather than as salts.

Supplemented DDA assays were also carried out in the presence of sodium chloride, again as described above.

The results are shown in Table 1 below. All tests were conducted in triplicate.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
<th>DDA (mm)</th>
<th>DDA + salt (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closantel</td>
<td>0.12</td>
<td>0.49</td>
<td>29.79</td>
<td>27.92 (±1.1)</td>
</tr>
<tr>
<td>Niclosamide</td>
<td>0.03</td>
<td>0.98</td>
<td>43.44</td>
<td>41.98 (±0.98)</td>
</tr>
<tr>
<td>Rafoxanide</td>
<td>0.12</td>
<td>0.98</td>
<td>21.46</td>
<td>22.92 (±3.34)</td>
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<tr>
<td>Oxyclozanide</td>
<td>0.49</td>
<td>1.95</td>
<td>52.6</td>
<td>50.73 (±1.41)</td>
</tr>
</tbody>
</table>

It can be seen from Table 1 that all four compounds are highly active as antibacterial agents against P. acnes NCTC 737. This indicates their likely utility as anti-acne agents, the propionibacteria being implicated in acne.

This high level of activity appears to be maintained in the presence of salt.

Example 2 – activity against other Propionibacterium spp
The activities (MIC by agar dilution) of closantel and niclosamide were determined against a panel of different propionibacterium strains. DMSO was used as the solvent. All tests were performed in triplicate.

The results are shown in Table 2 (closantel MICs) and Table 3 (niclosamide MICs) below; the resistance phenotype for each of the test species/strains is also indicated.

### Table 2

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Resistance phenotype</th>
<th>MIC (µg/ml)</th>
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<tbody>
<tr>
<td>Propionibacterium acnes NCTC 737</td>
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</tr>
<tr>
<td>P. granulosum NCTC 11865</td>
<td>None</td>
<td>0.49</td>
</tr>
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<td>Tet/MLS</td>
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</tr>
<tr>
<td>P. acnes PRP-003</td>
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</tr>
<tr>
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<td>P. granulosum PRP-005</td>
<td>MLSK</td>
<td>0.25</td>
</tr>
<tr>
<td>P. granulosum PPR-006</td>
<td>MLS</td>
<td>0.49</td>
</tr>
<tr>
<td>P. acnes PPR-007</td>
<td>Clin</td>
<td>0.12</td>
</tr>
<tr>
<td>P. acnes PRP-008</td>
<td>Clin</td>
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<td>Test organism</td>
<td>Resistance phenotype</td>
<td>MIC (µg/ml)</td>
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</tr>
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<td>0.12</td>
</tr>
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<tr>
<td><em>P. acnes</em> PRP-102</td>
<td>Tet/MLS</td>
<td>0.12</td>
</tr>
</tbody>
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[Abbreviations: 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).]

Table 3

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Resistance phenotype</th>
<th>MIC (µg/ml)</th>
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</thead>
<tbody>
<tr>
<td><em>Propionibacterium acnes</em> NCTC 737</td>
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<td>0.03</td>
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<tr>
<td><em>P. granulosum</em> NCTC 11865</td>
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<td><em>P. acnes</em> PRP-002</td>
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<td>MLSK</td>
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</tr>
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<tr>
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<td>MLSK</td>
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<tr>
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<td><em>P. granulosum</em> PRP-021</td>
<td>MLS</td>
<td>0.06</td>
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<tr>
<td>Test organism</td>
<td>Resistance phenotype</td>
<td>MIC (µg/ml)</td>
</tr>
<tr>
<td>----------------------------</td>
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<td>MLS</td>
<td>0.06</td>
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<td>Tet/MLS</td>
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</tr>
<tr>
<td><em>P. granulosum</em> PRP-055</td>
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<td>0.03</td>
</tr>
<tr>
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</tr>
<tr>
<td><em>P. acnes</em> PRP-102</td>
<td>Tet/MLS</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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Both closantel and niclosamide can be seen to possess an excellent level of activity against the wide range of propionibacterium strains tested. This further indicates the utility of such halogenated salicylanilides either to treat or to prevent infections associated with such bacteria, in particular acne. The results are likely to be of particular clinical value for the antibiotic resistant test strains.

**Example 3 – topical anti-acne formulations**

The results from Examples 1 and 2 show that halogenated salicylanilides can be effective antibacterial agents against the bacteria associated with acne. This can be of use in preparing antibacterial formulations, in particular for topical application to the skin, for prophylactic or therapeutic use in any context where such bacteria are thought
to be involved as possible sources of infection. More specifically, it can be of use in preparing anti-acne formulations, again suitably for topical use.


The formulation may be prepared and administered using known techniques. It may for example take the form of a cream, lotion, ointment or gel.

The concentration of the salicylanilide or derivative may be in the ranges described above, and will be determined based on its antibacterial activity and the intended use of the formulation.
Claims

1. A halogenated salicylanilide (other than a brominated salicylanilide), or a pharmaceutically acceptable derivative thereof, 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 propionibacteria.

2. A halogenated salicylanilide (other than a brominated salicylanilide), or a pharmaceutically acceptable derivative thereof, for use in the treatment of acne or acne lesions.

3. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to claim 1 or claim 2, wherein the salicylanilide or derivative is topically applied.

4. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to claim 1 or claim 2, wherein the salicylanilide or derivative is delivered orally.

5. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to any one of the preceding claims, wherein the salicylanilide is substituted with one or more halogen atoms selected from chlorine and iodine atoms.

6. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to claim 5, wherein the salicylanilide is a chlorinated salicylanilide.

7. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to claim 6, wherein the salicylanilide is substituted with two or more chlorine atoms.
8. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to any one of the preceding claims, wherein the salicylanilide is substituted with two or more iodine atoms.

9. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to any one of the preceding claims, wherein the salicylanilide is selected from closantel, niclosamide, rafoxanide, oxyclozanide and mixtures thereof.

10. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to claim 9, wherein the salicylanilide is selected from closantel, niclosamide and mixtures thereof.

11. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to claim 10, wherein the salicylanilide is niclosamide.

12. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to any one of the preceding claims, wherein the salicylanilide or derivative is used in a formulation at a concentration of up to 5% w/v.

13. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to any one of the preceding claims, wherein the salicylanilide or derivative is used in a formulation at a concentration of 0.05% w/v or greater.

14. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to any one of the preceding claims, wherein the salicylanilide or derivative is used in a formulation which additionally contains one or more agents selected from anti-acne agents, keratolytics, comedolytics, agents capable of normalising keratinocyte and/or sebocyte function, anti-inflammatory, anti-proliferatives, antibiotics, anti-androgens, sebostatic/sebostimulatory agents, anti-pruritics, immunomodulators, agents which promote wound healing, additional antimicrobial agents and mixtures thereof.
15. A halogenated salicylanilide or pharmaceutically acceptable derivative thereof, for use according to any one of the preceding claims, wherein the salicylanilide or derivative is used in a formulation which is in the form of a cream, paste, gel, ointment, lotion, foam or other viscous or semi-viscous fluid.

16. A halogenated salicylanilide (other than a brominated salicylanilide), or a pharmaceutically acceptable derivative thereof, for use in the treatment of a condition which is caused by, transmitted by and/or exacerbated by propionibacteria, wherein the use is substantially as herein described.

17. A halogenated salicylanilide (other than a brominated salicylanilide), or a pharmaceutically acceptable derivative thereof, for use in the treatment of acne or acne lesions, wherein the use is substantially as herein described.

18. Use of a halogenated salicylanilide (other than a brominated salicylanilide), or a pharmaceutically acceptable derivative thereof, in the preparation of a medicament 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 propionibacteria.

19. Use of a halogenated salicylanilide (other than a brominated salicylanilide), or a pharmaceutically acceptable derivative thereof, in the preparation of a medicament for use in the treatment of acne or acne lesions.

20. Use according to claim 18 or claim 19, wherein the medicament is a formulation which is suitable and/or intended and/or adapted for topical application.

21. Use according to claim 18 or claim 19, wherein the medicament is a formulation which is suitable and/or intended and/or adapted for oral delivery.

22. Use according to any one of claims 18 to 21, wherein the salicylanilide is substituted with one or more halogen atoms selected from chlorine and iodine atoms.
23. Use according to claim 22, wherein the salicylanilide is a chlorinated salicylanilide.

24. Use according to any one of claims 18 to 23, wherein the salicylanilide is selected from closantel, niclosamide, rafoxanide, oxyclozanide and mixtures thereof.

25. Use according to claim 24, wherein the salicylanilide is niclosamide.

26. Use according to any one of claims 18 to 25, wherein the medicament is substantially as herein described.

27. 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 halogenated salicylanilide (other than a brominated salicylanilide) or derivative thereof.

28. A method according to claim 27, wherein the salicylanilide or derivative is applied topically.

29. A method of treatment of a human or animal patient suffering from or at risk of suffering from a condition which is caused by, transmitted by and/or exacerbated by (in particular caused or transmitted by) propionibacteria, the method involving administering to the patient a therapeutically (which term includes prophylactically) effective amount of a halogenated salicylanilide (other than a brominated salicylanilide) or a pharmaceutically acceptable derivative thereof.

30. A method of treatment of a patient suffering from or at risk of suffering from acne or acne lesions, the method involving administering to the patient a therapeutically (which term includes prophylactically) effective amount of a halogenated salicylanilide (other than a brominated salicylanilide) or a pharmaceutically acceptable derivative thereof.
31. A method according to claim 29 or claim 30, wherein the salicylanilide or derivative is administered topically.

32. A method according to claim 29 or claim 30, wherein the salicylanilide or derivative is delivered orally.

33. A method according to any one of claims 29 to 32, wherein the salicylanilide is selected from closantel, niclosamide, rafinoxide, oxyclozanide and mixtures thereof.

34. A method according to claim 33, wherein the salicylanilide is niclosamide.

35. An antibacterial or anti-acne formulation containing a halogenated salicylanilide (other than a brominated salicylanilide) or pharmaceutically acceptable derivative thereof, together with a pharmaceutically acceptable vehicle, the formulation being adapted for topical application.

36. An antibacterial or anti-acne formulation containing a halogenated salicylanilide (other than a brominated salicylanilide) or pharmaceutically acceptable derivative thereof, together with a pharmaceutically acceptable vehicle, the formulation being adapted for oral delivery.

37. An antibacterial or anti-acne formulation according to claim 35 or claim 36, which is substantially as herein described.

38. A product which incorporates an antibacterial or anti-acne formulation according to any one of claims 35 to 37.

39. Use of a halogenated salicylanilide (other than a brominated salicylanilide), or a derivative thereof, as an anti-propionibacterial and/or anti-acne agent.

40. Use according to claim 39, wherein the salicylanilide is selected from closantel, niclosamide, rafinoxide, oxyclozanide and mixtures thereof.

41. Use according to claim 40, wherein the salicylanilide is niclosamide.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**
INV. A61K31/167 A61P17/10

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

**B. FIELDS SEARCHED**
Minimum documentation searched (classification system followed by classification symbols)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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<tr>
<th>Category</th>
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<td>&quot;Substituted salcylanilides as inhibitors of two-component regulatory systems in</td>
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<td>bacteria&quot; JOURNAL OF MEDICINAL CHEMISTRY, vol. 41, 1998, pages 2939-2945,</td>
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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 document 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

**I** 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

**SH** document member of the same patent family

Date of the actual completion of the international search: 29 September 2008

Date of mailing of the international search report: 09/10/2008

Name and mailing address of the ISA
European Patent Office, P.O. 5018 Patentboulevard 2 NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-2016

Authorized officer
Baurand, Petra

Form PCT/A/210 (second sheet) (April 2005)
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<td>ROTMISTROV M M; MOHILEVYCH N F; STAVS’KA S S: &quot;THE NATURE OF THE ANTI MICROBIAL ACTION OF 4-1 5 DI CHLORO SALICYLANILIDE&quot; MIKROBIOLOHICHNYI ZHURNAL, vol. 34, no. 1, 1972, pages 8-9, XP009105847 the whole document</td>
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