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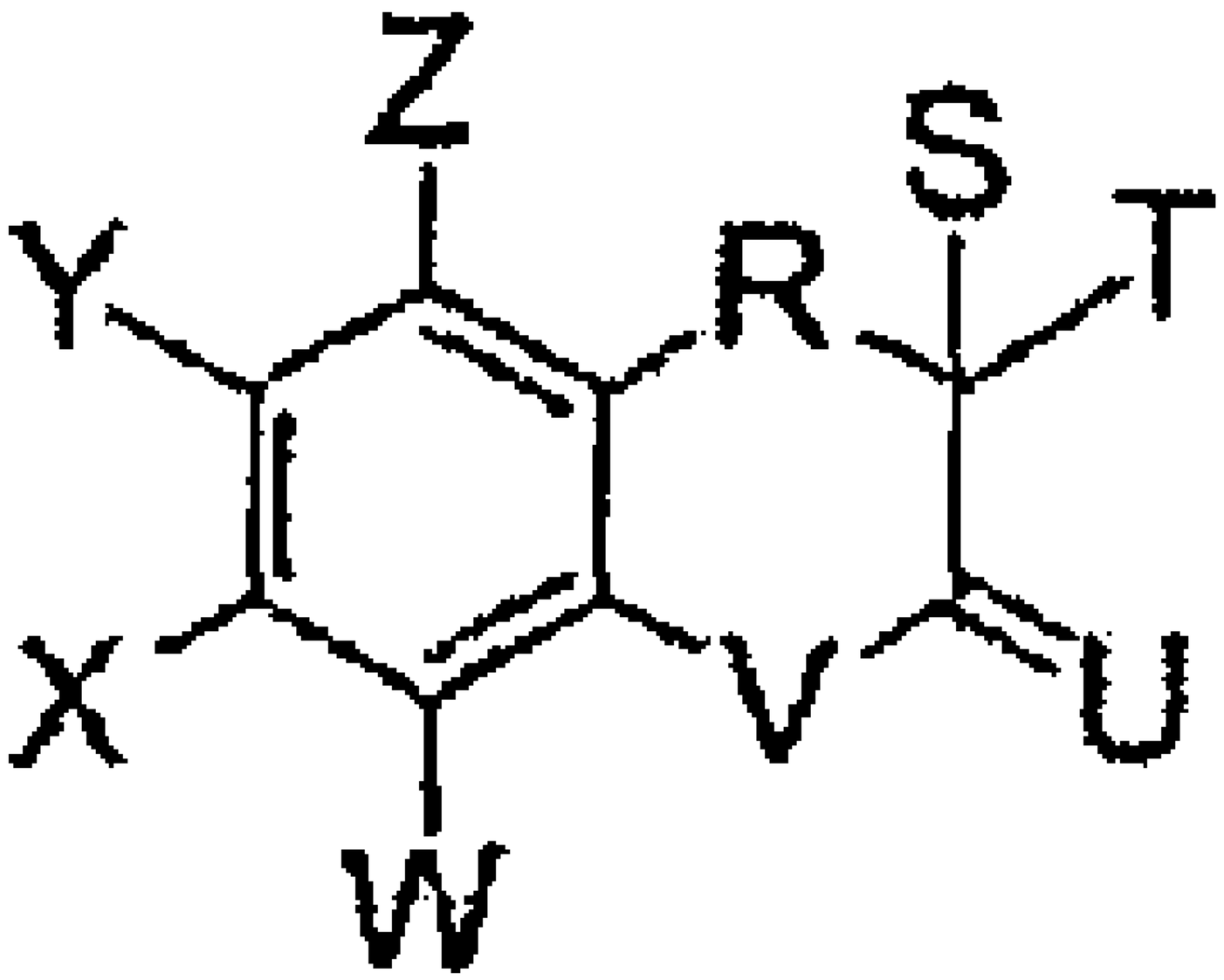
(54) **Titre : BENZOTHIOMORPHOLINE ET DERIVES DE DE BENZOMORPHOLINE COMME INHIBITEURS DE BIOFILM**
(54) **Title: BENZOTHIOMORPHOLINE AND BENZOMORPHOLINE DERIVATIVES AS BIOFILM INHIBITORS**



31

(57) **Abrégé/Abstract:**
Novel compounds and the novel use of a class of previously identified small molecules for the inhibition of biofilm formation is disclosed. Inhibition of biofilm formation can play a very important role in contributing to pathogenicity. Bacteria in the biofilm state

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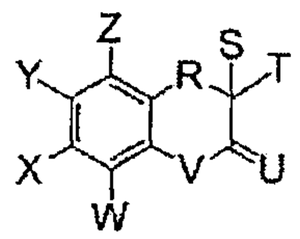
(57) **Abrégé(suite)/Abstract(continued):**
have been shown to be 10- 10,000-fold less susceptible to antibiotic treatment. Estimations made by the Centers for Disease Control (CDC) and the National Institutes of Health (NH) attribute 65% to 80% of human infections as biofilm mediated. Consequently, biofilm formation is often responsible for chronic infections due to bacterial persistence despite antibiotic treatment. The compounds have the general formula: (See Formula 31) wherein: R is O; S and T are independently selected from H, OH, Me, Et, /Pr or methylene; U is O or S; V is CH_2, NH, NMe, Nbenzyl, O or S; W is CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H, or $CH_2OP(O)(ONa)_2$; X is H or F, Y is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$; and Z is H or F.

ABSTRACT

Novel compounds and the novel use of a class of previously identified small molecules for the inhibition of biofilm formation is disclosed. Inhibition of biofilm formation can play a very important role in contributing to pathogenicity. Bacteria in the biofilm state have been shown to be 10- 10,000-fold less susceptible to antibiotic treatment. Estimations made by the Centers for Disease Control (CDC) and the National Institutes of Health (NIH) attribute 65% to 80% of human infections as biofilm mediated. Consequently, biofilm formation is often responsible for chronic infections due to bacterial persistence despite antibiotic treatment. The compounds have the general formula:



31

wherein: R is O; S and T are independently selected from H, OH, Me, Et, *i*Pr or methylene; U is O or S; V is CH_2, NH, NMe, Nbenzyl, O or S; W is CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H, or $CH_2OP(O)(ONa)_2$; X is H or F; Y is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$; and Z is H or F.

BENZOTHIOMORPHOLINE AND BENZOMORPHOLINE DERIVATIVES AS BIOFILM INHIBITORS

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FIELD OF THE INVENTION

Small molecule therapeutics for inhibition of biofilm formation.

BACKGROUND

Inhibition of biofilm formation can play a very important role in contributing to pathogenicity. Bacteria in the biofilm state have been shown to be 10-10,000-fold less susceptible to antibiotic treatment. Estimations made by the Centers for Disease Control (CDC) and the National Institutes of Health (NIH) attribute 65% to 80% of human infections as biofilm mediated. Consequently, biofilm formation is often responsible for chronic infections due to bacterial persistence despite antibiotic treatment. Considering that the formation of biofilms undermines the utility of existing antibiotics, our research team is interested in the development of new solutions that specifically address biofilm formation. To date, very few compounds have been shown to selectively inhibit biofilm formation in a non-microbicidal manner.

It has been suggested that the development of biofilm inhibitors may restore susceptibility to antibiotics in pathogenic infections, thus renewing the utility of existing therapies, particularly therapies employing antibiotics that show low human toxicity. Treatment strategies employing co-dosing of antibiotics and compounds disrupting biofilm formation may therefore provide a new avenue for combatting antibiotic resistance.

Additionally, small molecule biofilm modulators have promising potential for development as coatings for indwelling medical devices. Medical implant devices are a major source of nosocomial infections. Compound coatings of this nature could therefore prevent initial bacterial colonization of these surfaces, and reduce the instances of persistent bacterial infections among inpatient populations.

By discovering new classes of biofilm inhibitor such as the compound described below, and by discovering the novel use of previously described compounds, we shall develop a suite of tools for drug therapy and medical device coating.

BRIEF DESCRIPTION OF THE INVENTION

Biofilm formation is very strongly linked with pathogenesis for many infective organisms. The invention encompasses novel compounds and the novel use a class of previously identified small molecules for the inhibition of biofilm formation.

The small molecule compounds of the invention include purified products and substituted derivatives of the Auromomycin Chromophore derived from a marine organism. The present disclosure is the first instance of the use of such compounds to inhibit biofilm formation.

Examination of a prefractionated library of microbially-derived marine natural products has led to the identification of a new biofilm inhibitor that is structurally unrelated to previously reported inhibitors and is one of the most potent inhibitors reported to date against *V. cholerae*. Combination of this compound with sub-MIC (minimum inhibitory concentration) concentrations of a number of clinically relevant antibiotics was shown to improve the biofilm inhibitory efficacy of this new compound compared to monotherapy treatments, and provides evidence for the potential therapeutic benefit of biofilm inhibitors in treating persistent biofilm-mediated infections.

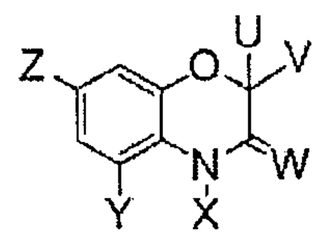
The invention includes compounds (and methods) for the inhibition of biofilm colonization that may be used with and without traditional antibiotics and offers a unique approach to the elimination of persistent biofilm-mediated bacterial infections.

The invention has a number of important applications including medical device coatings to prevent colonization and co-therapies with antibiotics for the treatment and prevention and treatment of various infections, including hospital acquired (nosocomial) infections, such as, for example, Staphylococcus aureus, Acinetobacter and Klebsiella infections. Other organisms of interest include any pathogenic organism that produces a biofilm, for example Vibrio cholera.

Medical devices are well known to be susceptible to biofilm colonization and in the present invention may be coated with a composition comprising a compound or the invention. Medical devices may include stents, any implanted device, whether permanent,

semi-permanent or temporary, prosthetic devices such as artificial bones such as hip bones, and structural implants such as plates, pins and screws.

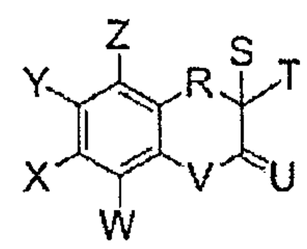
In one embodiment the invention includes a method for inhibiting biofilm formation by an organism on a surface the method comprising contacting the organism or the surface with a compound having a fused heterocyclic ring structure. The compound may have the following formula:



2

wherein: U and V are independently selected from H, OH, Me, Et, or *i*Pr, or together represent methylene; W is O or S; X is H, Me or Benzyl; Y is selected from CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H or $CH_2OP(O)(ONa)_2$; and Z is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$.

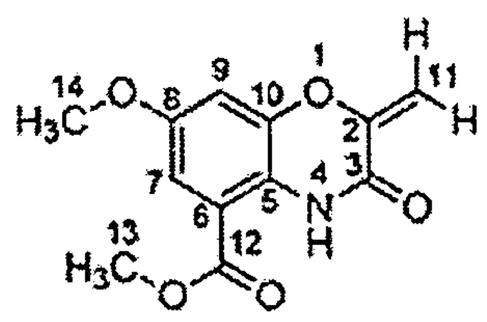
Or alternatively the compound may have the following formula:



31

wherein: R is O; S and T are independently selected from H, OH, Me, Et, or *i*Pr or together represent methylene; U is O or S; V is CH_2, NH, NMe, Nbenzyl, O or S; W is CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H, or $CH_2OP(O)(ONa)_2$; X is H or F; Y is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$; and Z is H or F.

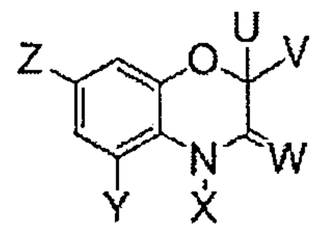
Or in one embodiment the compound may have the following formula:



compound 5

In some embodiments the compound exhibits no bactericidal activity and no mammalian cell cytotoxicity. The method may further comprise contacting the organism or the surface with an antibiotic. The antibiotic may be is selected from (but is not limited to) beta-lactam antibiotics, cephalosporins, glycopeptides, lipopeptides, macrolides, monobactams, nitrofurnas, oxazolidonones, quinolones, sulphonamides, tetracyclines and sulfur drugs. The surface may be a biological tissue or an inorganic surface such as the surface of a medical device.

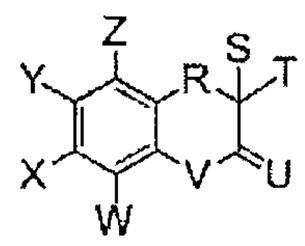
According to an aspect of the invention, there is provided use of a compound for inhibiting biofilm formation wherein the compound has the following formula:



2

wherein: U and V are independently selected from H, OH, Me, Et, or *i*Pr, or together represent methylene; W is O or S; X is H, Me or Benzyl; Y is selected from CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H or $CH_2OP(O)(ONa)_2$; and Z is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$.

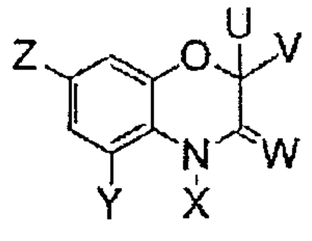
According to another aspect of the invention, there is provided use of a compound for inhibiting biofilm formation wherein the compound has the following formula:



31

wherein: R is O; S and T are independently selected from H, OH, Me, Et, or *i*Pr or together represent methylene; U is O or S; V is CH_2, NH, NMe, Nbenzyl, O or S; W is CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H, or $CH_2OP(O)(ONa)_2$; X is H or F; Y is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$; and Z is H or F.

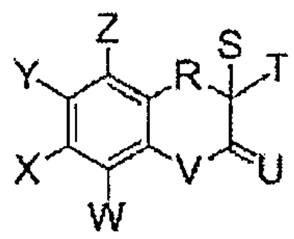
According to a further aspect of the invention, there is provided a compound for inhibiting biofilm formation, the compound having the following formula:



2

wherein: U and V are independently selected from H, OH, Me, Et, or *i*Pr, or together represent methylene; W is O or S; X is H, Me or Benzyl; Y is selected from CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H or $CH_2OP(O)(ONa)_2$; and Z is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$.

According to a yet further aspect of the invention, there is provided a compound for inhibiting biofilm formation, the compound having the following formula:



31

wherein: R is O; S and T are independently selected from H, OH, Me, Et, or *i*Pr or together represent methylene; U is O or S; V is CH_2, NH, NMe, Nbenzyl, O or S; W is CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H, or $CH_2OP(O)(ONa)_2$; X is H or F; Y is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$; and Z is H or F.

According to another aspect of the invention, there is provided a composition comprising the compound as described above and an antibiotic.

Other embodiments include a composition for inhibiting biofilm formation, the composition comprising a heterocyclic compound having the formula above. The composition may further comprise an antibiotic, for example including a beta-lactam antibiotics, , cephalosporins, glycopeptides, lipopeptides, macrolides, monobactams, nitrofurnas, oxazolidonones, quinolones, sulphonamides, tetracyclines and sulfur drugs.

DESCRIPTION OF THE FIGURES AND DRAWINGS

Figure 1. A generic representation of the first generation analogues of lead compound 5.

Figure 2. A generic representation of the first generation analogues with functionalization at the U, V, W and X positions.

Figure 3. A generic representation of the first generation analogues with functionalisation at the Y and Z positions.

Figure 4. A representation of the second generation substrates that could be expected to be synthesized. The compounds will contain one or more of the functionalities as depicted above.

Figure 5. Screening of the resulting peak library from prefraction 1671D revealed a strong region of biofilm inhibition at minutes 26-28 that corresponded to a single peak in the chromatogram

DEFINITIONS AND REPRESENTATIONS CONCERNING THE DISCLOSURE

MIC = minimum inhibitory concentration

BIC = biofilm inhibitory concentration in terms of biofilm coverage

When the disclosure refers to "a surface", for example "biofilm formation on a surface" the surface may be any surface and is not limited to a specific surface, but may include a biological surface such as a biological tissue surface or any kind (a mucous membrane, epidermis etc) or such as an experimental synthetic surface such as a polymer surface (such as in a Petri dish) or a synthetic surface coated with organic matter such as a cell culture of protein matrix. The nature of the surface is not an essential part of the invention, but the method of inhibiting biofilm formation is.

Note that although *V. cholera* was used as a standard test organism, the application of the present work is not limited to treatment of disease caused by *V. cholera* but may be applied to any pathogenic organism that produces a biofilm.

The term "pathogen" in this disclosure is used broadly to mean any organism that is known to cause a pathology by infection of an animal subject. The term "disease" is used to mean any state of an animal that deviates from normal healthy physiology and that is clinically detectable.

The term "binds" or "binding" in connection with the interaction between a one compound or molecule and another compound or molecule, such as a target and a potential binding compound, indicates that the potential binding compound associates with the target to a statistically significant degree as compared to association with proteins generally. Thus, the term "specific binding" refers to binding between two molecules or compounds that is statistically significantly higher than non-specific binding to another molecule. Preferably a

binding compound interacts with a specified target with a dissociation constant (k.sub.d) of 1 mM or less, for example 0.1-100 nM. A binding compound can bind with "low affinity", "very low affinity", "extremely low affinity", "moderate affinity", "moderately high affinity", or "high affinity" as described herein. In the context of compounds binding to a target, the term "greater affinity" indicates that the compound binds more tightly than a reference compound, or than the same compound in a reference condition, i.e., with a lower dissociation constant. In particular embodiments, the greater affinity is at least 2, 3, 4, 5, 8, 10, 50, 100, 200, 400, 500, 1000, or 10,000-fold greater affinity. Also in the context of compounds binding to a biomolecular target, the term "greater specificity" indicates that a compound binds to a specified target to a greater extent than to another biomolecule or biomolecules that may be present under relevant binding conditions, where binding to such other biomolecules produces a different biological activity than binding to the specified target. Typically, the specificity is with reference to a limited set of other biomolecules. In particular embodiments, the greater specificity is at least 2, 3, 4, 5, 8, 10, 50, 100, 200, 400, 500, or 1000-fold greater specificity.

The term "derivative" or "derivative compound" or “analogue” refers to a compound having a chemical structure that contains a common core chemical structure as a parent or reference compound, but differs by having at least one structural difference, e.g., by having one or more substituents added and/or removed and/or substituted, and/or by having one or more atoms substituted with different atoms. Unless clearly indicated to the contrary, the term "derivative" does not mean that the derivative is synthesized using the parent compound as a starting material or as an intermediate, although in some cases, the derivative may be synthesized from the parent.

The term “fragment” refers to a part of a larger whole, for example a fragment of a molecule may be any dissociated part of that molecule, regardless of size.

The term “specie” or “group” when used to describe an “R” group in a chemical formula, is used to mean any chemical compound, sub-compound or substituent that may chemically interact with (covalently, ionically or by Van der Waal’s forces) another molecule or group such as shown on a chemical formula.

The terms "formulation, "drug formulation or “pharmaceutical formulation,” refers to a drug combined with a non-drug such as a carrier material designed not to have a pharmaceutical activity, such as pharmaceutical excipient, filler, or carrier material that may

be used to modify or improve the drug release, improve its physical and/or chemical stability, dosage form performance, processing, manufacturing, etc.

When a "terminus" or "terminal group" is discussed as having a substituent, side-chain, group or moiety attached, that substituent, side-chain, group or moiety may equally be present at one or more termini or at side locations along the length of the molecule.

The terms "drug" or "therapeutic agent" mean any substance meant to affect the physiology of a subject. Examples of drugs are described in well known literature references such as the Merck Index and the Physicians Desk Reference.

The term "therapeutically effective amount" means an amount of a therapeutic agent, or a rate of delivery of a therapeutic agent, effective to facilitate a desired therapeutic effect. The precise desired therapeutic effect will vary according to the condition to be treated, the formulation to be administered, and a variety of other factors that are appreciated by those of ordinary skill in the art.

The term "diagnostic agent" means any chemical moiety that may be used for diagnosis or in a diagnostic test. For example, diagnostic agents include imaging agents containing radioisotopes, contrasting agents containing for example iodine, enzymes, fluorescent substances and the like.

The term "treatment" means the application of a process to an individual in order to alter a physiological state, whether or not the process includes a curative element.

It should be noted that the invention encompasses compounds, methods, uses and treatments wherein compounds of the invention, and their derivatives and analogues, sub-components and fragments derived therefrom, may be employed to inhibit biofilm formation in any organism, either animal or plant.

In this specification, reference is made to particular features of the invention (including for example components, ingredients, elements, devices, apparatus, systems, groups, ranges, method steps, test results, etc). It is to be understood that the disclosure of the invention in this specification includes all appropriate combinations of such particular features. For example, where a particular feature is disclosed in the context of a particular embodiment or a particular claim, that feature can also be used, to the extent appropriate, in the context of other particular embodiments and claims, and in the invention generally. The embodiments disclosed in this specification are exemplary and do not limit the invention. Other embodiments can be utilized and changes can be made. As used in this specification,

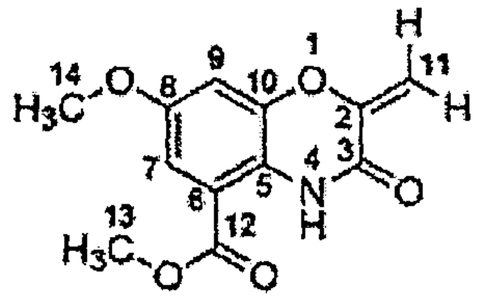
the singular forms “a”, “an”, and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to “a part” includes a plurality of such parts, and so forth.

The term "comprises" and grammatical equivalents thereof are used in this specification to mean that, in addition to the features specifically identified, other features are optionally present. Where reference is made in this specification to a method comprising two or more defined steps, the defined steps can be carried out in any order or simultaneously. Where reference is made herein to "first" and "second" features, this is generally done for identification purposes; unless the context requires otherwise, the first and second features can be the same or different, and reference to a first feature does not mean that a second feature is necessarily present (though it may be present).

DETAILED DESCRIPTION OF THE INVENTION

In the present study, a 1,248-member prefractionated marine natural product library was evaluated for biofilm inhibitory activity using a recently-developed image-based screening platform. Of the prefractions screened, 7 hits showed non-microcidal biofilm inhibitory activity with normalized OD_{600} values greater than 0.7 and normalized percent biofilm coverage values less than 20% (reported as 0.2). Of these, prefraction 1671D showed the strongest effect on biofilm formation. Prefraction 1671D is a semi-purified mixture that contains mixture of several individual compounds. Prefraction 1671D was therefore subjected to one-compound-one-well ‘peak library’ fractionation using the inventors’ standard protocol that automatically separates the constituents of prefractions of interest based on HPLC retention times, and connects specific constituents to observed biological activities through secondary screening.

Screening of the resulting peak library from prefraction 1671D revealed a strong region of biofilm inhibition at minutes 26-28 that corresponded to a single peak in the chromatogram (see Figure 5). This activity was coupled with a striking biofilm macrocolony phenotype, providing strong justification for purification and full structural characterization of the active constituent. It was from 1671D that “compound 5” was isolated.



compound **5**

BIOFILM INHIBITORY ACTIVITY

In order to determine the biofilm inhibitory activity (IC_{50}) of identified compounds, a two-fold dilution series was examined using the inventors' standard imaging platform to afford an IC_{50} of 60.1 μM against *V. cholerae*.The data from both the epifluorescence and the confocal images indicate that on treatment with compound **5**, micro-colonies initially become less organized and less tightly packed. This is the first time we have observed this type of biofilm inhibition phenotype in our screening program, and suggests that the biofilm matrix may be being disrupted by a reduction in the production of matrix components involved in controlling biofilm architecture.

In order to further validate the results obtained in the primary screen, we analyzed biofilm formation under static conditions using chambered cover glasses in 1 mL growth medium.

Results revealed that compound **5** had no bactericidal activity against any of the tested strains. Additionally, compound **5** showed no mammalian cell cytotoxicity against HeLa cells, up to the highest tested concentration (250 μM), indicating that compound **5** possesses selective activity for biofilm inhibition without directly impacting either bacterial or host cell survival.

There are very few examples of agents capable of causing inhibition of biofilm formation in *V. cholerae*. Compound **5** is one of only a small number of biofilm inhibitors with activity against *V. cholerae* and is the first example of an inhibitor possessing this fused heterocyclic ring system.

Initial development of lead compound **5**

First generation approach

Having identified lead compound **5** as a biofilm inhibitor, subsequent work has focused upon the generation of a library of synthetic analogues that surpass the activity of the initial target. The first generation molecules have the generic description as depicted in compound **2** and will have six molecular entities examined to determine their necessity in the biological activity of structure **5** (figure 1).

Note that compounds **13** and **30**, two carboxylic acid derivatives of compound **5** have been previously synthesized (see *Tetrahedron Letters* **1986**, *27*, 1351-1354) and as such are not new chemical entities (figure 3). The other compounds and substituted derivatives are believed to be entirely novel chemical entities.

Despite their previous identification, however, neither compound 13 or 30 has been previously used as, or formulated for, or reported as a biofilm inhibitor, with the only biological data present in the literature being concerned with the two entities ability to act as DNA cleaving reagents (see above referenced paper for further details).

Second generation strategy

With some first generation analogues of compound **5** proving to be superior improved candidates for pharmaceutical use, a second generation library of molecules is synthesized. A generic molecule **31** is depicted below in which the possible moieties for each functional site are listed. The purpose of the second generation library is to further improve both the activity and pharmaceutical efficacy of the lead compound in question. The susceptibility for aromatic hydrogen atoms to undergo oxidation warrants the second generation compound to incorporate the analogous fluorine units as to prevent such a process from occurring.

CO-DOSING OF BIOFILM INHIBITORS WITH SUB-MICS OF ANTIBIOTICS

It is well-documented that sub-MIC (minimum inhibitory concentration) doses of antibiotics are capable of inducing bacterial biofilm formation *in vitro*. In 2005, a study reported in *Nature* (Hoffman et al., 2005, Aminoglycoside antibiotics induce bacterial biofilm formation. Nature 436, 1171–1175) illustrated the biofilm-inducing properties of sub-MIC concentrations of tobramycin, and the following years resulted in dozens of additional publications reporting similar results. To date, tetracycline has been shown to induce biofilm formation in five different bacterial species, and rifamycin in three different bacterial species. These studies raise concerns that therapeutic treatments of infections where prescribed

antibiotics are not administered at sufficiently high doses may be contributing to the severity and persistence of infections by inducing biofilm formation. This is of concern for treatment of bacterial infections, since bacterial biofilms are inherently less responsive to antibiotic treatment. Thus, it has been suggested that the development of cotherapeutic agents to suppress biofilm induction could serve as a valuable solution to this problem by restoring antibiotic susceptibility.

Three commercially available antibiotics, tetracycline, ceftazidime, and ciprofloxacin, were chosen for evaluation using this strategy due to their orthogonal bacterial targets and their utility as therapeutics against *V. cholerae* infections. Dilution series for each antibiotic were evaluated using our image-based screen in order to determine both sub-MIC and sub-BIC (biofilm inhibitory concentration in terms of biofilm coverage) concentrations. A dilution series of compound **5** was then co-treated with a single fixed concentration of each antibiotic (a concentration qualifying as both sub-MIC and sub-BIC). This allowed us to evaluate whether the biofilm inhibition efficacy of compound **5** was affected by the addition of sub-MIC and sub-BIC levels of antibiotics.

These results indicate that the addition of low concentrations of antibiotics significantly enhance the biofilm inhibitory activity of compound **5**. Addition of sub-BIC quantities of tetracycline improved biofilm clearance by halving the concentration of compound **5** required to cause a 50% decrease in biofilm coverage. Sub-BIC concentrations of ceftazidime and ciprofloxacin were also found to improve the biofilm-clearing efficacy of compound **5**, suggesting that this strategy is broadly applicable to antibiotics with different modes of action.

Any type of antibiotic may be employed with the invention, including, but not limited to penicillins and other beta-lactam antibiotics, penicillin combinations, cephalosporins, glycopeptides, lipopeptides, macrolides, monobactams, nitrofurnas, oxazolidonones, quinolones, sulphonamides, tetracyclines, sulfur drugs, and, for example, tobramycin rifamycin, ceftazidime, and ciprofloxacin.

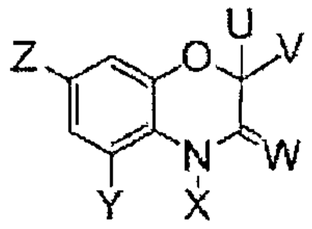
CONCLUSION

This study reports the discovery of a new structural class of biofilm inhibitors, discovered through the application of an image-based, high-throughput screening platform to our in-house prefractionated marine natural products library. Using this target-independent

phenotypic screening platform in concert with standard antibiotic and cytotoxicity assays we have demonstrated that this compound is a selective, non-bacteridical inhibitor of *V. cholerae* biofilms, and possesses a unique phenotype, causing diffuse microcolony formation. By shifting the focus of therapeutic discovery from antibiotic development to a more subtle inhibition of biofilm colonization we have identified a compound with potential to restore the efficacy of traditional antibiotics that is orthogonal to existing therapeutic options, and offers a unique approach to the elimination of persistent biofilm-mediated bacterial infections.

Claims

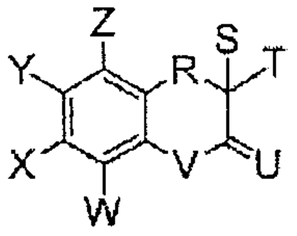
1. Use of a compound for inhibiting biofilm formation wherein the compound has the following formula:



2

wherein: U and V are independently selected from H, OH, Me, Et, or *i*Pr, or together represent methylene; W is O or S; X is H, Me or Benzyl; Y is selected from CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H or $CH_2OP(O)(ONa)_2$; and Z is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$.

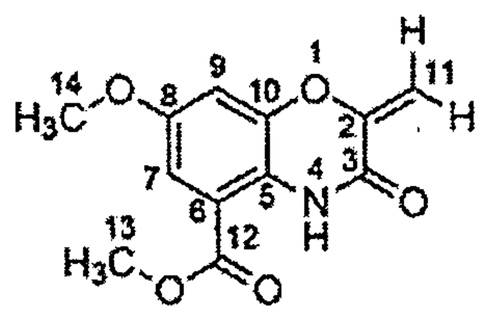
2. Use of a compound for inhibiting biofilm formation wherein the compound has the following formula:



31

wherein: R is O; S and T are independently selected from H, OH, Me, Et, or *i*Pr or together represent methylene; U is O or S; V is CH_2, NH, NMe, Nbenzyl, O or S; W is CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H, or $CH_2OP(O)(ONa)_2$; X is H or F; Y is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$; and Z is H or F.

3. The use of claim 1 or 2 wherein the compound has the following formula:



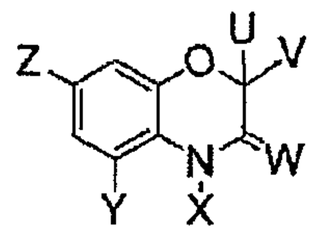
compound 5

4. The use according to any one of claims 1 to 3 wherein the compound exhibits no bactericidal activity and no mammalian cell cytotoxicity.

5. The use according to any one of claims 1 to 4 wherein the compound is used with an antibiotic.

6. The use of claim 5 wherein the antibiotic is selected from beta-lactam antibiotics, cephalosporins, glycopeptides, lipopeptides, macrolides, monobactams, nitrofumas, oxazolidonones, quinolones, sulphonamides, tetracyclines and sulfur drugs.

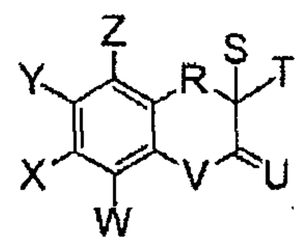
7. A compound for inhibiting biofilm formation, the compound having the following formula:



2

wherein: U and V are independently selected from H, OH, Me, Et, or *i*Pr, or together represent methylene; W is O or S; X is H, Me or Benzyl; Y is selected from CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H or $CH_2OP(O)(ONa)_2$; and Z is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$.

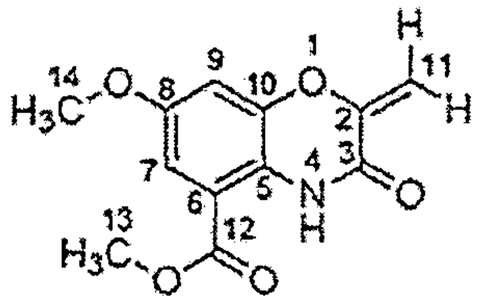
8. A compound for inhibiting biofilm formation, the compound having the following formula:



31

wherein: R is O; S and T are independently selected from H, OH, Me, Et, or *i*Pr or together represent methylene; U is O or S; V is CH_2, NH, NMe, Nbenzyl, O or S; W is CO_2Me, CO_2H, CH_2OH, CH_2NH_2, CH_2NMe_2, H, or $CH_2OP(O)(ONa)_2$; X is H or F; Y is OMe, OH, H, OAc, $OP(O)(ONa)_2$, $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ or $OCH_2C_6H_4CF_3$; and Z is H or F.

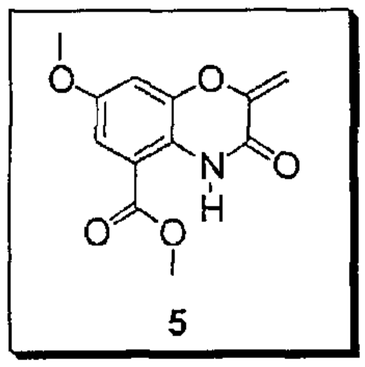
9. The compound according to claim 7 or 8 wherein the compound has the following formula:

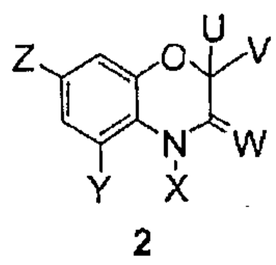


compound 5

10. A composition comprising the compound for inhibiting biofilm formation according to any one of claims 7 to 9 and an antibiotic.

11. The composition of claim 10 wherein the antibiotic is selected from beta-lactam antibiotics, cephalosporins, glycopeptides, lipopeptides, macrolides, monobactams, nitrofurnas, oxazolidonones, quinolones, sulphonamides, tetracyclines and sulfur drugs.

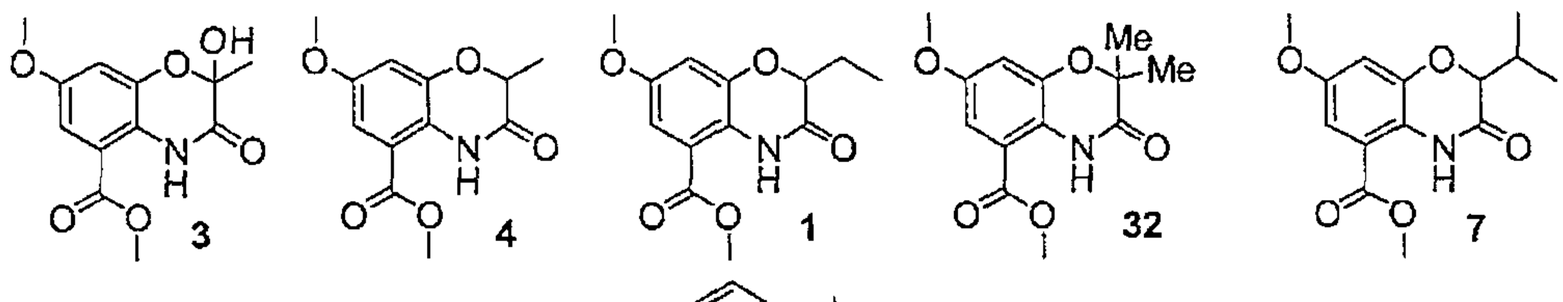


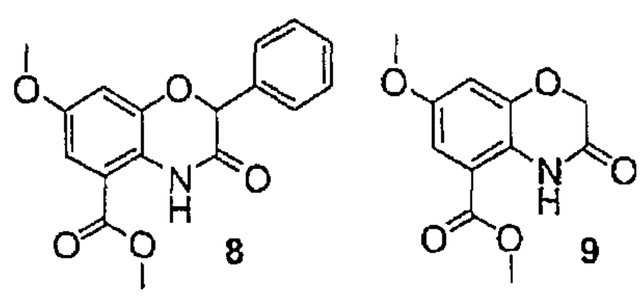


U and V = H, OH, Me, Et, *i*Pr, methylene
W = O, S
X = H, Me, Benzyl
Y = CO_2Me. CO_2H, CH_2OH CH_2NH_2, CH_2NMe_2, H $CH_2OP(O)(ONa)_2$
Z = OMe, OH, H, OAc, $OP(O)(ONa)_2$ $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ $OCH_2C_6H_4CF_3$

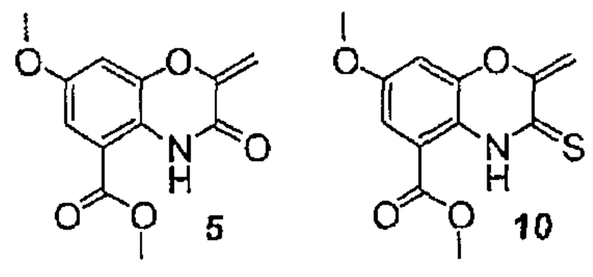
Figure 1

Functionalization of the U and V positions





Functionalization of the W position



Functionalization of the X position

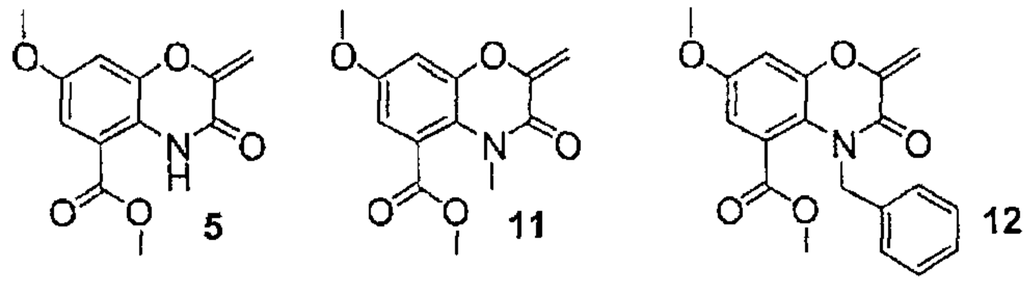
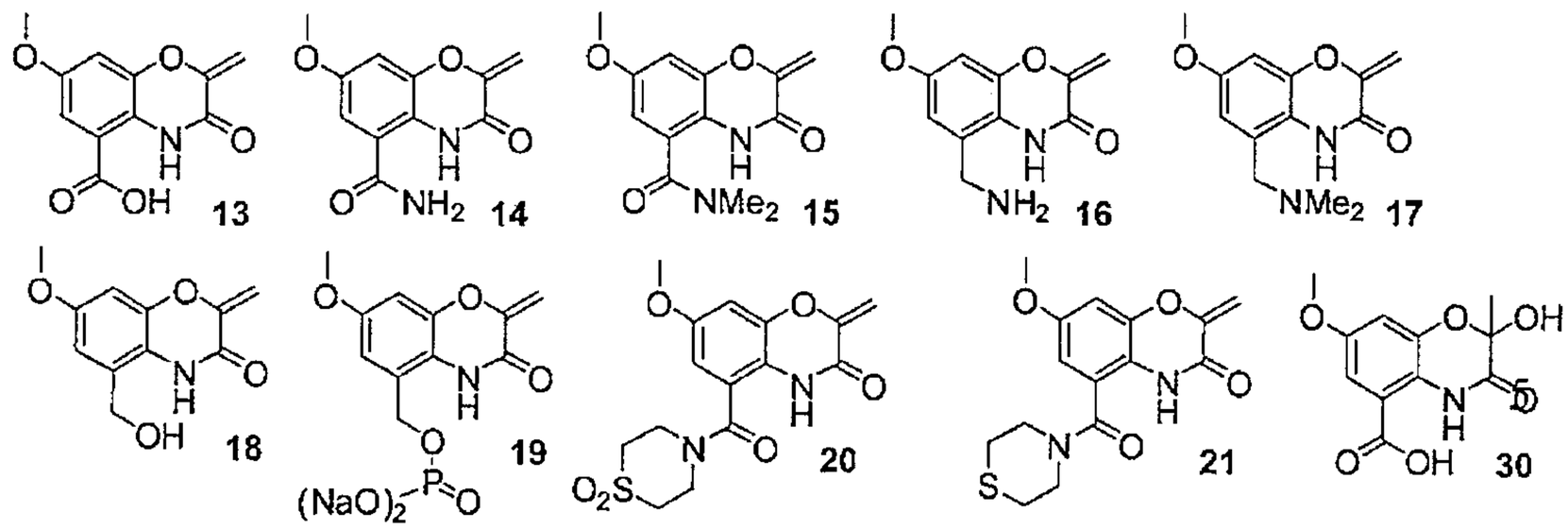


Figure 2

Functionalization of the Y position



Functionalization of the Z position

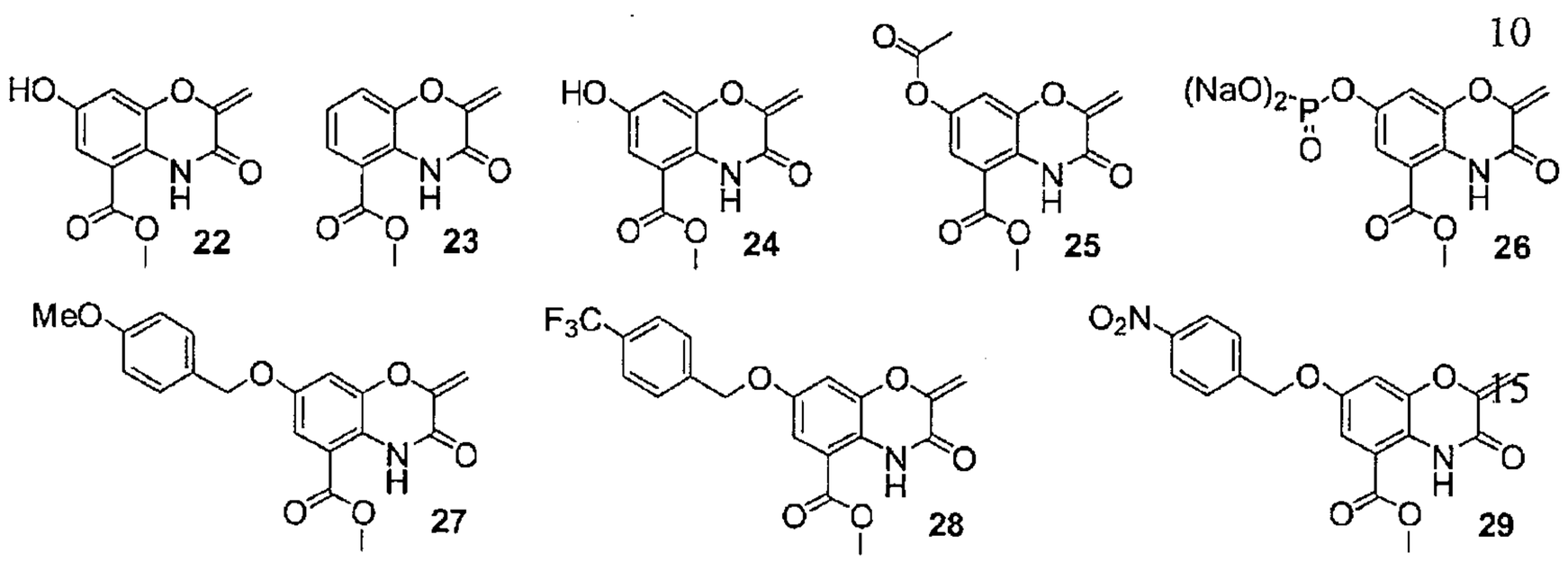
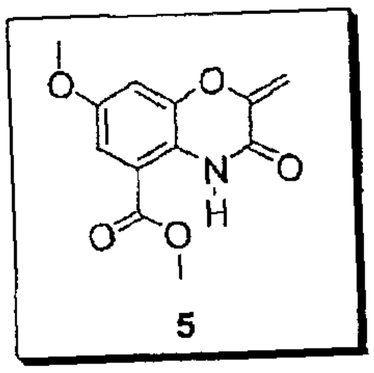
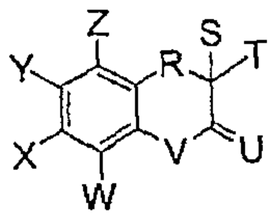


Figure 3



5



31

S and T = H, OH, Me, Et, *i*Pr, methylene

U = O, S

V = C, NH, NMe, NBenzyl, O, S

W = CO_2Me. CO_2H, CH_2OH CH_2NH_2, CH_2NMe_2, H $CH_2OP(O)(ONa)_2$

X = H, F

Y = OMe, OH, H, OAc, $OP(O)(ONa)_2$ $OCH_2C_6H_4OMe$, $OCH_2C_6H_4NO_2$ $OCH_2C_6H_4CF_3$

Z = H, F

Figure 4

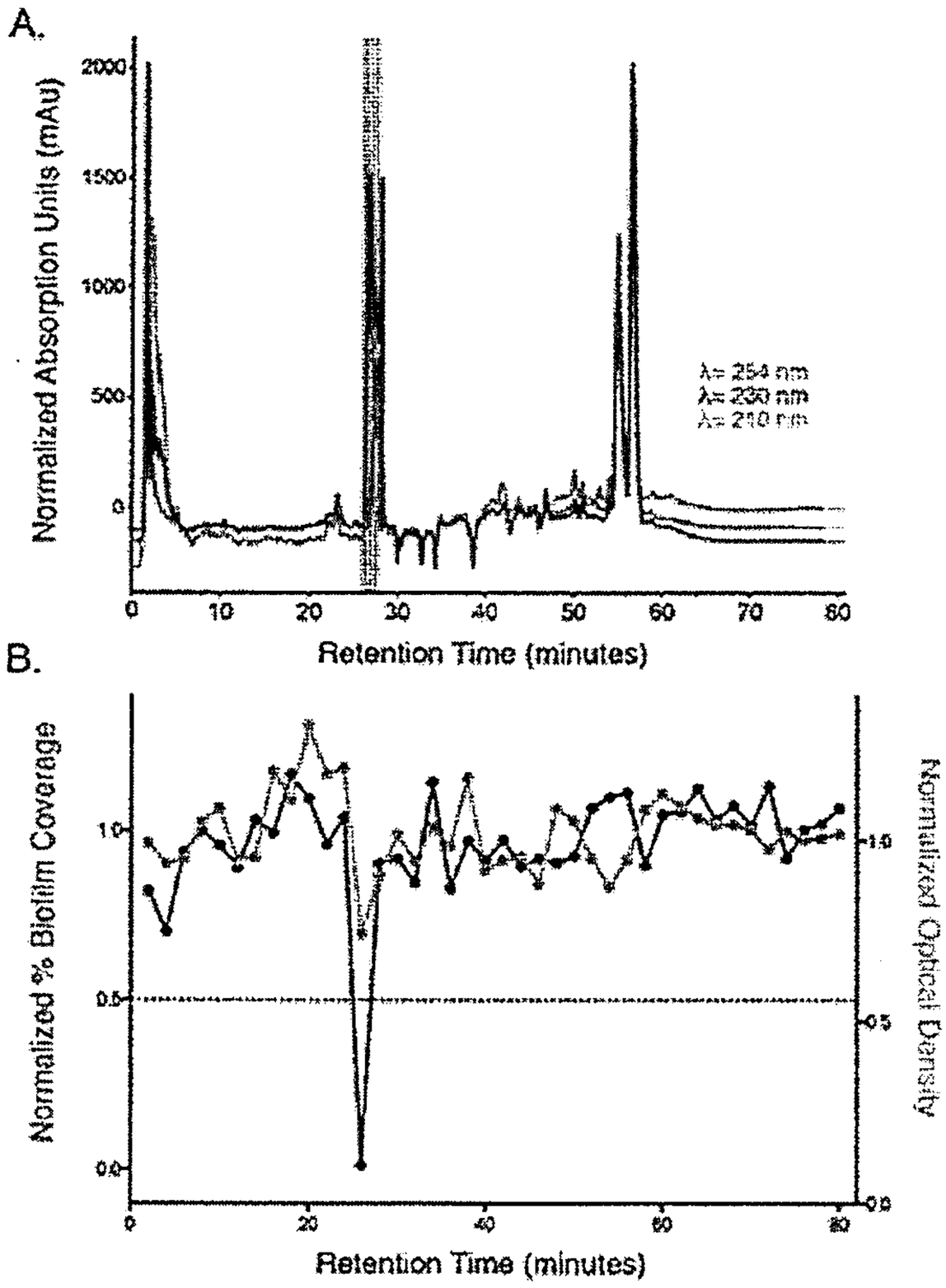
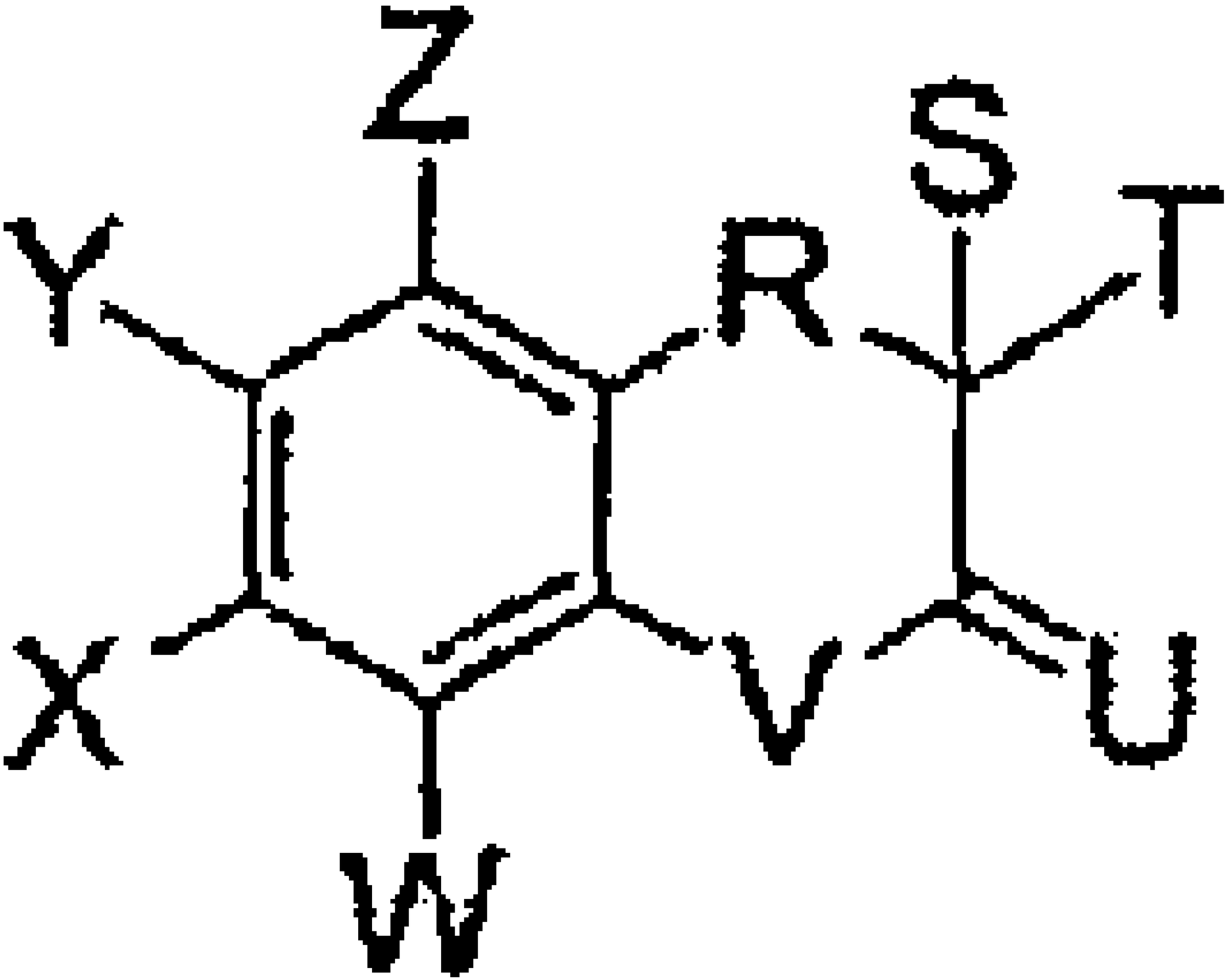

A.
Normalized Absorption Units (mAu)
2000
1500
1000
500
0
λ= 254 nm
λ= 230 nm
λ= 210 nm
0
10
20
30
40
50
60
70
80
Retention Time (minutes)
B.
Normalized % Biofilm Coverage
1.0
0.5
0.0
Normalized Optical Density
1.0
0.5
0.0
0
20
40
60
80
Retention Time (minutes)

Figure 5


Z
Y
R
S
T
X
U
W