Screening for novel quorum-sensing inhibitors to interfere with the formation of *Pseudomonas aeruginosa* biofilm

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The objective of this study was to screen for novel quorum-sensing inhibitors (QSIs) from traditional Chinese medicines (TCMs) that inhibit bacterial biofilm formation. Six of 46 active components found in TCMs were identified as putative QSIs based on molecular docking studies. Of these, three compounds inhibited biofilm formation by *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* at a concentration of 200 µM. A fourth compound (emodin) significantly inhibited biofilm formation at 20 µM and induced proteolysis of the quorum-sensing signal receptor TraR in *Escherichia coli* at a concentration of 3–30 mM. Emodin also increased the activity of ampicillin against *P. aeruginosa*. Therefore, emodin might be suitable for development into an antivirulence and antibacterial agent.

Received 9 July 2010

Accepted 15 August 2011

INTRODUCTION

Pseudomonas aeruginosa, an opportunistic human pathogen, may cause acute infections in hospitalized patients, can be isolated from the environment, particularly from soil and water, and it regularly contaminates medical devices (Stover *et al.*, 2000). It is also the predominant cause of chronic lung infection in cystic fibrosis patients (Frederiksen *et al.*, 1997) and has recently been recognized as one of the main causes of chronic wound infections (Gjødtsbøl *et al.*, 2006). *P. aeruginosa* can infect patients by producing a wide range of virulence factors, the expression levels of which are tightly regulated. Key to this regulation is cell density-dependent cell-to-cell signalling, which is termed quorum sensing (QS) (Rumbaugh *et al.*, 2000). As in many other bacteria, QS controls secretion of virulence factors (Mittal *et al.*, 2006), biofilm formation (Waters *et al.*, 2008) and the exchange of DNA (Fuqua & Winans, 1996) in *P. aeruginosa*. The biofilm mode of growth is recognized as an important bacterial trait that is relevant to infections (Costerton *et al.*, 1994). Many infections involve

the formation of bacterial biofilms, which are bacterial communities that settle and proliferate on surfaces and are covered by exopolymers (Lewis, 2007). Once established, biofilms are difficult to eradicate and become a source of secondary infection (Jones *et al.*, 2009). Moreover, bacteria embedded in biofilms are more tolerant than planktonic cells of antibiotics (Donlan & Costerton, 2002; Drenkard, 2003). The dose of antibiotics needed in this situation will often exceed the highest deliverable dose, which makes efficient treatment impossible.

QS, as a regulatory mechanism, enables bacteria to make collective decisions with respect to the expression of a specific set of genes that involve the production, release and subsequent detection of chemical signalling molecules, such as *N*-acylhomoserine lactones (AHLs) that are commonly used by Gram-negative bacteria. When the concentration of AHLs reaches a certain threshold level, binding to a receptor molecule (for example, LuxR) is promoted and the activated LuxR–AHL complex forms dimers or polymers, which, in turn, act as transcriptional regulators of target genes in the QS regulon (Parsek & Greenberg, 2000; Vannini *et al.*, 2002). *P. aeruginosa* uses AHLs to coordinate the expression of a battery of virulence genes and induction of biofilm formation via a cascade of

Abbreviations: AHL, *N*-acylhomoserine lactone; 3-oxo-C8-HSL, *N*-(3-oxo-octanoyl)-L-homoserine lactone; QS, quorum sensing; QSI, quorum-sensing inhibitor; TCM, traditional Chinese medicine.

regulatory events (Passador *et al.*, 1993), and so the QS system is involved in establishing infections as well as elevating tolerance to antibiotics (Drenkard, 2003).

The traditional approach for the treatment of infectious diseases is to kill or inhibit the growth of bacteria using antibiotics, which has selected for resistance to these drugs, and this has particularly been the case in *P. aeruginosa*. In response to the rise of antibiotic resistance, the continued development of new drugs and the judicious use of our current arsenal of antibiotics is required (Bergstrom *et al.*, 2004). In this context, the development and use of QS-inhibition-based drugs to attenuate bacterial pathogenicity is attractive (Boyen *et al.*, 2009; Jones *et al.*, 2009). Indeed, possibly because of their anti-biofilm effects, some quorum-sensing inhibitors (QSIs) like patulin and garlic extracts have even been found to make *P. aeruginosa* more susceptible to antibiotics, for example, tobramycin (Hentzer *et al.*, 2003; Rasmussen *et al.*, 2005a, b).

Like all plants, traditional Chinese medicinal herbs grow in an environment that has high bacterial density and so they have co-existed with QS bacteria during their evolution. Accordingly, the herbs may have evolved protective mechanisms against bacterial infections, and may even produce QSIs, which reduce the pathogenic capability of bacteria (Zhu *et al.*, 1998). Components of some traditional Chinese medicines (TCMs), derived from these herbs, have been identified to be effective in the treatment of various inflammatory and infectious diseases such as gastritis, stomatitis and pneumonia (Ma *et al.*, 2005). Thus, it was considered interesting to screen compounds from known TCMs to test whether they have QSI activity.

The discovery of inhibitors for a target protein can be greatly facilitated by using computer-aided drug design. In particular, virtual database screening approaches offer the potential to identify novel chemical entities with a high probability of binding to a target protein (Furci *et al.*, 2007). The 3D structures of transcriptional regulators from *Agrobacterium tumefaciens*, *Escherichia coli* and *P. aeruginosa* involved in QS have been elucidated (Vannini *et al.*, 2002; Yao *et al.*, 2006; Bottomley *et al.*, 2007). On the basis of the TraR crystal structure from the plant-associated

bacterium *A. tumefaciens*, mutational analyses indicated that TraR is directly involved in the transcriptional activation in response to QS (Vannini *et al.* 2002). As an autoinducer-dependent transcriptional activator of plasmid conjugal transfer genes, TraR positively regulates interbacterial conjugal transfer of the Ti plasmid (Zhu & Winans, 2001). The complex of TraR with the QS ligand *N*-(3-oxo-octanoyl)-homoserine lactone binds to promoter elements, called *tra* boxes, upstream of at least five different *tra* operons on the Ti plasmid and activates gene expression. A similar protein in *P. aeruginosa* is LasR, which controls the expression of virulence and biofilm-associated genes. The amino acid residues in the LasR and TraR active sites show significant identity (70%) and the 3D model of the LasR active site closely resembles the X-ray structure of the TraR active site (Müh *et al.* 2006). Thus, inhibitors of TraR may have evolved in plants to combat the plant pathogen *A. tumefaciens*, but they may also inhibit LasR in the human pathogen *P. aeruginosa*. Accordingly, our approach was to first identify putative inhibitors of *A. tumefaciens* TraR based on structure analysis in combination with computer-aided drug design and biological assays, and then to test any potential QSIs for their ability to inhibit biofilm formation and antimicrobial drug sensitivity.

METHODS

Compound preparation. All the compounds (purity $\geq 98\%$) used in this study were purchased from the Guangdong Institute for Drug Control (Guangzhou, China) and dissolved in DMSO. The QS signal molecule *N*-(3-oxo-octanoyl)-L-homoserine lactone (3-oxo-C8-HSL, AHL) was purchased from Sigma. Information on the six active components extracted from TCMs is listed in Table 1.

Ligand docking. The 3D structure of the *A. tumefaciens* QS transcriptional activator protein TraR, obtained from the Protein Data Bank (PDB ID code 1H0M), was used in the DOCK program. Only chain A was used for the model; the other chains (B, C, D, E, F, G) and all the water molecules were removed. In the DOCK package, the binding pocket was described by spheres. Each sphere touched the molecular surface at two points and had its radius along the surface of one of the points. The spheres were generated by the program SPHGEN, which is distributed as an accessory of DOCK. To define the ligand-

Table 1. Compounds extracted from TCMs

Compound number	Compound name	Molecular mass (Da)	TCM	Docking score (kcal mol ⁻¹)
	3-Oxo-C8-HSL			-46.20
C1	Rhein	284.21	<i>Rheum palmatum</i> L.	-7.78
C2	Chrysophanol	254.23	<i>Rheum officinale</i> Baill.	-2.96
C3	Nodakenetin	408.40	<i>Peucedanum decursivum</i> (Miq.) Maxim	10.96
C4	Shikonin	288.31	<i>Lithospermum erythrorhizon</i> Sieb.	6.08
C5	Emodin	270.23	<i>Rheum palmatum</i> L.	-15.46
C6	Fraxin	370.31	<i>Fraxinus chinensis</i> Roxb.	-20.16
Control	Halogenated furanone 30			-28.94

binding pocket in chain A of TraR, 3-oxo-C8-HSL, the autoinducer of TraR, was used as a ligand to select spheres. The spheres within 10 Å of the ligand were selected. The sphere-selector module was used for sphere selection.

On the basis of the reported application of TCMs for the treatment of diseases, 46 compounds from these TCMs were selected to construct a database for screening potential novel TraR (and so QS) inhibitors. Then, the 46 compounds and halogenated furanone C30 (as a known QS inhibitor control) were rigidly docked into the TraR binding pocket to see whether they might competitively inhibit QS ligand binding. The box size was set at 6 Å, the grid space was 0.3 Å and the energy cut-off distance was 9999 Å. Maximal orientations were set at 30 000. Default settings were used in docking. Before docking, the SYBYL program-package (SYBYL 6.9; Tripos Associates) was used to add hydrogen atoms and charges to the protein. All of the hydrogen atoms of the protein were added and refined in the presence of explicit solvent with a progressive energy-minimization protocol using AMBER7 FF99 force field. The termination gradient was 0.01 kcal mol⁻¹ and the maximal iteration was 1000 (SYBYL 6.9; Tripos Associates). Docking studies were performed with the DOCK 5.3.0 program (Meng *et al.*, 1993).

Bacterial strains and culture conditions. *P. aeruginosa* PAO1 was routinely cultured in Luria–Bertani (LB) medium at 37 °C with 160 r.p.m. agitation in a shaking incubator. Medium was solidified with 1.2 % agar when required. *Stenotrophomonas maltophilia* GIMT1.118 was cultured in medium (peptone 2.5 g, beef extract 1.5 g, NaCl 2.5 g, agar 10 g, ddH₂O 500 ml, pH 7.2) at 37 °C with 160 r.p.m. agitation in a shaking incubator. The *traR* gene (encoding TraR) was amplified from the plasmid pCF218 of *A. tumefaciens* WCF47(pCF372/pCF218) with primers TraRf (5'-CGCGGATCCCAT-GCAGCACTGGCTG-3') and TraRr (5'-CCGGAATTCTCAGATGA-GTTTCCGC-3'). After being further digested with *Bam*HI and *Eco*RI, the *traR* gene was cloned into the vector pET-17b (Novagen). The cloned plasmid (pET_{TraR}) was electroporated into *E. coli* strain BL21(DE3) and the *traR* gene was expressed according to the manufacturer's instructions.

Assay of *P. aeruginosa* growth. Antibacterial activity was tested by paper disc diffusion assay. *P. aeruginosa* was incubated in LB, 100 µl of this culture (~10⁵ c.f.u. ml⁻¹) was spread onto an LB plate and three filter paper discs (8 mm in diameter) were placed on the plate. Different solutions, including 20 µl test compound (0.2, 2, 20, 200 mM) plus 2 µl ampicillin (1.2 mg ml⁻¹), 20 µl DMSO plus 2 µl ampicillin (1.2 mg ml⁻¹) and 20 µl test compound (0.2, 2, 20, 200 mM) plus 2 µl sterile water, were loaded onto the discs, respectively. The plate was cultured at 37 °C for 24 h and growth inhibition zones were measured (Choo *et al.*, 2006).

Assay of biofilm formation by *P. aeruginosa* and *S. maltophilia*. The biofilm assay was performed by the method described previously with further modification (You *et al.*, 2007). Briefly, 1 ml *P. aeruginosa* or *S. maltophilia* cells, which were cultured overnight and diluted to an OD₆₀₀ of 1.0, were mixed with 200 µM of the different test compounds made up in 1 % (v/v) glycerol in 2 ml microfuge tubes that were incubated at 37 °C for 3 days without shaking. The liquid in each tube was then removed and the bacteria adhering to the sides of the tubes were washed with sterile distilled water three times and air-dried. The remaining biofilm was stained with 1 ml 0.5 % (w/v) crystal violet solution for 20 min and the excess dye in tubes was removed by washing with sterile distilled water three times. The quantitative analysis of biofilm was measured by adding 1 ml 95 % (v/v) ethanol into the tube and waiting for 15 min. Then, the A₅₇₀ nm of dissolved crystal violet was determined. To view biofilm formation under a microscope, cover glasses were put into 50 ml conical flasks containing bacteria, in the presence or absence of

test compounds (200 µM), and incubated at 37 °C for 3 days without shaking. The cover glasses were recovered, washed gently with sterile distilled water, Gram stained, and examined under a microscope.

Assay of TraR degradation in *E. coli*. The assay of TraR degradation in *E. coli* was performed by the method described previously with further modification (Zeng *et al.*, 2008). *E. coli* BL21(DE3)(pET_{TraR}) was incubated in LB broth at 37 °C with shaking until the OD₆₀₀ of cells reached 0.3. Then, 400 µM isopropyl-β-D-thiogalactoside was added, followed by further incubation at 37 °C for 4 h. Subsequently, 1.5 ml cultures were harvested by centrifugation (10 000 g) at 4 °C for 2 min. The cells were washed three times with an equal volume of sterile distilled water. The cells were resuspended in 50 µl sterile distilled water containing 100 µg ampicillin ml⁻¹ and 100 µg chloramphenicol ml⁻¹ together with different test compounds (up to 30 mM), DMSO vehicle (4 %, v/v) or AHL (10 mg ml⁻¹), and incubated at 30 °C for up to 4 h. Protein extracts were then produced from these cells, which were examined by SDS-PAGE.

RESULTS

Screening for TCM compounds with potential anti-QS activity

The DOCK 5.3.0 program was applied to screen for putative novel QS inhibitors of *A. tumefaciens* TraR from the database of compounds found in TCMs constructed by us. The results suggested that 9 compounds from the 46 candidates might be potential inhibitors of the QS system; i.e. they might potentially bind to the TraR protein and inhibit binding of the QS ligand, the AHL. In this study, six of these compounds with different docking scores were further selected for bioassay (Table 1). The predicted orientation of the potential QSI, compound 5 (emodin), bound to TraR was generated using the Chimera program (Pettersen *et al.*, 2004) (Fig. 1). The interactions between compound 5, the halogenated furanone C30 (a known QSI) and TraR are depicted in Fig. 2.

Assay of *P. aeruginosa* growth

The paper disc diffusion assay was performed to determine the antibacterial activity of compound 5 (emodin) against *P. aeruginosa*. An inhibition zone of 10.5 mm in diameter (including the paper disc diameter of 8 mm) was observed on the discs containing ampicillin (1.2 mg ml⁻¹), and an inhibition zone of 9.8 mm was observed on the discs containing compound 5 alone at a concentration of 200 mM. No inhibition zone was noted on the discs at a concentration of 0.2, 2 and 20 mM. However, inhibition zones of 10.8, 11.4, 12.8 and 14.2 mm in diameter (including the paper disc diameter of 8 mm) were observed on the discs containing both ampicillin (1.2 mg ml⁻¹) and different concentration of compound 5 (0.2, 2, 20 and 200 mM).

The diameter of the inhibition zones was bigger in the presence of both compound 5 and ampicillin than in the presence of ampicillin alone. The results showed that whilst

Fig. 1. (a) Map of the TraR receptor and the AHL QS ligand crystal structure. (b) Superposition of QS ligand and emodin. (c) Schematic presentation of the putative interaction between TraR and emodin.

emodin had little direct effect on the growth of *P. aeruginosa* at a concentration of 0.2, 2 and 20 mM, and only a small activity against *P. aeruginosa* at 200 mM, emodin appeared to make *P. aeruginosa* more susceptible to ampicillin, an antibiotic to which *P. aeruginosa* is resistant.

Effect of putative QSI compounds on biofilm formation of *P. aeruginosa* and *S. maltophilia*

The results showed that compounds 2, 3, 4 and 5 could inhibit biofilm formation of *P. aeruginosa* and *S. maltophilia*. Of the four compounds, compound 5 (emodin) showed the most potent inhibitory activity (Fig. 3a). Further assays for the effect of compound 5 on biofilm formation of *P. aeruginosa* and *S. maltophilia* were carried out at different concentrations of compound 5. The results indicated that

compound 5 at 20 μM could significantly inhibit biofilm formation ($P < 0.05$; Fig. 3b). Almost all of the *P. aeruginosa* and *S. maltophilia* cells incubated with compound 5 (200 μM) detached and dispersed from the glass surface. In contrast, the control cells adhered to the glass surface, continued to proliferate, and also congregated together (Fig. 3c, d).

Effect of putative QSI compounds on the degradation of TraR protein *in vivo*

The intensity of the SDS-PAGE band was related to the concentration of the corresponding protein expressed in *E. coli*. The results showed that the concentration of TraR protein expressed in *E. coli* BL21(DE3)(pETtrar) decreased after being treated with compound 5 for 4 h (Fig. 4a). Further studies were performed to examine the effect of different concentrations of compound 5 on the degradation of TraR (Fig. 4b). TraR concentration decreased with increased concentration of compound 5. When the concentration of compound 5 reached 30 mM, the concentration of TraR decreased rapidly within 0.5 h. The effect of AHL on the degradation of TraR was also monitored. The results showed that the degradation of TraR was inhibited in the presence of AHL (Fig. 4c).

DISCUSSION

Inhibition of the bacterial QS system, rather than a bactericidal or bacteriostatic strategy, might be applied in many fields such as medicine, agriculture and food technology. This approach is very attractive because it is not directly involved in the inhibition of bacterial growth and does not impose harsh selective pressure for the development of resistance. Accordingly, there is a particular interest in finding new chemical entities that inhibit bacterial QS (Zeng *et al.*, 2008; Ganin *et al.*, 2009). A number of virtual screening techniques have been developed and have become an integral part of drug discovery, and are available in the literature to identify potent drug leads. In this study, such techniques have been

Fig. 2. Possible mode of binding of emodin and halogenated furanone C30 to TraR. (a) Interaction between TraR and emodin; (b) interaction between TraR and the known QSI halogenated furanone C30. The energy score consists of van der Waals and electrostatic components with the van der Waals predominant.

Fig. 3. Effect of different putative QSI compounds on biofilm formation of *P. aeruginosa* and *S. maltophilia*. (a) Treatment of *P. aeruginosa* (white bars) and *S. maltophilia* (black bars) with various compounds at 200 μM; (b) concentration-dependent effect of emodin (0–200 μM) on biofilm formation of *P. aeruginosa* (white bars) and *S. maltophilia* (black bars); (c) micrograph of a *P. aeruginosa* biofilm; (d) micrograph of a *P. aeruginosa* biofilm following treatment with 200 μM emodin. Bar, 10 μm.

applied to explore the effects of novel QS inhibitors extracted from TCMs on the inhibition of bacterial QS.

To date, the only known anti-QS compounds of non-bacterial origin are halogenated furanones from the red alga *Delisea pulchra* (Manfield *et al.*, 1999). Anti-QS activity has also been noted in a number of traditional medicinal plants (Adonizio *et al.*, 2006). Rhubarb (named Dahuang in Chinese), a medicinal plant, displays diverse pharmacological activities such as bacteriostatic, antiviral, antifungal and antitumour activities (Huang & Zhen, 2001; Kim *et al.*, 2004; Basu *et al.*, 2005; Huang *et al.*, 2005). Emodin, one of the free anthraquinone compounds extracted from rhubarb, is the major active constituent (Ma *et al.*, 2008) that inhibits the expression of the tumour necrosis factor alpha (TNF- α) gene (Chen *et al.*, 2008) and tumour metastasis *in vitro* and *in vivo* (Kwak *et al.*, 2006). It also promotes the apoptosis of human breast cancer BCap-37 cells (Huang *et al.*, 2008). Some studies have also reported the effect of emodin on cell death in human prostate, lung, liver, cervical and blood cancer cells (Cha *et al.*, 2005; Su *et al.*, 2005; Jing *et al.*, 2006; Fu *et al.*, 2007; Muto *et al.*, 2007). In this study, compound 5 (emodin) and ampicillin acted jointly against *P. aeruginosa* more effectively than either of them did alone, suggesting that

compound 5 enhanced the activity of ampicillin against *P. aeruginosa*. The results were similar to the marked synergies between the compounds extracted from TCMs and antibiotics reported by Fujita *et al.* (2005) and Chang *et al.* (2007).

It has been well documented that biofilm growth and formation is a severe problem in medicine and industry (Costerton *et al.*, 1995). The strategy to interrupt the QS system has been increasingly regarded as an important method to control the formation and growth of biofilm (Lynch *et al.*, 2002). A QS-deficient biofilm of *P. aeruginosa* was almost entirely eliminated with antibiotics compared with the wild-type biofilm, in which only cells in the top layer were killed (Bjarnsholt *et al.*, 2005). Shih & Huang (2002) obtained similar results by comparing wild-type biofilm and QS-deficient biofilm treated with kanamycin. In this study, compound 5 (emodin) inhibited the adhesion of *P. aeruginosa* to a glass surface and biofilm formation (Fig. 3). The results were consistent with the findings of Rasmussen & Givskov (2006). It is likely that compound 5 (emodin) penetrated into the biofilm and interfered with bacterial intercellular communications, by which the QS system of *P. aeruginosa* was disturbed and repressed. Moreover, previous studies have found that

Fig. 4. Effect of different putative QSI compounds on TraR protein degradation *in vivo*. The *A. tumefaciens* TraR protein was overproduced in *E. coli*. (a) Cells were treated with different putative QSI compounds. C1–6 (compounds 1–6) at 30 mM or DMSO control for 4 h. (b) Cells treated with emodin (C5) (0.3–30 mM) for different lengths of time. (c) Cells treated with emodin (30 mM) or DMSO or emodin (30 mM) plus AHL (10 mg ml⁻¹).

QS-deficient *P. aeruginosa* has reduced tolerance to such antimicrobial compounds as tobramycin and H₂O₂ (Bjarnsholt *et al.*, 2005; Rasmussen *et al.*, 2005b). In addition, the results also showed that compound 5 can inhibit formation of *S. maltophilia* biofilm (Fig. 3). Interestingly, *S. maltophilia* does not have a traditional AHL-based QS system, as does *P. aeruginosa* (Fouhy *et al.* 2007), and so if compound 5 specifically targets TraR-like AHL-binding proteins to inhibit biofilm formation, it is not clear how it is able to inhibit biofilm formation in this species. It may be that the role of compound 5 is more of an antibacterial one for *S. maltophilia*, and so it is more similar to moxifloxacin, which, at concentrations not able to inhibit growth, was able to inhibit the adhesion and biofilm formation of *S. maltophilia* (Pompilio *et al.*, 2010).

The QS system might be interfered with by decreased AHL biosynthesis, which might be due to decreased AHL transcription that is related to the biosynthase gene mediated by upstream regulators (Yan *et al.*, 2009), degradation of AHL molecules by bacterial lactonases and use of small molecules to block the activation of AHL

receptor protein (Rasmussen & Givskov, 2006). Since the halogenated furanones from *D. pulchra* provided the first examples of QS inhibitors (Givskov *et al.*, 1996), various compounds from fruit, herb and spice extracts have been tested as QS inhibitors (Vattem *et al.*, 2007). In our experiments, compound 5 (emodin) accelerated TraR proteolysis in *E. coli* (Fig. 4a) and could therefore reduce the concentration of TraR protein in *E. coli* (Fig. 4b). When the concentration of compound 5 (emodin) increased, the TraR protein was hydrolysed more efficiently, which was consistent with the result reported by Zhu *et al.* (1998) and Zhu & Winans (2001). However, TraR proteolysis was inhibited to a certain extent in the presence of the AHL molecule (Fig. 4c), which might be related to a competitive binding of compound 5 and AHL to TraR. In addition, because of the similarities of TraR turnover in both *E. coli* and *A. tumefaciens* (Zhu & Winans, 2001), it is highly probable that these results support a mechanism of action of compound 5 in *A. tumefaciens*. Because the amino acid residues in the *P. aeruginosa* QS regulator LasR and *A. tumefaciens* TraR active sites show significant identity (70 %) and the 3D model of the LasR active site closely resembles the X-ray structure of the TraR-active site, the observation that compound 5 (emodin) accelerated TraR proteolysis suggests that it may accelerate LasR proteolysis in *P. aeruginosa*, which would explain its anti-biofilm effects (Müh *et al.*, 2006). The result was consistent with that of halogenated furanones on LuxR protein turnover (Manefield *et al.*, 2002) and that of *P. aeruginosa* on TraR degradation (Zeng *et al.*, 2008). It has also been reported that emodin can inhibit androgen receptor (AR) transcriptional activity, resulting in AR degradation through a proteasome-mediated pathway and suppressing prostate cancer cell growth *in vitro* (Cha *et al.*, 2005).

In summary, emodin was shown to significantly inhibit biofilm formation in *P. aeruginosa*, and induce proteolysis of a known AHL-binding protein, and could be used as a potential QS inhibitor for the control of biofilm formation and growth.

ACKNOWLEDGEMENTS

This work was supported by grants from National High-tech R&D Program of China (863 Program) (2007AA09Z448, 2008AA09Z402), Science and Technology Plan Project of Guangdong (2009B0203 11003), Science and Technology Plan Project of Guangzhou (2008J1-C241) and the Central Level, Scientific Research Institutes Special Fund for Basic R & D (2010TS01). We gratefully thank College of Life Sciences, Sun Yat-Sen University, Guangzhou, PR China for providing the Sybyl 6.9 and thank the Kuntz Group for providing the DOCK program.

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