Towards quorum-quenching catalytic antibodies†

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The development of a novel method to attenuate bacterial virulence is reported, which is based upon the use of designed transition-state analogues to select human catalytic antibodies capable of degrading bacterial quorum-sensing molecules.

Antibiotic drugs have played an essential role in the global increase in life expectancy and quality of life that has occurred over the last century.[1] However, the emergence and increasing prevalence of multi-drug-resistant bacterial strains are eroding such gains.[1,2] Existing antibiotics generally inhibit bacterial cellular processes that are *essential* for microbial survival.[2,3] An inherent problem with this approach is that it creates a selection pressure for drug-resistant mutations.[4,5] Bacterial *antivirulence therapies* seek to avoid the development of treatment-induced resistance. In this context, bacterial quorum-sensing systems offer an attractive target.[6]

Quorum sensing is the intercellular signalling mechanism, mediated by small molecules, which many bacteria use to co-ordinate gene expression with population density.[7] Quorum sensing is used by many bacterial pathogens to regulate virulence; however, it is not essential for survival.[8] Thus, disruption of quorum sensing (so-called 'quorum quenching') should attenuate pathogenicity without imposing the same selection for resistance, compared to existing antibiotic treatments.[9]

N-acylated-L-homoserine lactones (AHLs) are used by many Gram-negative bacteria for intercellular communication.[10] For example, *Pseudomonas aeruginosa* utilizes BHL and OdDHL (Fig. 1) to regulate virulence.[10]

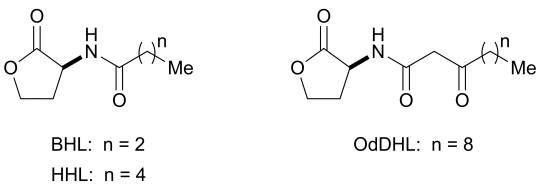
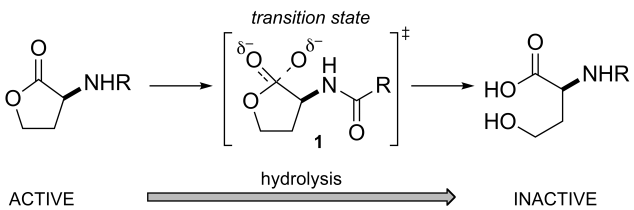


Fig. 1 Examples of AHL small molecules employed by Gram-negative bacteria in quorum sensing. BHL = *N*-butyryl-L-homoserine lactone; HHL = *N*-hexanoyl-L-homoserine lactone; OdDHL = *N*-(3-oxo-dodecanyl)-L-homoserine lactone.

Several methods have been employed previously to block AHL-dependent quorum sensing,[11] including enzymatic degradation of AHL,[12] AHL sequestration by antibodies[13] and the synthesis of AHL mimics that block natural signals.[14] Nature is known to have evolved quorum-quenching enzymes that are capable of hydrolyzing both the amide and lactone moieties of AHL signalling molecules.[15] For example, a class of enzymes known as paraoxanases has been identified in several mammals, which are capable of inactivating OdDHL and thus attenuating *P. aeruginosa* quorum sensing in cell cultures and *in vivo*.[16] Recently, the concept of quorum quenching using antibody catalysis[17] has been introduced.[18] This approach uses small molecules as haptens to elicit antibodies capable of catalyzing AHL hydrolysis and thus inhibit quorum sensing. A haptenic small molecule that closely resembles the transition state of a reaction should elicit antibodies that act as catalysts of the reaction.[19] However, previous reports on the application of antibody catalysis in quorum quenching[18] have employed haptens that were *not* specifically designed to be structural mimics of the transition state for AHL hydrolysis. Consequently, antibodies procured to such haptens catalyzed AHL hydrolysis with only very moderate levels of activity. Herein we report on the synthesis of sulfones *deliberately designed* to resemble the transition-state structure for AHL-ring hydrolysis (Scheme 1).



Scheme 1 Catalytic hydrolysis of AHL (active in quorum sensing) to products (inactive in quorum sensing) *via* the predicted transition state of the rate-determining step. Note that the transition-state structure is only representative and is highly simplified.

Our studies began with the design of a suitable transition-state analogue for the rate-determining step of lactone ring hydrolysis in AHL molecules. Hydrolysis proceeds *via* approach of water or hydroxide towards the lactone carbonyl, forming a pseudo-tetrahedral transition-state structure,[20] with a degree of negative charge delocalized over the two *exo* carbon–oxygen bonds (**1**, Scheme 1). We identified sulfone **2** as a stable transition-state mimic (Fig. 2). Many stable electronic surrogates[21] of transition states for amide and ester hydrolysis have been investigated;[22] however, to the best of our knowledge, the use of a sulfone has yet to be exploited.[23] Three sulfones were targeted for synthesis: transition-state analogues for BHL (**2a**), OdDHL (**2b**) and biotinoylated HHL (**2c**, Fig. 2).[24]

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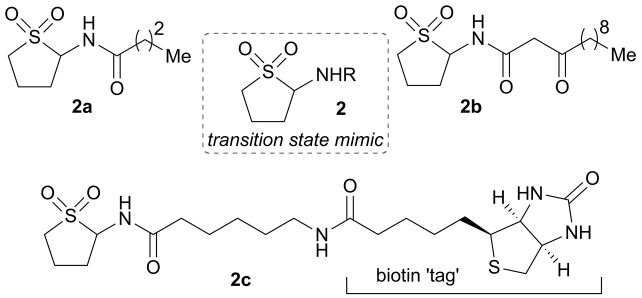
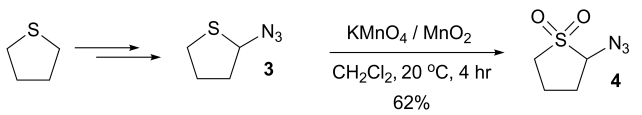


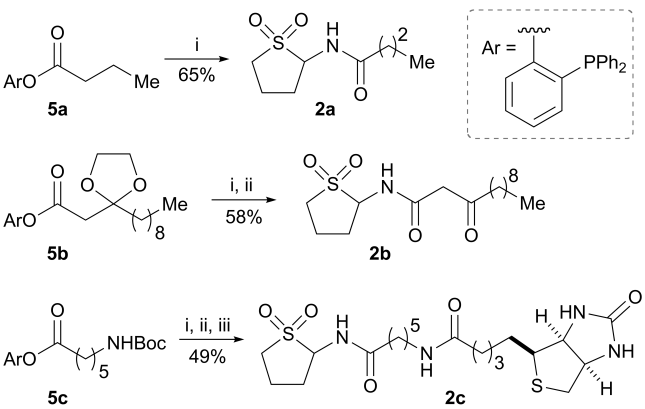
Fig. 2 Target AHL hydrolysis transition-state analogues (**2**).

The successful formation of the amide bond in **2** was anticipated to be the key reaction in their synthesis, since the obvious disconnection gives an unstable amino-sulfone.[25] Therefore, to avoid the free amine, traceless Staudinger ligation methodology was investigated.[26] 2-Azido-tetrahydrothiophene **3** was readily synthesized from tetrahydrothiophene according to the method of Still *et al.*[27] Oxidation to the corresponding sulfone using potassium permanganate supported on manganese dioxide[28] provided the desired azido-sulfone **4** (Scheme 2).



Scheme 2 Synthesis of azido-sulfone **4**.

Phosphine coupling partners **5a–c** were synthesized in two steps from *o*-iodophenol (see ESI†). Reaction with azide **4** generated the amide products **2a** and **2b** in good yields (Scheme 3). The phosphines and azide gave aza-ylide intermediates that were trapped intramolecularly by the phenolic esters, thereby avoiding formation of the unstable amine. Biotin-tagged derivative **2c** was synthesized by a similar route involving biotin coupling as the final step (Scheme 3).



Scheme 3 Summary of analogue synthesis: (i) azide **4**, DMF, 70 °C, then H_2O; (ii) TFA, CH_2Cl_2; (iii) Biotin, EDC·HCl, NEt_3, DMAP, CH_2Cl_2.

In order to determine if the sulfone (**2**) was an 'accurate' mimic of the transition state for AHL ring hydrolysis, we purified a known AHL lactonase, AiiA.[29] AiiA was purified as a maltose binding protein (MBP) fusion. The ability of **2a** to inhibit MBP–AiiA-dependent hydrolysis of HHL *in vitro* was monitored using isothermal titration calorimetry (ITC). To the best of our knowledge, this is the first reported use of ITC to measure AHL degradation. Using ITC we obtained k_{cat} and $K_{M(HHL)}$ values for MBP–AiiA (in the absence of **2a**) of 0.027 s^{-1} and 0.49 mM, respectively (Fig. 3). These k_{cat} and K_M values are lower than those reported for AiiA by previous workers,[29d,e] perhaps reflecting the fact that our ITC titrations were carried out using much lower AHL concentrations (sub-millimolar) than previously, or the improved assay technique. Crucially, as the concentration of **2a** present was increased, $K_{M(HHL)}$ values increased without any significant effect upon k_{cat}, which suggested that **2a** was acting as a potent *competitive inhibitor* of AiiA with a K_i of 3 μM. It should be noted that the transition-state analogues **2a–c** are not themselves biologically active as AHL mimetics (they did not display either AHL agonist or antagonist activity *in vivo* in responsive organisms (*Serratia marcescens*, *Pseudomonas aeruginosa* and *Erwinia carotovora*) at concentrations below 1 mM).

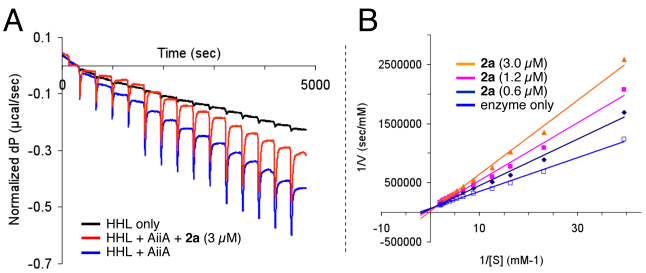


Fig. 3 Competitive inhibition of AiiA activity by **2a**. (A) Raw ITC output showing that **2a** inhibits AiiA-dependent hydrolysis of HHL; (B) Lineweaver–Burk plot showing that **2a** is a potent competitive inhibitor. [S] = HHL (substrate) concentration.

The fact that **2a** acts as competitive inhibitor of AiiA implies that compounds of the general form **2** are indeed good transition-state mimics for AHL lactone ring degradation and should therefore act as haptens for the selection of antibody catalysts for this hydrolysis process.

In summary, we have reported the design and synthesis of predicted transition-state mimics for AHL lactone hydrolysis and demonstrated their ability to act as competitive inhibitors of the AHL hydrolase AiiA. Initial proof-of-principle work indicates that these mimics, once immobilized, can be used to select for binders from a human antibody phage display library.[30] The ability of these transition-state binders to degrade bacterial quorum-sensing molecules is currently being investigated. To the best of our knowledge, this work represents the first report aiming to use *deliberately designed* transition-state analogues to biopan for catalytic antibodies capable of degrading AHL molecules. If proved successful it may lead to the development of a new method for the treatment of bacterial infections.

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