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Pyrogallol and its analogs can antagonize bacterial quorum sensing in *Vibrio harveyi*

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Abstract—Bacteria can coordinate community-wide behaviors through quorum sensing, that is, the secretion and sensing of autoinducer (AI) molecules. Bacterial quorum sensing is implicated in the regulation of pathologically relevant events such as biofilm formation, bacterial virulence, and drug resistance. Inhibitors of bacterial quorum sensing could therefore be useful therapeutics. Herein we report for the first time the discovery of several pyrogallol compounds as single digit micromolar inhibitors of bacterial quorum sensing in *Vibrio harveyi*.

Bacteria can sense the presence and concentration of and communicate with other bacteria in the same environment through the secretion and detection of small molecules called autoinducers (AI). This process is referred to as quorum sensing,[1,2] which is known to regulate many pathologically relevant processes such as virulence factor production, biofilm formation, and drug resistance. For example, biofilm formation in *Pseudomonas aeruginosa*[3,4] and *Vibrio cholerae*,[5] antibiotic production in *Lactococcus lactis*,[6] bioluminescence production in *Vibrio harveyi*[7] are all affected by bacterial quorum sensing. Several quorum sensing pathways have been identified.[8,9] Among them, the autoinducer-2 (AI-2) pathway is referred to as a universal pathway since it functions in both Gram-positive and Gram-negative bacteria while the acylated homoserine lactones (AHL, AI-1) pathway functions only in Gram-negative bacteria[8] and autoinducing peptides (AIP) functions only in Gram-positive bacteria.[9]

Since bacterial quorum sensing is implicated in pathologically relevant characteristics, conceivably its inhibition can be a useful approach for the development of therapeutics against bacterial infection.[10–17] Though quorum sensing inhibitors/antagonists alone are not expected to have bactericidal effect, their ability to attenuate virulence, drug resistance, and biofilm formation can bring in clinical benefits because those are the problems that are hard to be resolved with currently available antibiotics. There have been extensive efforts in developing quorum sensing inhibitors[18–21] and inhibitors of enzymes responsible for autoinducer synthesis.[14–18,21–26] However, most such efforts have been focused on the AHL pathway in Gram-negative bacteria and the AIP-mediated pathway in Gram-positive bacteria.[12,24,27–33] For the AI-2 pathway, there have been a few reports of AI-2 analogs as agonists and partial agonists[8,9,34,35] and two reports of AI-2 antagonists with IC_{50} in the high micromolar range.[36,37] Herein, we describe for the first time that pyrogallol and several of its analogs can inhibit AI-2 mediated quorum sensing in *V. harveyi* with IC_{50} values in the single digit micromolar range.

The AI-2 molecule is unique in that it exists in different forms (Scheme 1). The boric acid complex **F** is the biologically active form in *V. harveyi*. The binding of AI-2 (**F**) to the LuxP receptor in *V. harveyi* triggers a cascade of events that lead to quorum sensing and bioluminescence.[38] In its binding to LuxP, the borate moiety in complex **F** is known to exist in the anionic tetrahedral form. In the binding site of LuxP, there are two arginine residues (215 and 310) nearby, which presumably afford significant stabilization through ionic interactions. Based on the concept of molecular mimicry, we envisioned that other diol-containing compounds, which can complex with boric acid,[39,40] should have the potential to bind

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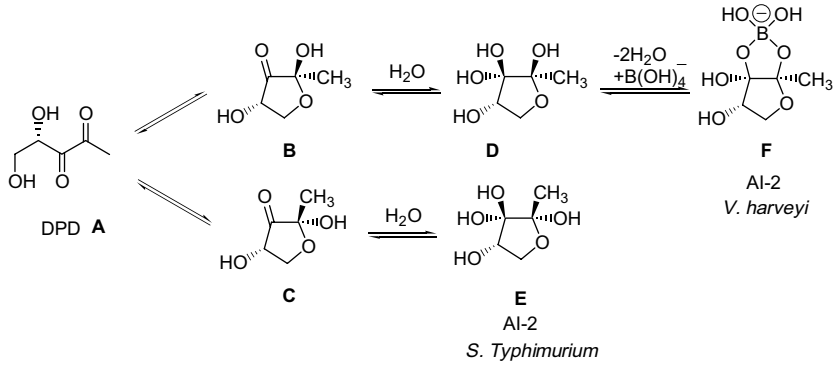

Scheme 1. DPD exists in different forms.

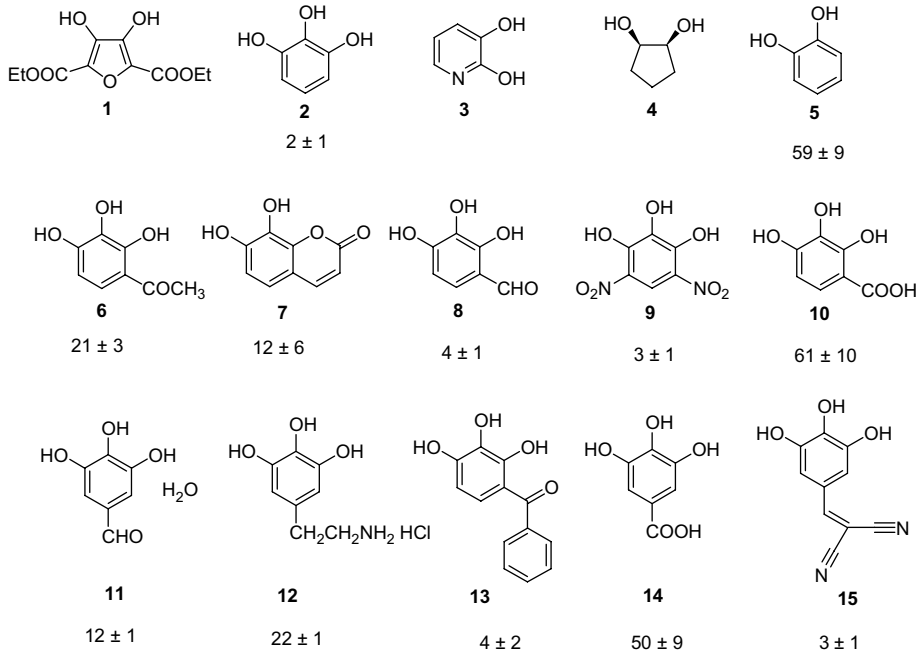

Figure 1. Diol compounds tested and their corresponding IC_{50} value (μM).

LuxP in their boric acid complex forms. Several non-aromatic cyclic polyols have been studied by others.[41–44] Most of them showed agonistic effects. Since our interest is in the search for antagonists, we decided to examine some novel structures that have significant differences from cyclic polyols that have been studied. Therefore, we studied some aromatic diol-containing compounds together with two five-membered ring diols (Fig. 1).

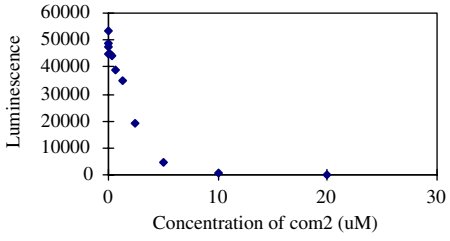

Figure 2a. Concentration-dependent inhibition of *V. harveyi* luminescence by pyrogallol, $IC_{50} = 2 \pm 1$ μM.

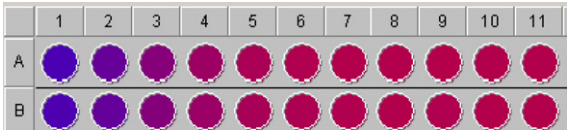

Figure 2b. Microplate reader results for pyrogallol (**2**). Each line represents one set of experiments. The concentrations of pyrogallol from left to right are 20, 10, 5, 2.5, 1.25, 0.64, 0.32, 0.16, 0.08, 0.04, 0.00 μM.

For the screening work, we chose MM32 strain of *V. harveyi*. MM32 lacks the LuxN receptor needed to respond to autoinducer AI-1 and the LuxS enzyme needed to synthesize DPD, which is a precursor of AI-2 in its boric acid complex form (Scheme 1).[45] Since the MM32 strain of *V. harveyi* produces no endogenous AI-2 signal, bioluminescence is measurable only following the addition of DPD, which was synthesized by following literature procedures.[43] Based on literature precedents, we chose to use 5 μM of DPD as the final concentration for the test in the presence of

1 mM of boric acid.[46] It should be noted that organic solvents could also affect the intensity of the luminescence. In this case, DMSO was used to solubilize the tested compounds in making stock solutions. Therefore, it is important that final DMSO concentrations be kept at minimum and constant in all tests.

The effect of the diols shown in Figure 1 on bacterial quorum sensing was examined by following literature procedures.[47] Briefly, diols of different concentrations were first added into autoinducer bioassay (AB) medium[47] prepared in 96-well plates. To these solutions, freshly synthesized DPD in solution (pH 7) was added for a final concentration of 5 μM. Boric acid was added to give a final concentration of 1 mM. (The optimal DPD and boric concentrations were determined based on literature precedents as well as our own experimental confirmation.)[17] After addition of bacteria in AB medium (bacteria was diluted 5000-fold after 16 h incubation), the micro plates were incubated at 30 °C with

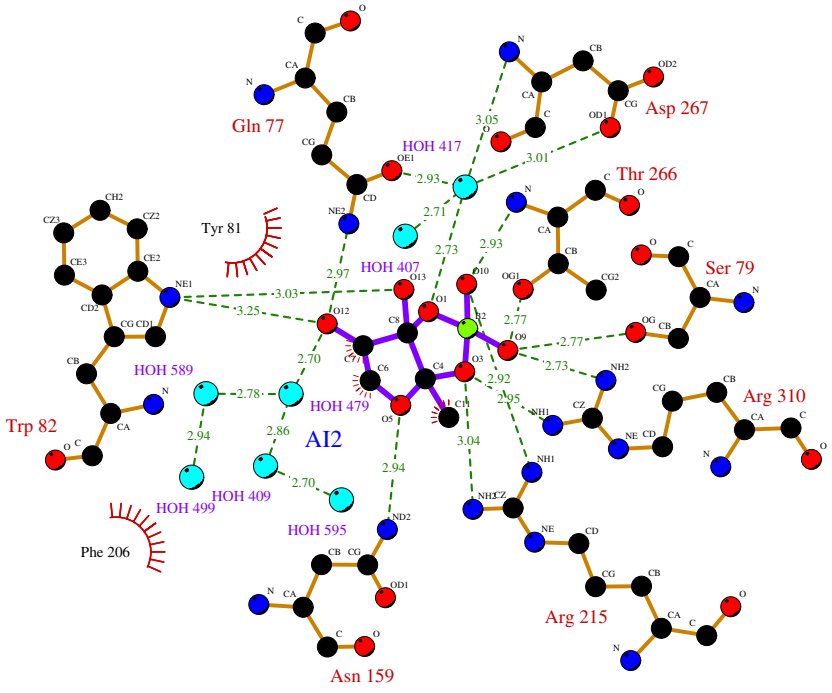

Figure 3a. AI-2-boric acid complex binding with LuxP as reported in the literature (AI-2).[38]

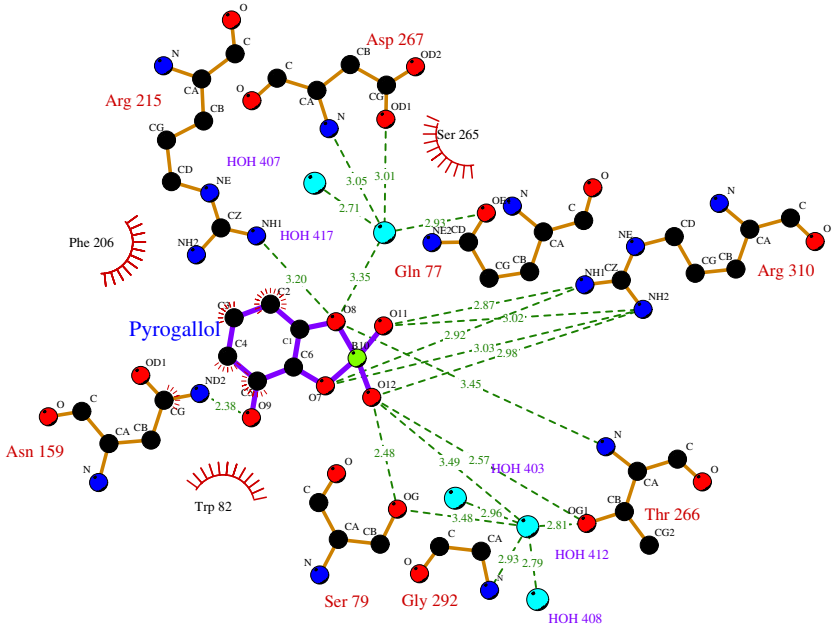

Figure 3b. Proposed binding of pyrogallol–boric acid complex with the LuxP receptor.

aeration for 3–4 h. Light production was measured every half hour by using a Perkin-Elmer luminescence microplate reader. Figure 2 shows a typical set of data reflecting the concentration-dependent luminescence intensity changes with added diols.

Among these 15 compounds tested, five showed IC_{50} at single digit micromolar concentrations. They are compound **2** (IC_{50}: 2 ± 1), compound **8** (IC_{50}: 4 ± 1), compound **9** (IC_{50}: 3 ± 1), compound **13** (IC_{50}: 4 ± 2), and compound **15** (IC_{50}: 3 ± 1). From the results presented, we can draw some general structure–activity conclusions. First, non-aromatic 5-membered ring *cis*-diols (**4**) showed no inhibitory effect. Such result is consistent with literature reports, which showed that many non-aromatic polyols do not inhibit AI-2 mediated quorum sensing. Second, catechols showed much lower activities than pyrogallols. For example, catechol **5** has an IC_{50} value about 59 μM, while compound **2** has an IC_{50} value of around 2 μM. Such results indicate that the third hydroxyl group on pyrogallol is important in binding interactions. Third, pyrogallol seems to have certain tolerance of non-ionizable substitution at the 4-position. Ionizable groups on the other hand tend to lower activities. For example, compounds **10** (IC_{50}: 61 ± 10) and **14** (IC_{50}: 50 ± 9) have a carboxyl group and **12** (IC_{50}: 22 ± 1) has an amino group, all of which are ionizable under normal physiological conditions. These compounds showed much lower activities than pyrogallol.

We also did some molecular modeling work to understand the structure–activity relationships following similar procedures reported previously.[48,49] In brief, the docked complexes were solvated by using the TIP3P water model,[50] subjected to 500 steps of molecular mechanics minimization and molecular dynamics simulations at 300 K for 1.5 ns using the SANDER module in AMBER 8 program.[51] The resulting structures were then analyzed using HBPLUS 3.06[52] and Ligplot 4.22[53] program to identify specific contacts between ligands and LuxP.

From Figures 3a and 3b, we can see that the boric acid moiety can engage in salt bridge and/or hydrogen bond interactions with Arg215, Arg310, Ser79, and Thr 266. It is similar for both AI-2-borate and pyrogallol-borate. In AI-2, the hydroxyl groups and oxygen atom of the furan ring can also form H-bonds with Asn 159, Gln 77, and Trp 82, while the third hydroxyl group of pyrogallol can also form a H-bond with Asn 159. This interaction could be the reason that pyrogallol binds LuxP receptor more tightly than catechol (IC_{50}: 59 ± 9 μM), which only has two hydroxyl groups on the aromatic ring (Fig. 4).

As stated earlier, in the pyrogallol scaffold ionizable groups tend to lower activities. For example, 5-hydroxydopamine (**12**, IC_{50}: 22 ± 1) has an amino group, which is ionizable under normal physiological conditions. This negative influence may be due to undesirable ionic interactions with Asp 136, which is positioned closely to the AI-2-borate binding site (Fig. 5). It is conceivable that side chain ionizable functional groups on pyrogallol, either positive or negative, may engage in either attractive or repulsive interactions with Asp136, which can move the entire complex away from an otherwise ideal binding position.

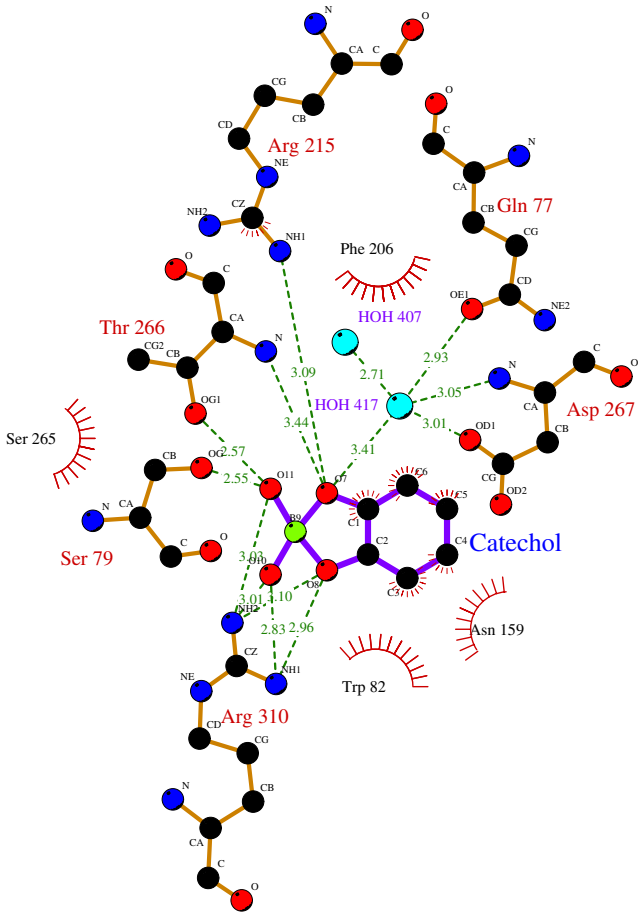

Figure 4. Proposed binding of catechol-borate with the LuxP receptor.

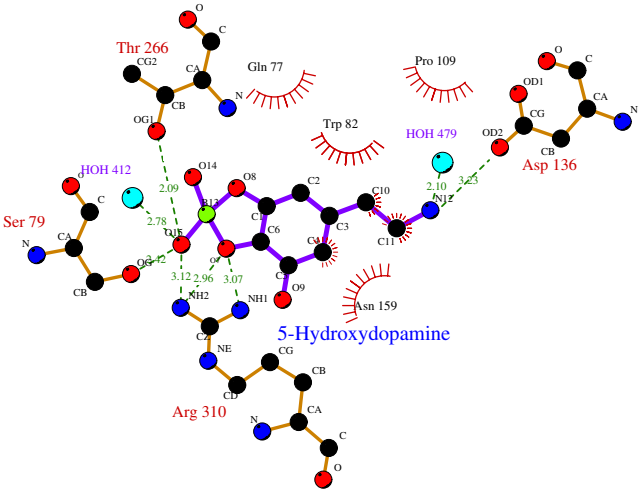

Figure 5. The proposed binding profile of 5-hydroxydopamine-borate in LuxP receptor.

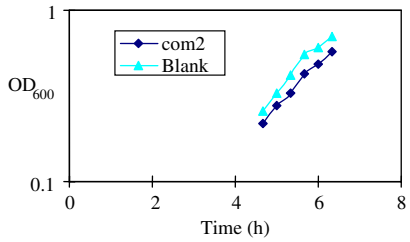

Figure 6. Growth curve for blank (doubling time: 77 min) and compound **2** at 20 μM (doubling time: 82 min).

With all the compounds that showed inhibitory activities, we also examined their effect on bacterial growth in order to eliminate general toxicity reflected in retarded bacterial growth as the reason for the observed reduced bioluminescence production. Briefly, bacteria were grown for 16 h with aeration (175 rpm) at 30 °C in 2 mL of AB medium with antibiotics (kanamycin 50 μg/mL and chloramphenicol 10 μg/mL). Then this bacterial culture was diluted 100-fold with 20 mL AB medium in a 250 mL flask and incubated at 30 °C (175 rpm). Certain concentrations of compounds were added. The OD_{600} value was determined every 20 min. The doubling time was calculated based on the OD_{600} value. Figure 6 shows one example of these compounds. Except for compound **12**, none of the others exhibited significant inhibition of bacterial growth when compared with the control group (no compound). Therefore, no general cytotoxicity was observed at the concentrations tested.

In conclusion, several pyrogallol compounds were found to exhibit AI-2 inhibition effect with IC_{50} values in the single digit micromolar range. The potency observed was much higher than the AI-2 antagonists reported in the literatures. These pyrogallol analogs will be very useful tools for research and good lead compounds for further structural optimization.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.081.

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