JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

A Multivalent Probe for AI-2 Quorum-Sensing Receptors

Amanda L. Garner, Junguk Park, Joseph S. Zakhari, Colin A. Lowery, Anjali Kumari Struss, Daisuke Sawada, Gunnar F. Kaufmann, and Kim D. Janda*

Departments of Chemistry and Immunology and Microbial Science, The Skaggs Institute for Chemical Biology, and The Worm Institute for Research and Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

Supporting Information

ABSTRACT: Multivalency is a common principle in the recognition of cellular receptors, and multivalent agonists and antagonists have played a major role in understanding mammalian cell receptor biology. The study of bacterial cell receptors using similar approaches, however, has lagged behind. Herein we describe our efforts toward the development of a dendrimer-based multivalent probe for studying AI-2 quorum-sensing receptors. From these studies, we have discovered a chemical probe specific for Lsr-type AI-2 quorum-sensing receptors with the potential for enabling the identification of new bacterial species that utilize AI-2 as a quorum-sensing signaling molecule.

Multivalency is a powerful underlying logic in biological recognition.[1−3] In chemical biology, multivalent ligands have been widely used to antagonize cellular receptors.[1−3] To employ such techniques, researchers often rely on polymeric scaffolds that are able to display multiple copies of a ligand or even multiple ligands.[1−4] In particular, the use of dendrimers, a class of monodisperse polymers, in drug-, antibody- and peptide-delivery systems has rapidly expanded within recent years due to the favorable properties that dendrimers possess (biocompatible, nonimmunogenic, nonmutagenic, nontoxic, water-soluble).[5−9] Additionally, decoration of the termini of dendrimers with targeting, solubilizing, and imaging groups has been reported.[7−10] With respect to imaging, while many dendritic probes have been used to study mammalian receptor biology using fluorescence microscopy,[11−15] no analogous experiments have been reported for bacteria.[16]

In bacterial quorum sensing, only one quorum-sensing signaling molecule has been discovered that facilitates both Gram-negative and Gram-positive bacterial communication, AI-2, a class of furanone-based quorum-sensing signaling molecules derived from 4,5-dihydroxy-2,3-pentanedione (DPD, **1**, Figure 1).[17,18] Although the DPD synthase, *luxS*, has been identified in over 55 bacterial species, the exact chemical structure of the AI-2 signaling molecule(s) along with their respective receptor(s) have been confirmed in only two species, *Vibrio harveyi* and *Salmonella typhimurium*.[18] Interestingly, the chemical form of this autoinducer is dependent on species: *V. harveyi* utilizes a boron-containing AI-2 molecule, (2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate ((*S*)-THMF-borate, **2**, Figure 1),[19] while *S. typhimurium* uses (*R*)-THMF (**3**, Figure 1).[20] Moreover, these Gram-negative bacterial species also use functionally different AI-2 receptors, which have low sequence homology (11%) and electrostatically and sterically diverse binding sites.[21,22] LuxP is a periplasmic binding protein that, together with LuxQ, a membrane sensor histidine kinase, controls quorum sensing in *V. harveyi* upon the binding of **2** in its positively charged binding pocket.[21] LsrB, on the other hand, is part of an adenosine triphosphate binding cassette (ABC) transporter and binds **3** in its net negatively charged binding pocket to faciltate AI-2 internalization and quorum sensing in *S. typhimurium*.[22] Intriguingly, LuxPQ receptor systems have only been identified in Vibrionales, while the Lsr receptor complex has been identified in many pathogenic bacterial species.[23−25] However, these studies relied solely on genetic and structural predictions,[23−25] and no probe has been reported to rapidly analyze bacteria for AI-2 binding receptors. Herein, we report the design and synthesis of a multivalent chemical probe that is capable of specifically recognizing Lsr-type AI-2 receptors.

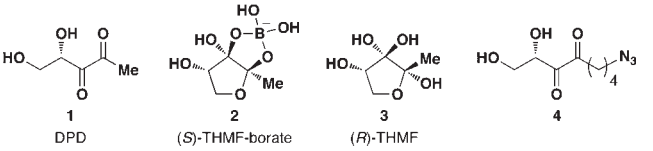


Figure 1. Structures of DPD, AI-2 quorum-sensing signaling molecules, and an azido-DPD analogue.

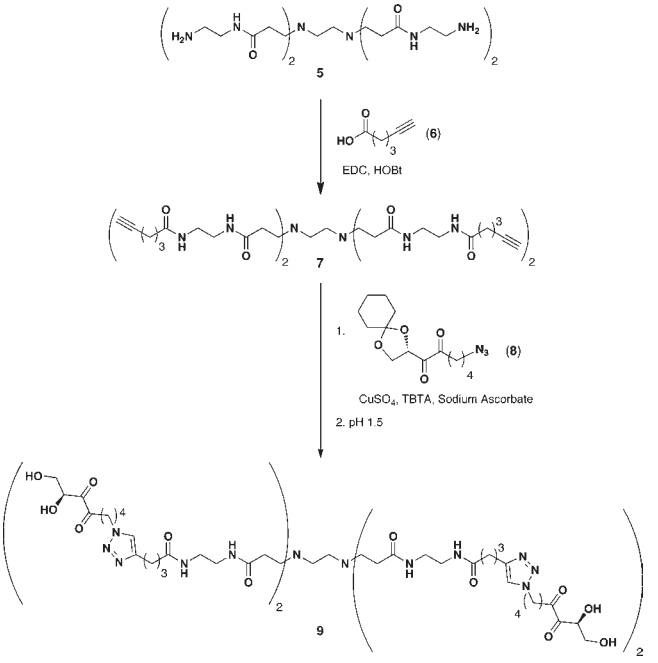
Our group has reported the synthesis of a series of alkyl-DPD analogues that demonstrated a molecular basis for highly discriminatory recognition in AI-2 quorum sensing.[26] Additionally, from these studies, an azidobutyl-containing DPD analogue (**4**, Figure 1) was found to be a quorum-sensing antagonist in both *V. harveyi* and *S. typhimurium*.[26] As this analogue is amenable to modification using "click chemistry", we were interested in exploiting the azido motif of **4** for the synthesis of DPD-based dendritic probes using Cu^{I}-catalyzed azide−alkyne cycloaddition chemistry.[27−29] It is important to note here that while reports of the use of functionalized dendrimers in cancer therapeutics are numerous,[5−10] only limited studies exploring their use as antibacterials have been reported,[9,30−33] none of which target quorum sensing specifically.[34]

The synthesis of our DPD-based dendritic probe is shown in Scheme 1, and began with commercially available PAMAM dendrimer **5** (generation 0.0). The *N*-terminal amines of **5** were first converted to alkynyl-amides **7** by coupling with 5-hexynoic acid (**6**) using standard coupling conditions. The alkynyl moieties

Received: August 10, 2011
Published: September 13, 2011

dx.doi.org/10.1021/ja207556d | *J. Am. Chem. Soc.* 2011, 133, 15934–15937

Scheme 1. Synthesis of DPD Dendrimer 9



were then utilized to install protected azido-DPD analogues **8** using Cu^{I}-catalyzed azide−alkyne cycloaddition chemistry. Click chemistry has been widely used both for dendrimer assembly using azide- or alkyne-modified dendrons and for terminal conjugation of various small molecules.[35−37] Following azide−alkyne cycloaddition, the purified dendrimers were dissolved in pH 1.5 buffer to afford deprotected alkyl-DPD-functionalized dendrimer **9**.[26]

To determine the effect of conjugating azidobutyl-DPD to the dendrimer, we first tested **9** for its activity as a quorum-sensing antagonist in *S. typhimurium*. Assays were conducted using *S. typhimurium* strain Met844, a Δ*luxS* strain with a *lacZ-lsr* fusion, as previously reported.[26] The *lacZ* fusion, which encodes for the biosynthesis of β-galactosidase under *lsr* promoter control, allows for the monitoring of AI-2-dependent *lsr* activation.[38] As Figure 2a shows, DPD dendrimer **9** exhibited dose-dependent inhibition of quorum sensing in *S. typhimurium* with an IC_{50} value of 29.5 μM, similar to that of **4** (20.3 μM).[26] Importantly, no effect on growth was observed at any of the concentrations examined (Figure 2b). We note that, although the inhibitory activity of DPD dendrimer **9** was not enhanced, as has previously been reported for other multivalent probes,[1−3] this may be readily interpreted in that **9** must be internalized to bind the periplasmic LsrB receptor and antagonize quorum sensing. Thus, only one DPD molecule may be required for binding LsrB; however, multiple ligand display was sought for probe versatility and future de novo receptor discovery (vide infra).

To examine the feasibility of larger dendrimers, generations 1.0 and 2.0, with 8 and 16 DPD molecules, respectively, were also examined. However, both carried a serious liability: they were toxic to the bacteria, with a likely basis being outer membrane disruption. Of additional note, a DPD dimer was also examined; however, no quorum-sensing antagonism was observed. To confirm our inhibition data, we also examined the down-regulation of quorum-sensing-relevant genes using RT-PCR, and a noted decrease in LsrB, LsrF, and LsrK expression was observed (see Supporting Information). Thus, DPD dendrimer **9** selectively

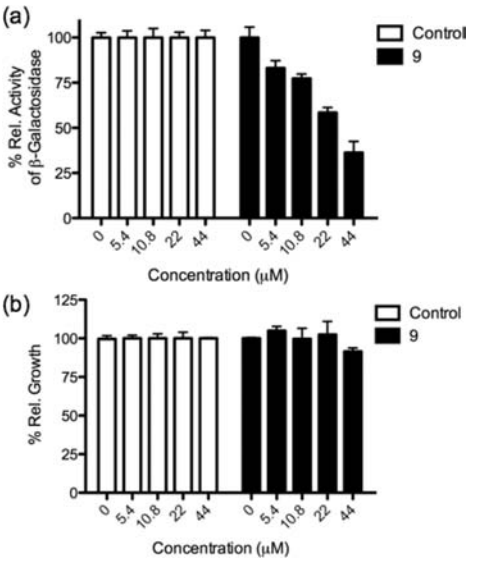


Figure 2. Quorum-sensing modulation (a) and growth impact (b) by DPD dendrimer **9**. The antagonist assay was performed in the presence of 50 μM **1**.

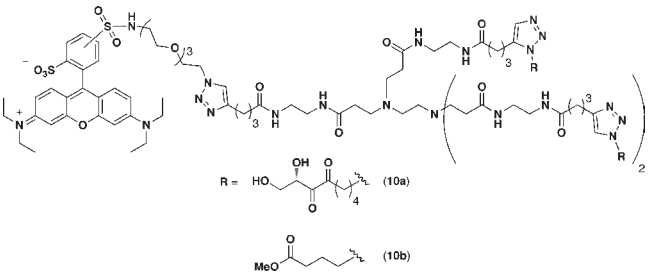


Figure 3. Structure of rhodamine-containing DPD and control ligand dendrimers.

inhibits quorum sensing in *S. typhimurium*. Based on these successful results in *S. typhimurium*, we were also interested in examining quorum-sensing antagonist activity of **9** in *V. harveyi*; however, no activity was noted in this species.

Based on our activity data and the fact that *S. typhimurium* and *V. harveyi* use functionally different quorum-sensing receptors, LsrB, part of an ABC transporter,[22] and LuxP, a periplasmic binding protein,[21] respectively, we decided to establish whether DPD dendrimer **9**-based constructs may be useful as specific probes for Lsr-type AI-2 quorum-sensing receptors. This hypothesis was also driven by the seminal studies of Chmielewski, who demonstrated the capacity of multivalent ligands to act as potent inhibitors of ABC transporter drug efflux systems.[39−41] To engage this logic, we synthesized DPD-rhodamine dendrimer **10a** and control dendrimer **10b** (Figure 3; see Supporting Information for synthetic details) for fluorescence imaging studies. Importantly, dendrimer **10a** retained antagonistic activity in *S. typhimurium*, supporting its use as a probe (see Supporting Information). Bacteria were stained with either dendrimer **10a** or **10b** (50 μM each), and the cells were analyzed using confocal microscopy for rhodamine fluorescence. As Figure 4a,b shows, dendrimer **10a** successfully stained *S. typhimurium*. Moreover, no fluorescence was observed after staining with dendrimer **10b**, indicating that the interaction between DPD-rhodamine dendrimer

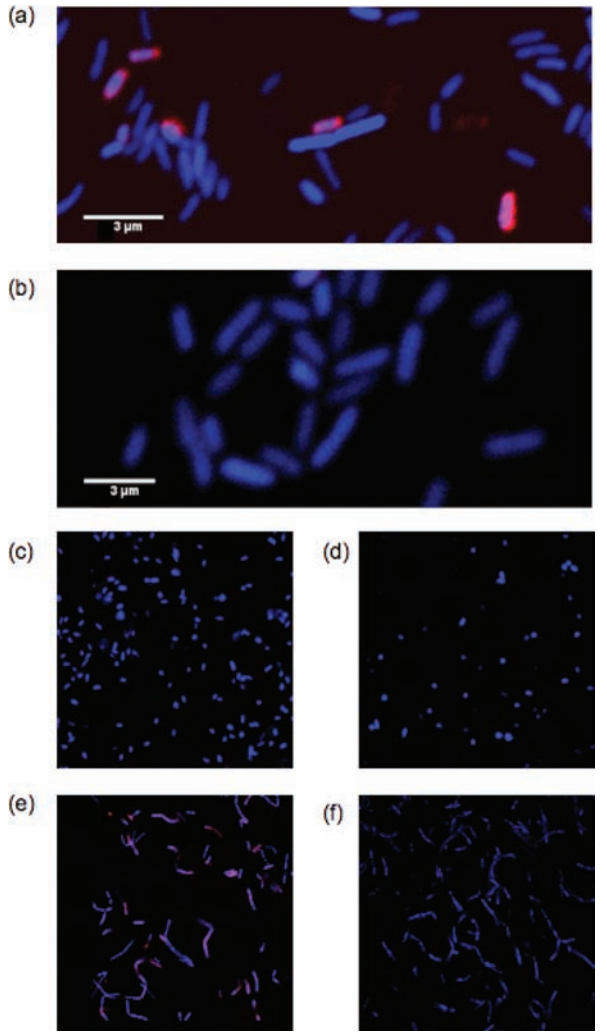


Figure 4. Fluorescence imaging in (a,b) *S. typhimurium*, (c,d) *V. harveyi*, and (e,f) *B. cereus* with dendrimers **10a** (a,c,e) and **10b** (b,d,f) (50 μM each). Images were obtained using a Zeiss LSM 710 laser scanning confocal microscope. Rhodamine fluorescence is shown in pink. Hoescht 33342 (blue), a DNA stain, was used as a control.

10a and LsrB is specific. As an additional control for Lsr specificity, we also performed imaging experiments in *V. harveyi*. As Figure 4c,d shows, no fluorescence was observed with either dendrimer **10a** or **10b**, providing additional evidence that dendrimer **10a** is specific for Lsr-type quorum-sensing receptors.

Finally, we sought to examine the versatility of our probe in another bacterial species known to contain an Lsr-type AI-2 receptor. Recent biochemical evidence has shown that *Bacillus cereus*, a Gram-positive bacterial species, possesses functional AI-2 receptors with 63% homology to LsrB.[25] *B. cereus* was stained with either dendrimer **10a** or **10b** as described above. As Figure 4e,f shows, DPD dendrimer probe **10a** was also capable of recognizing the *B. cereus* AI-2 receptor. Thus, dendrimer probe **10a** is able to specifically bind Lsr-type AI-2 receptors in both Gram-negative and Gram-positive bacterial species. This finding could be broadly useful, as Lsr system orthologues have been genetically identified in bacterial species belonging to the Enterobacteriaceae, Rhizobiaceae, Bacillaceae, Pasteurellaceae, and Rhodobacteriaceae families, while LuxPQ systems have only been identified in Vibrionales, indicating the potential importance of Lsr systems for AI-2 quorum sensing.[23−25]

In conclusion, we have developed a multivalent chemical probe for Lsr-type AI-2 receptors. More specifically, using this multivalent scaffold approach, an AI-2 quorum-sensing antagonist in *S. typhimurium* and an imaging agent for bacterial species utilizing Lsr-type AI-2 receptors have been identified. Although only modest antagonism was achieved with probe **9**, efficacy could be readily improved upon, possibly by examining other dendrimer scaffolds or altering the linker length. These considerations notwithstanding, this multivalent scaffold was key in obtaining an effective imaging agent, as similar imaging experiments failed with a rhodamine-conjugated DPD molecule. Thus, an understanding of these multivalent receptor−ligand interactions should be valuable for designing new tools for discovering additional bacterial species that utilize Lsr-type AI-2 receptor. Furthermore, since the dendrimer ligands are modular, both fluorescence and affinity tags can be employed to facilitate such studies. Most importantly, however, since our probe is capable of binding Lsr-type receptors in both Gram-negative and Gram-positive bacteria, it may facilitate our understanding of interspecies bacterial communication.

■ ASSOCIATED CONTENT

Supporting Information. Synthetic protocols for and characterization of dendrimers **9**, **10a**, and **10b**; assay and imaging protocols; complete ref 31. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
kdjanda@scripps.edu

■ ACKNOWLEDGMENT

We thank Prof. Bonnie Bassler (Princeton University) for kindly providing *S. typhimurium* strain Met844. We gratefully acknowledge financial support from the NIH (Grant AI077644 to K.D.J. and Grants AI080715 and AI085324 to G.F.K.) and the Skaggs Institute for Chemical Biology.

■ REFERENCES

(1) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 696.
(2) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 2348.
(3) Mammen, M.; Choi, S.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754.
(4) Rolland, O.; Turrin, C.; Caminade, A.; Majoral, J. *New J. Chem.* **2009**, *33*, 1809.
(5) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117.
(6) Grayson, S. M.; Frechet, J. M. J. *Chem. Rev.* **2001**, *101*, 3819.
(7) Gillies, E. R.; Fréchet, J. M. J. *Drug Discovery Today* **2005**, *10*, 35.
(8) Patri, A. K.; Majoros, I. J.; Baker, J. R., Jr. *Curr. Opin. Chem. Biol.* **2002**, *6*, 466.
(9) Boas, U.; Heegaard, P. M. H. *Chem. Soc. Rev.* **2004**, *33*, 43.
(10) Svenson, S.; Chauharr, A. S. *Nanomedicine* **2008**, *3*, 679.
(11) Quintana, A.; Raczka, E.; Piehler, L.; Lee, I.; Myc, A.; Majoros, I. J.; Patri, A. K.; Thomas, T.; Mule, J.; Baker, J. R., Jr. *Pharm. Res.* **2002**, *19*, 1310.

(12) Choi, Y.; Thomas, T.; Kotlyar, A.; Islam, M. T.; Baker, J. R., Jr. *Chem. Biol.* **2005**, *12*, 35.
(13) Xu, H.; Regino, C. A. S.; Koyama, Y.; Hama, Y.; Gunn, A. J.; Bernardo, M.; Kobayashi, H.; Choyke, P. L.; Brechbiel, M. W. *Bioconjugate Chem.* **2007**, *18*, 1474.
(14) Shi, X.; Wang, S. H.; Shen, M.; Antwerp, M. E.; Chen, X.; Li, C.; Petersen, E. J.; Huang, Q.; Weber, W. J.; Baker, J. R. *Biomacromolecules* **2009**, *10*, 1744.
(15) Thomas, T.; Shukla, R.; Kotlyar, A.; Kukowska-Latallo, J.; Baker, J. R., Jr. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 700.
(16) Live cell labeling of an acylhomoserine lactone quorum-sensing receptor has been reported:Rayo, J.; Amara, N.; Krief, P.; Meijler, M. M. *J. Am. Chem. Soc.* **2011**, *133*, 7469.
(17) Schauder, S.; Shokat, K.; Surette, M. G.; Bassler, B. L. *Mol. Microbiol.* **2001**, *41*, 463.
(18) Waters, C. M.; Bassler, B. L. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 319.
(19) Chen, X.; Schauder, S.; Potier, N.; Van Dorsselaer, A.; Pelczer, I.; Bassler, B. L.; Hughson, F. M. *Nature* **2002**, *415*, 545.
(20) Miller, S. T.; Xavier, K. B.; Campagna, S. R.; Taga, M. E.; Semmelhack, M. F.; Bassler, B. L.; Hughson, F. M. *Mol. Cell* **2004**, *15*, 677.
(21) Neiditch, M. B.; Federle, M. J.; Miller, S. T.; Bassler, B. L.; Hughson, F. M. *Mol. Cell* **2005**, *18*, 507.
(22) Taga, M. E.; Semmelhack, J. L.; Bassler, B. L. *Mol. Microbiol.* **2001**, *42*, 777.
(23) Sun, J.; Daniel, R.; Wagner-Dobler, I.; Zeng, A. *BMC Evol. Biol.* **2004**, *4*, 36.
(24) Rezzonico, F.; Duffy, B. *BMC Microbiol.* **2008**, *8*, 154.
(25) Pereira, C. S.; de Regt, A. K.; Brito, P. H.; Miller, S. T.; Xavier, K. B. *J. Bacteriol.* **2009**, *191*, 6975.
(26) Lowery, C. A.; Park, J.; Kaufmann, G. F.; Janda, K. D. *J. Am. Chem. Soc.* **2008**, *130*, 9200.
(27) Rostovtsov, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
(28) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
(29) Meldal, M.; Tornoe, C. W. *Chem. Rev.* **2008**, *108*, 2952.
(30) Johansson, E. M. V.; Kolomiets, E.; Rosenau, F.; Jaeger, K.-E.; Darbre, T.; Reymond, J.-L. *New J. Chem.* **2007**, *31*, 1291.
(31) Johansson, E. M. V.; et al. *Chem. Biol.* **2008**, *15*, 1249.
(32) Kolomiets, E.; Swiderska, M. A.; Kadam, R. U.; Johansson, E. M. V.; Jaeger, K.-E.; Darbre, T.; Reymond, J.-L. *ChemMedChem* **2009**, *4*, 562.
(33) Hou, S.; Zhou, C.; Liu, Z.; Young, A. W.; Shi, Z.; Ren, D.; Kallenbach, N. R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5478.
(34) Quorum sensing control using other polymers has been reported:Kato, N.; Kobayashi, A.; Motohashi, H.; Ozonoe, Y.; Morohoshi, T.; Ikeda, T. *Prog. Colloid Polym. Sci.* **2009**, *136*, 155. Piletska, E. V.; Stavroulakis, G.; Karim, K.; Whitcombe, M. J.; Chianella, I.; Sharma, A.; Eboigbodin, K. E.; Robinson, G. K.; Piletsky, S. A. *Biomacromolecules* **2010**, *11*, 975. Piletska, E. V.; Stavroulakis, G.; Larcombe, L. D.; Whitcombe, M. J.; Sharma, A.; Primrose, S.; Robinson, G. K.; Piletsky, S. A. *Biomacromolecules* **2011**, *12*, 1067.
(35) Wu, P.; Malkoch, M.; Hunt, J. N.; Vestberg, R.; Kaltgrad, E.; Finn, M. G.; Fokin, V. V.; Sharpless, K. B.; Hawker, C. J. *Chem. Commun.* **2005**, 5775.
(36) Malkoch, M.; Schleicher, K.; Drockenmuller, E.; Hawker, C. J.; Russell, T. P.; Wu, P.; Fokin, V. V. *Macromolecules* **2005**, *38*, 3663.
(37) Franc, G.; Kakkar, A. *Chem. Commun.* **2008**, 5267.
(38) Taga, M. E.; Miller, S. T.; Bassler, B. L. *Mol. Microbiol.* **2003**, *50*, 1411.
(39) Pires, M. M.; Hrycyna, C. A.; Chmielewski, J. *Biochemistry* **2006**, *45*, 11695.
(40) Pires, M. M.; Emmert, D.; Hrycyna, C. A.; Chmielewski, J. *Mol. Pharmacol.* **2009**, *75*, 92.
(41) Namanja, H. A.; Emmert, D.; Pires, M. M.; Hrycyna, C. A.; Chmielewski, J. *Biochem. Biophys. Res. Commun.* **2009**, *388*, 672.