Microwave Synthesis and Evaluation of Phenacylhomoserine Lactones as Anticancer Compounds that Minimally Activate Quorum Sensing Pathways in *Pseudomonas aeruginosa*

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The bacterial quorum sensing (QS) signal molecule 3-*oxo*-dodecanoyl-L-homoserine lactone (OdDHL) is produced by the opportunistic pathogen *Pseudomonas aeruginosa* and controls expression of virulence factors associated with life threatening infections in immune compromised individuals. OdDHL has also demonstrated anticancer activity, yet its ability to enhance pathogenicity of *P. aeruginosa* compromises further consideration as a potential anticancer agent. In search of acylhomoserine lactones that selectively inhibit cancer cell growth, a library of phenacylhomoserine lactone analogues has been prepared by microwave synthesis and evaluated for cancer growth inhibition and quorum sensing activation. Comparative SAR analysis demonstrates that both anticancer and QS signaling systems require long acyl side chains with a 3-*oxo* substitution for maximum activity. Compound **12b**, 3-*oxo*-12-phenyldodecanoyl-L-homoserine lactone, was identified as a lead compound with strong cancer growth inhibitory activity that minimizes activation of QS signaling pathways in a *P. aeruginosa* reporter assay.

Introduction

The acylhomoserine lactone (AHL[a]), 3-*oxo*-dodecanoyl-L-homoserine lactone (OdDHL, Figure 1), has been shown to inhibit the growth of tumorigenic but not nontumorigenic cells.[1] In colorectal and prostate cancer cells, OdDHL down-regulates thymidylate synthase (TS) and enhances the efficacy of the widely used cancer chemotherapeutic agents, 5-fluorouracil (5-FU) and Taxol.[2] The effects of OdDHL are highly structure-specific as elimination of the 3-*oxo* moiety attenuates activity. A racemic mixture of enantiomers is 50% as active as the L-isomer, indicating specificity.[2]

In nature, Gram-negative bacteria that enzymatically produce AHLs are capable of coordinating expression of population-sensitive genes through signaling networks known as quorum sensing (QS).[3] OdDHL is a QS signal produced by *Pseudomonas aeruginosa*.[4] Activation of QS pathways is frequently associated with characteristics that compromise the host organism (e.g., increased virulence, resistance).[5] Five percent of immune-compromised patients (e.g., cancer) die from nosocomial *P. aeruginosa* infection.[6] Moreover, OdDHL has been detected in clinical isolates of *P. aeruginosa*.[7]

OdDHL has promise as a cancer treatment modality. However, its ability to promote *P. aeruginosa* infection could be life-threatening in a clinical setting. Development of second-generation compounds with diminished QS signaling activity is essential to identifying the most promising candidates for drug development. In this respect, the aliphatic hydrocarbon side chain of OdDHL lacks a structural platform that can be easily modified. To address these limitations, we have prepared a library of phenacylhomoserine lactones (PHLs). Phenyl substitution retains the lipophilic properties of traditional AHLs while introducing a structural platform amenable to modification. We have evaluated our library of phenacylhomoserine lactone analogues (PHLA) for cancer growth inhibitory and QS signaling activities. By analyzing comparative structure−activity relationships (SAR), we have identified a lead structure that potently inhibits cancer cell growth and minimally activates QS.

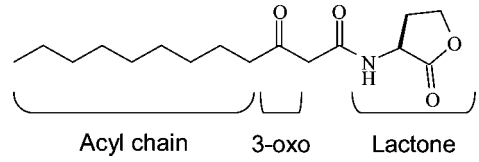

Figure 1. Structure of 3-*oxo*-dodecanoyl-L-homoserine lactone (OdDHL). Structural motifs subject to modification are listed. Figure adapted from Chhabra et al.[10]

Chemistry

To dissociate the inhibitory effects of AHLs on cancer cell growth from activation of quorum sensing signaling, we have synthesized a library of phenyl-substituted AHL analogues that vary in acyl chain length and oxidation state at carbon 3. Methods we have developed for synthesis of our library of PHLAs, through the combined use of a solid-phase coupling reagent and microwave chemistry, improve upon those previously described.[8−11] Other syntheses of AHL analogues that use solid-phase support and/or microwave irradiation require multiple, time-consuming steps that can include several rounds of purification.[8,9,12,13] Our method provides PHLAs as final products in one or two rapid steps from commercially available starting materials (Schemes 1 and 2). Use of a resin-linked

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[a] Abbreviations: 5-FU, 5-fluorouracil; AHL, acylhomoserine lactone; dFBS, dialyzed fetal bovine serum; DMAP, dimethylaminopyridine; L-HSL, L-homoserine lactone; PHL, phenacylhomoserine lactone; PHLA, phenacylhomoserine lactone analogue; PRF, phenol red free; OdDHL, 3-*oxo*-dodecanoyl-L-homoserine lactone; QS, quorum sensing; SRB, sulforhodamine B; W, microwave irradiation; GFP, green fluorescence protein.

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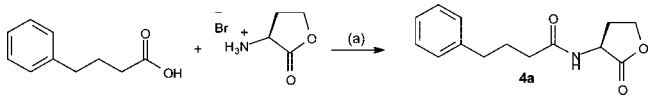
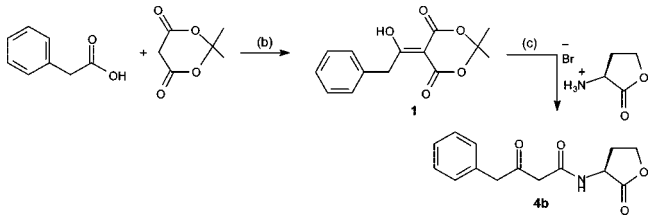
Scheme 1[a]



[a] (a) PS-carbodiimide (4 equiv); W (1000 W, 1.5 min).

Scheme 2[a]



[a] (b) PS-carbodiimide (5 equiv), DMAP (1.05 equiv); 22°C overnight (c) W (100 W, 2 × 10 min).

carbodiimide coupling reagent provides a platform for quick and simple purification via filtration, as the major reaction byproduct, urea, remains bound to the resin. Microwave irradiation affords dramatic reduction in reaction time.

N-phenacyl-L-homoserine lactones (Table 1, compounds **2** and **3a**−**12a**) were synthesized by resin-bound carbodiimide-mediated direct coupling of commercially available phenacyl carboxylic acids with L-homoserine lactone HBr (L-HSL) under conditions of microwave irradiation (Scheme 1). High energy microwave reaction (1000 W) allowed for very short reaction times (30 s to 1.5 min) to completion. Removal of resin-bound byproduct via filtration yields the final product. Short chain analogues (<8 carbons) were recrystallized from EtOAc with hexanes. Products not recrystallized were readily purified by preparative TLC. Final purification was achieved by RP-HPLC and structures confirmed by ^{1}H NMR and ESI-MS. Compounds **2, 3a** and **4a** (Table 1) have been previously reported.[13]

N-(3-*oxo*-Phenacyl)-L-homoserine lactones (Table 1, compounds **3b**−**12b**, **13**, and **14**) were synthesized by preparation of the corresponding active 3-*oxo*-phenacyl esters followed by coupling to L-HSL (Scheme 2). The 3-*oxo*-phenacyl active esters were prepared by overnight phenacylation of Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) in the presence of DMAP and resin-bound carbodiimide. The isolated phenacyl−Meldrum's acid derivatives **1** were used without further purification. Coupling to L-HSL was achieved in the presence of triethylamine in CH_3CN using microwave irradiation. Extended low energy microwave reactions (100 W, 2 × 10 min) were required to prevent formation of extensive side products. Regardless, microwave irradiation decreases reaction time to 20 min from 5 h.[10] Final products were readily isolated by gradient flash chromatography or preparative TLC. Final purification was achieved by RP-HPLC and structures confirmed by ^{1}H NMR and ESI-MS. Compound **4b** has been previously reported.[13]

Results and Discussion

The current study was undertaken to dissect the broad structural requirements for inhibition of cancer cell growth versus QS activation. The ultimate goal of this study was to identify potential lead compounds that retain anticancer properties while minimizing QS signaling activity. OdDHL, the naturally produced *P. aeruginosa* AHL, has demonstrated growth inhibitory activity at μM concentrations in a variety of cancer cell lines.[1,2] OdDHL-mediated growth inhibition is selective for tumorigenic human cells.[1]

To determine the structural basis for the distinct biological activities of OdDHL, a series of phenyl-substituted AHLs was synthesized and characterized. The phenyl substituent, which introduces a structural platform amenable to second- and third-generation modifications, retains the intrinsic lipophilicity of natural AHLs, a property that enables these small molecules to cross membranes through unregulated diffusion and act on intercellular targets.[3,14] Moreover, the previously reported PHLA, **4b**, has been shown to possess QS inhibitory activity, providing additional rationale for a comparative SAR analysis of a library of PHLAs.[12]

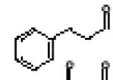
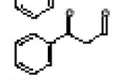
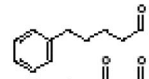
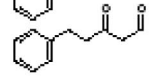
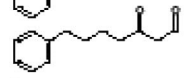
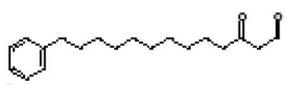
PHLAs were evaluated for their ability to inhibit the growth of three human cancer cell lines: H630 (colorectal−parental), H630-1 (colorectal−5-FU resistant), and PC3 (prostate). Results from growth inhibition assays demonstrate that PHLAs containing an acyl chain length shorter than eight carbons have no activity (Table 1) regardless of carbon 3 oxidation state. PHLAs with IC_{50} values greater than 100 μM were deemed inactive because growth inhibitory activity at higher concentrations may be a consequence of nonspecific detergent effects. Compounds with long acyl chain length (10−14 carbons) maintained the greatest growth inhibitory activity. Presence of a carbonyl at carbon 3 drastically enhances activity, confirming previous reports that 3-*oxo* substitution is required for anticancer effects of AHLs.[2]

QS signaling activity was assessed using a LasR-dependent, green fluorescence protein (GFP) reporter assay that mimics natural OdDHL signaling in *P. aeruginosa*.[15] Activation of QS in response to PHLA is reported as percent activation relative to OdDHL control (Figure 2). OdDHL was examined at 0.3 μM, which achieves half-maximal induction of the *rsaL::gfp* fusion reporter. PHLAs were evaluated at 33 μM or 100× the concentration of OdDHL. A dose-dependent decrease in activity was observed for analogues that activated QS to greater than 100% of control (data not shown). SAR analysis of QS signaling activity demonstrates that compounds with acyl chains between 8−11 carbons retain greatest capacity for QS signaling. A drop in activity is seen for compounds **12b**−**14**. As with cancer cell growth inhibition, short chain PHLAs lack functionality while presence of a 3-*oxo* substitution enhances QS signaling.

Drug discovery is based upon identification of a lead compound that can be accelerated through the developmental pipeline. For compounds from this study to qualify as leads, we established several criteria: (1) ready accessibility to starting materials, (2) inexpensive and straightforward synthesis and purification, (3) significant cancer growth inhibitory activity, (4) structurally specific cancer growth inhibitory activity (i.e., inactive non-3-*oxo*- or phenacyl-D-homoserine lactone analogue), and (5) selectivity for anticancer versus QS signaling activity. All compounds in the PHLA library meet criterion 1 as starting materials were commercially available. The microwave-assisted syntheses described herein qualify all compounds for criterion 2. As described, compounds with acyl chain length 10−14 carbons and 3-*oxo* substitution (**10b**−**14**) maintain highest cancer cell growth inhibitory activity. Compounds **10a** and **11a**, which lack a 3-*oxo* substitution, inhibit growth of PC3 cancer cells. Yet compound **12a** does not inhibit growth of cancer cells, indicating that the activity of **12b** is highly structure-specific. Comparative SAR analysis of biological activities points to **12b** as the compound that retains the greatest selectivity between cancer growth inhibitory and QS signaling activities (Figure 3).

To confirm **12b** as the lead compound from these studies, control structures were synthesized and evaluated for comparison. OdDHL was used for reference. Table 2 demonstrates that

Table 1. Structures, Yields, and IC_{50} Values of PHLA Library in Three Human Cancer Cell Lines

No	Compound	R	%Yield	H630	IC_{50} (μM) H630-1	PC3
2	2-phenylethanoyl-L-HSL[a]		27.6[b]	>100	>100	>100
3a	3-phenylpropanoyl-L-HSL[a]		23.4[b]	>100	>100	>100
3b	3-oxo-**3a**		25.0	>100	>100	>100
4a	4-phenylbutanoyl-L-HSL[a]		8.9[b]	>100	>100	>100
4b	3-oxo-**4a**[a]		29.5	>100	>100	>100
5a	5-phenylpentanoyl-L-HSL		27.4[b]	>100	>100	>100
5b	3-oxo-**5a**		39.9	>100	>100	>100
6a	6-phenylhexanoyl-L-HSL		16.1[b]	>100	>100	>100
6b	3-oxo-**6a**		21.2	>100	>100	>100
7a	7-phenylheptanoyl-L-HSL		19.3[b]	>100	>100	>100
7b	3-oxo-**7a**		52.8	>100	80±17	83±21
8a	8-phenyloctanoyl-L-HSL		14.4[b]	>100	>100	85±13
8b	3-oxo-**8a**		45.0	44±9	24±6	9±3
9a	9-phenylnonanoyl-L-HSL		61.0	>100	>100	67±6
9b	3-oxo-**9a**		42.1	65±6	27±5	9±4
10a	10-phenyldecanoyl-L-HSL		32.0	>100	>100	75±18
10b	3-oxo-**10a**		41.4	42±8	10±6	2±1
11a	11-phenylundecanoyl-L-HSL		38.9	70±14	66±8	66±15
11b	3-oxo-**11a**		52.1	11±1	10±2	4±3
12a	12-phenyldodecanoyl-L-HSL		25.8	>100	>100	>100
12b	3-oxo-**12a**		58.6	16±2	13±4	4±2
13	3-oxo-13-phenyltridecanoyl-L-HSL		41.2	23±6	14±6	2±0
14	3-oxo-14-phenyltetradecanoyl-L-HSL		45.8	33±6	27±10	4±2

[a] Compounds previously reported.[13] [b] Recrystallized yield.

lead compound **12b** preserves cancer growth inhibitory activity when compared to OdDHL in three cancer cell lines, while the enantiomer, **D-12b**, is inactive.

Conclusions

The overall goal of this study was to identify lead compounds with cancer growth inhibitory activity that minimize activation of QS signaling in *P. aeruginosa*. Comparative SAR analysis of a library of PHLAs for cancer growth inhibitory and QS signaling activity identified **12b** as a lead structure for further biological evaluation and structural modification. Compound **12b** inhibits the growth of cancer cells at low μM concentrations with little activation of QS signaling pathways. The activity of **12b** is structure-

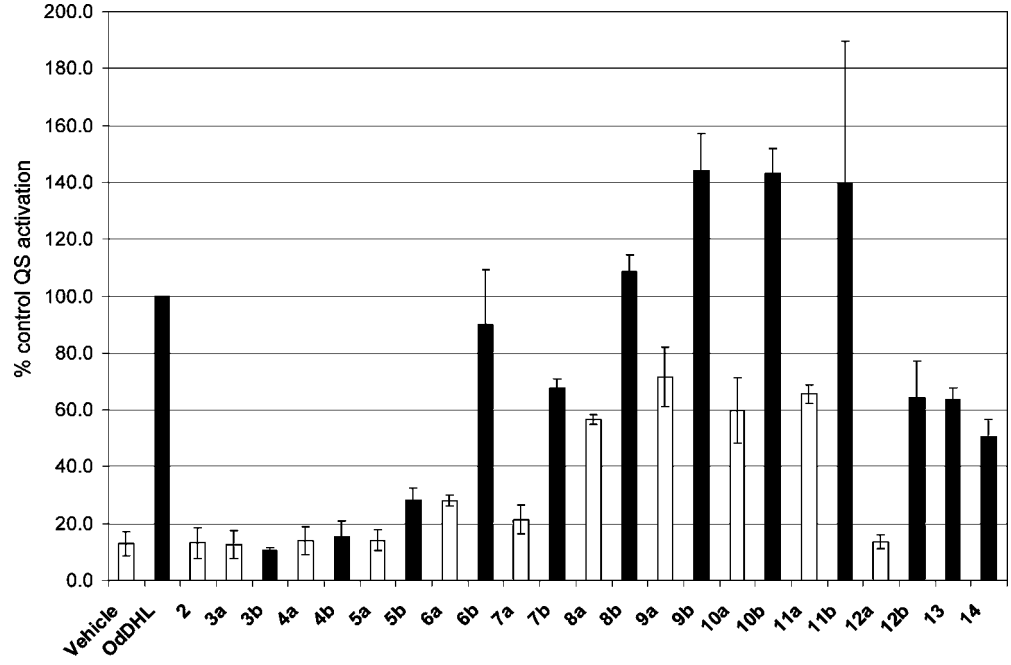


Figure 2. Activation of QS. Expression of GFP in response to OdDHL or PHLA. Data presented as percent QS activation relative to OdDHL and represents the average of three independent experiments. Open bars represent series **a**, which lack a 3-*oxo* substitution. Closed bars represent series **b**, which possess a 3-*oxo* substitution.

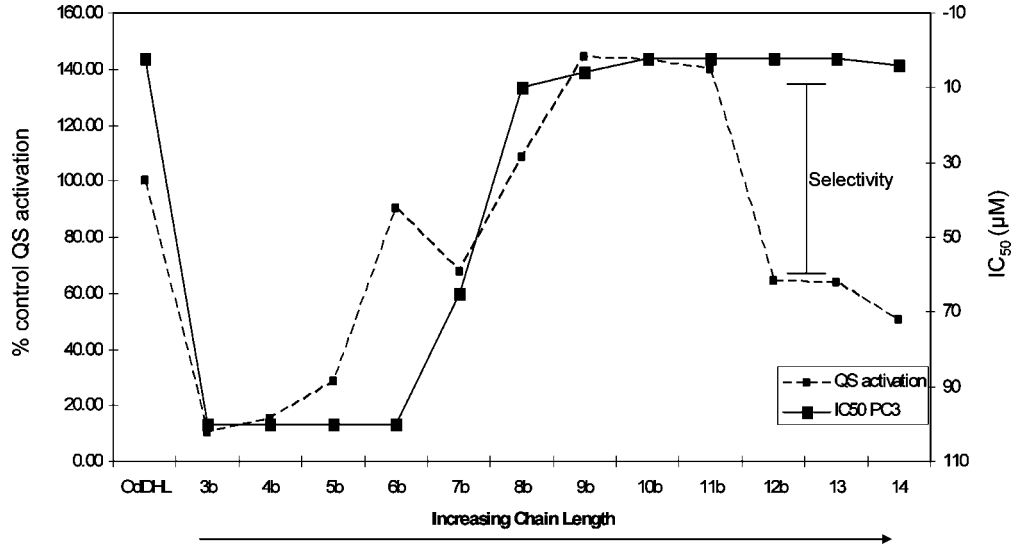


Figure 3. Comparative SAR analysis. Comparison of PC3 cancer cell growth inhibitory and QS signaling activities for series **b** PHLAs. IC_{50} data presented on inverse axis. Selectivity for anticancer activity is determined by distance of QS activation line from IC_{50} line. Greater positive distance (QS activation line below IC_{50} line) indicates greater selectivity for anticancer activity.

Table 2. Comparison of Anticancer Activity of Lead and Control Structures

	IC_{50} (μM)		
compd	H630	H630−1	PC3
OdDHL	34 ± 10	12 ± 4	5 ± 3
12b	16 ± 2	13 ± 4	4 ± 2
D-12b	>100	>100	>100

specific as elimination of the 3-*oxo* substitution or use of the D-HSL enantiomer attenuates activity. The phenyl substituent of **12b** offers a platform amenable to modification. Such modifications could address any residual QS activation and/or enhance solubility to yield third-generation analogues with improved drug characteristics. OdDHL increases the activity of 5-FU and Taxol.[2] Thus, continued development of this series could yield an agent that enhances current chemotherapeutic regimens while suppressing nosocomial *P. aeruginosa* infections that plague immune-compromised patients. This study presents the first comparative analysis of the distinct biological effects of AHLs on cancer and bacterial QS.

Experimental Methods

Chemistry. Synthetic procedures were adapted from methods previously described.[10,11] All starting materials were purchased from commercial sources (Aldrich, Milwaukee, WI; Karl Industries, Aurora, OH; Lancaster Synthesis, Pelham, NH; Oakwood Products, West Columbia, SC). Analytical thin layer chromatography (TLC) was conducted on silica plates (Analtech). Developed plates were examined under UV light and stained with ninhydrin. Preparative TLC was conducted on 1000 μm silica plates (Analtech). Flash

chromatography was performed with a Flashmaster Personal (Argonaut Technologies). Analytical reverse-phase high-performance liquid chromatography (RP-HPLC) was conducted on an Agilent LC system using a Luna C18(2) 4.6 mm × 250 mm, 5 μm column (Phenomenex, Torrance, CA). NMR spectra were recorded on a Bruker AMX 400 MHz, Varian VNMRS 400 MHz, or Bruker AMX 600 MHz instrument at room temperature. Electrospray mass spectra (ESI-MS) were recorded on a Bruker Esquire-LC ion trap mass spectrometer.

Synthesis of *N*-Phenacyl-L-homoserine Lactones: General Method A. To a mixture of PS-carbodiimide resin (0.620 mmol, 1.24 mmol/g, Argonaut Technologies), phenacyl carboxylic acid (0.230 mmol), and DCM (5.0 mL) in a 10 mL reactivial (Pierce) was added a solution of (*S*)-(−)-α-amino-γ-butyrolactone HBr (0.155 mmol, Aldrich) in MeOH (0.5 mL). After microwave irradiation (1000 W, 1.5 min), PS-carbodiimide resin was removed by vacuum filtration and solvents were removed in vacuo. The reaction product was: (1) recrystallized from EtOAc with Hex or (2) purified by preparative TLC (EtOAc:Hex, 80:20 v/v). Purity was confirmed by HPLC (linear gradient, 30 min, 10−100% CH_3CN in H_2O + 0.1% TFA).

***N*-2-Phenylethanoyl-L-homoserine Lactone (2).**[13] Melting point 130.7−132.0 °C. RP-HPLC: t_r = 11.40 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.563 (d, 1H, NH, J = 7.9 Hz), 7.329−7.200 (m, 5H, Ar−H), 4.594−4.510 (m, 1H), 4.339 and 4.334 (dt, 1H, J = 8.85 Hz), 4.239−4.163 (m, 1H), 3.466 (s, 2H), 2.439−2.352 (m, 1H), 2.190−2.075 (m, 1H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{12}H_{13}NNaO_3$, 242.1; found, 242.1; $[2M + Na]^+$ calcd for $C_{24}H_{26}N_2NaO_6$, 461.2; found, 461.1.

***N*-3-Phenylpropanoyl-L-homoserine Lactone (3a).**[13] Melting point 150.0−150.8 °C. RP-HPLC: t_r = 13.25 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.357 (d, 1H, NH, J = 7.7 Hz), 7.295−7.148 (m, 5H, Ar−H), 4.573−4.491 (m, 1H), 4.338 and 4.333 (dt, 1H, J = 8.85 Hz), 4.236−4.160 (m, 1H), 2.820 (t, 2H, J = 7.7 Hz), 2.419 and 2.415 (dt, 2H, J = 7.6 Hz and 8.15 Hz), 2.388−2.323 (m, 1H), 2.154−2.035 (m, 1H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{13}H_{15}NNaO_3$, 256.1; found, 256.3; $[M + K]^+$ calcd for $C_{13}H_{15}KNO_3$, 272.1; found, 272.2 1; $[2M + Na]^+$ calcd for $C_{26}H_{30}N_2NaO_6$, 489.2; found, 489.1.

***N*-4-Phenylbutanoyl-L-homoserine Lactone (4a).**[13] Melting point 76.2−77.3 °C. RP-HPLC: t_r = 15.14 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.307 (d, 1H, NH, J = 7.7 Hz), 7.307−7.146 (m, 5H, Ar−H), 4.575−4.490 (m, 1H), 4.341 and 4.336 (dt, 1H, J = 8.9 Hz and 8.7 Hz), 4.240−4.160 (m, 1H), 2.572 (t, 2H, J = 7.6 Hz), 4.424−4.339 (m, 1H), 2.211−2.083 (m, 1H), 2.129 (t, 2H, J = 7.6 Hz), 1.798 (m, 2H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{14}H_{17}NNaO_3$, 270.1; found, 270.1; $[2M + Na]^+$ calcd for $C_{28}H_{34}N_2NaO_6$, 517.2; found, 517.0.

***N*-5-Phenylpentanoyl-L-homoserine Lactone (5a).** Melting point 132.5−134.0 °C. RP-HPLC: t_r = 16.85 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.299 (d, 1H, NH, J = 8.0 Hz), 7.304−7.134 (m, 5H, Ar−H), 4.562−4.473 (m, 1H), 4.335 and 4.329 (dt, 1H, J = 9.0 Hz), 4.235−4.154 (m, 1H), 2.571 (t, 2H, J = 7.2 Hz), 2.419−2.313 (m, 1H), 2.184−2.064 (m, 1H), 2.137 (t, 2H, J = 7.0 Hz), 1.613−1.471 (m, 4H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{15}H_{19}NNaO_3$, 284.1; found, 284.1; $[M + K]^+$ calcd for $C_{15}H_{19}KNO_3$, 300.1; found, 300.1; $[2M + Na]^+$ calcd for $C_{30}H_{38}N_2NaO_6$, 545.3; found, 545.0.

***N*-6-Phenylhexanoyl-L-homoserine Lactone (6a).** Melting point 91.6−92.6 °C. RP-HPLC: t_r = 18.57 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.276 (d, 1H, NH, J = 7.9 Hz), 7.291−7.129 (m, 5H, Ar−H), 4.558−4.470 (m, 1H), 4.334 and 4.329 (dt, 1H, J = 9.0 Hz and 8.85 Hz), 4.235−4.154 (m, 1H), 2.557 (t, 2H, J = 7.55 Hz), 2.410−2.314 (m, 1H), 2.169−2.048 (m, 1H), 2.100 (t, 2H, J = 7.25 Hz), 1.609−1.488 (m, 4H), 1.329−1.234 (m, 2H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{16}H_{21}NNaO_3$, 298.1; found, 298.2; $[2M + Na]^+$ calcd for $C_{32}H_{42}N_2NaO_6$, 573.3; found, 572.9.

***N*-7-Phenylheptanoyl-L-homoserine Lactone (7a).** Melting point 127.8−129.3 °C. RP-HPLC: t_r = 20.28 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.270 (d, 1H, NH, J = 8.0 Hz), 7.293−7.130 (m, 5H, Ar−H), 4.558−4.470 (m, 1H), 4.334 and 4.329 (dt, 1H, J = 8.85 Hz), 4.232−4.156 (m, 1H), 2.559 (t, 2H, J = 7.6 Hz), 2.417−2.312 (m, 1H), 2.185−2.063 (m, 1H), 2.092 (t, 2H, J = 7.4 Hz), 1.601−1.440 (m, 4H), 1.321−1.248 (m, 4H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{17}H_{23}NNaO_3$, 312.2; found, 312.2; $[2M + Na]^+$ calcd for $C_{34}H_{46}N_2NaO_6$, 601.3; found, 601.0.

***N*-8-Phenyloctanoyl-L-homoserine Lactone (8a).** Melting point 99.3−100.4 °C. RP-HPLC: t_r = 21.98 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.266 (d, 1H, NH, J = 8.0 Hz), 7.288−7.128 (m, 5H, Ar−H), 4.554−4.471 (m, 1H), 4.332 and 4.328 (dt, 1H, J = 8.9 Hz), 4.231−4.153 (m, 1H), 2.559 (t, 2H, J = 7.9 Hz), 4.414−4.312 (m, 1H), 2.182−2.060 (m, 1H), 2.091 (t, 2H, J = 7.5 Hz), 1.603−1.441 (m, 4H), 1.318−1.213 (br s, 6H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{18}H_{25}NNaO_3$, 326.2; found, 326.3; $[M + K]^+$ calcd for $C_{18}H_{25}KNO_3$, 342.2; found, 342.2; $[2M + Na]^+$ calcd for $C_{36}H_{50}N_2NaO_6$, 629.4; found, 629.0.

***N*-9-Phenylnonanoyl-L-homoserine Lactone (9a).** RP-HPLC: t_r = 23.69 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.257 (d, 1H, NH, J = 8.1 Hz), 7.290−7.126 (m, 5H, Ar−H), 4.552−4.467 (m, 1H), 4.333 and 4.328 (dt, 1H, J = 8.8 Hz), 4.230−4.155 (m, 1H), 2.557 (t, 2H, J = 7.7 Hz), 2.412−2.313 (m, 1H), 2.181−2.055 (m, 1H), 2.089 (t, 2H, J = 7.5 Hz), 1.600−1.435 (m, 4H), 1.334−1.209 (br m, 8H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{19}H_{27}NNaO_3$, 340.2; found, 340.3.

***N*-10-Phenyldecanoyl-L-homoserine Lactone (10a).** RP-HPLC: t_r = 25.39 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.267 (d, 1H, NH, J = 8.0 Hz), 7.292−7.125 (m, 5H, Ar−H), 4.556−4.470 (m, 1H), 4.333 and 4.328 (dt, 1H, J = 8.8 Hz), 4.233−4.152 (m, 1H), 2.556 (t, 2H, J = 7.8 Hz), 2.422−2.315 (m, 1H), 2.185−2.056 (m, 1H), 2.089 (t, 2H, J = 7.4 Hz), 1.602−1.436 (m, 4H), 1.256 (m, 10H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{20}H_{29}NNaO_3$, 354.2; found, 354.3.

***N*-11-Phenylundecanoyl-L-homoserine Lactone (11a).** RP-HPLC: t_r = 26.48 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.280−8.206 (m, 1H, NH), 7.303−7.125 (m, 5H, Ar−H), 4.549−4.459 (m, 1H), 4.361−4.290 (m, 1H), 4.231−4.147 (m, 1H), 2.411−2.317 (m, 1H), 2.188−1.989 (br m, 3H), 1.676−1.378 (br m, 6H), 1.245−1.131 (br m, 12H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{21}H_{31}NNaO_3$, 368.2; found, 368.3.

***N*-12-Phenyldodecanoyl-L-homoserine Lactone (12a).** RP-HPLC: t_r = 28.67 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.267 (d, 1H, NH, J = 8.1 Hz), 7.300−7.125 (m, 5H, Ar−H), 4.560−4.468 (m, 1H), 4.333 and 4.328 (dt, 1H, J = 8.8 Hz), 4.236−4.154 (m, 1H), 2.554 (t, 2H, J = 7.6 Hz), 2.418−2.328 (m, 1H), 2.193−2.060 (m, 1H), 2.090 (t, 2H, J = 7.45 Hz), 1.600−1.440 (m, 4H), 1.300−1.208 (m, 14H). ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{22}H_{33}NNaO_3$, 382.2; found, 382.3.

Synthesis of *N*-(3-*oxo*-Phenacyl)-L-homoserine Lactones: General Method B. PS-carbodiimide resin (0.504 mmol, 1.26 mmol/g, Argonaut Technologies), phenacyl carboxylic acid (0.252 mmol), 2,2-dimethyl-1,3-dioxane-4,6-dione (0.252 mmol), 4,4-dimethylaminopyridine (0.265 mmol), and DCM (5.0 mL) were added to a 10 mL reactivial (Pierce). After stirring at room temperature overnight, PS-carbodiimide resin was removed by vacuum filtration and solvents were removed in vacuo. The resulting residue was dissolved in EtOAc (10 mL) and washed with 0.1 N HCl (10 mL). The organic layer was washed with H_2O (3 × 20 mL) and dried ($MgSO_4$) to yield a yellow oil. Without further purification, the yellow oil product was dissolved in CH_3CN (5 mL) and transferred to a 10 mL reactivial (Pierce) with triethylamine (0.237 mmol) and: (1) (*S*)-(−)-α-amino-γ-butyrolactone HBr (0.198 mmol) for L-isomer or (2) (*R*)-(+)-α-amino-γ-butyrolactone HCl (0.198 mmol) for D-isomer. After microwave irradiation (100 W, 2 × 10 min), solvents were removed in vacuo. The resulting residue was redissolved in EtOAc (10 mL + 0.5 mL H_2O) and washed sequentially with $NaHCO_3$ (1 × 10 mL), 1.0 M $KHSO_4$ (1 × 10 mL), and sat. NaCl (1 × 10 mL). The organic layer was dried ($MgSO_4$), concentrated and purified by gradient flash chromatography (20 g Isolute Flash SI II silica column cartridge, 0−80% EtOAc in Hex, 10 mL/min, 47 min). Purity was confirmed by HPLC (linear gradient, 30 min, 10−100% CH_3CN in H_2O + 0.1% TFA).

***N*-(3-*oxo*-3-Phenylpropanoyl)-L-homoserine Lactone (3b).** RP-HPLC: t_r = 11.17 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.687 (d, 1H, NH, J = 8.0 Hz), 7.677−7.473 (m, 5H, Ar−H), 4.625−4.538 (m, 1H), 4.348 and 4.344 (dt, 1H, J = 8.9 Hz), 4.249−4.171 (m, 1H), 3.958 (s, 2H), 2.480−2.393 (m, 1H), 2.208−2.086 (m, 1H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{13}H_{13}NNaO_4$, 270.1; found, 270.1.

***N*-(3-*oxo*-4-Phenylbutanoyl)-L-homoserine Lactone (4b).**[13] RP-HPLC: t_r = 12.60 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.602 (d, 1H, NH, J = 7.5 Hz), 7.335-7.147 (m, 5H, Ar−H), 4.590−4.533 (m, 1H), 4.346 and 4.343 (dt, 1H, J = 8.9 Hz), 4.233−4.179 (m, 1H), 3.853 (s, 2H), 3.427 (s, 2H), 2.446−2.386 (m, 1H), 2.190−2.106 (m, 1H). ESI-MS (m/z): $[M + Na]^+$ calcd for $C_{14}H_{15}NNaO_4$, 284.1; found, 284.2; $[2 M + Na]^+$ calcd for $C_{28}H_{30}N_2NaO_8$, 545.2; found, 545.1.

***N*-(3-*oxo*-5-Phenylpentanoyl)-L-homoserine Lactone (5b).** RP-HPLC: t_r = 14.71 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.584 (d, 1H, NH, J = 7.8 Hz), 7.273−7.142 (m, 5H, Ar−H), 4.574−4.514 (m, 1H), 4.338 and 4.335 (dt, 1H, J = 8.8 Hz), 4.227−4.170 (m, 1H), 3.368 (s, 2H), 2.859 (t, 2H, J = 7.65 Hz), 2.767 (t, 2H, J = 7.65 Hz), 2.439−2.377 (m, 1H), 2.178−2.095 (m, 1H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{15}H_{17}NNaO_4$, 298.1; found, 298.1; $[2M + Na]^+$ calcd for $C_{30}H_{34}N_2NaO_8$, 573.2; found, 573.0.

***N*-(3-*oxo*-6-Phenylhexanoyl)-L-homoserine Lactone (6b).** RP-HPLC: t_r = 16.17 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.559 (d, 1H, NH, J = 7.9 Hz) 7.290−7.148 (m, 5H, Ar−H), 4.569−4.513 (m, 1H), 4.337 and 4.335 (dt, 1H, J = 8.8 Hz), 4.227−4.174 (m, 1H), 3.336 (d, 2H, J = 1.1 Hz), 2.550−2.506 (m, 4H), 2.431−2.374 (m, 1H), 2.168−2.086 (m, 1H), 1.782−1.720 (m, 2H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{16}H_{19}NNaO_4$, 312.1; found, 312.1; $[2M + Na]^+$ calcd for $C_{32}H_{38}N_2NaO_8$, 601.3; found, 601.0.

***N*-(3-*oxo*-7-Phenylheptanoyl)-L-homoserine Lactone (7b).** RP-HPLC: t_r = 17.82 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.555 (d, 1H, NH, J = 7.8 Hz), 7.277−7.131 (m, 5H, Ar−H), 4.567−4.511 (m, 1H), 4.334 and 4.331 (dt, 1H, J = 8.8 Hz), 4.222−4.170 (m, 1H), 3.223 (d, 2H, J = 1.6 Hz), 2.566−2.526 (m, 4H), 2.429−2.374 (m, 1H), 2.175−2.096 (m, 1H), 1.550−1.434 (m, 4H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{17}H_{21}NNaO_4$, 326.1; found, 326.2; $[2M + Na]^+$ calcd for $C_{34}H_{42}N_2NaO_8$, 629.3; found, 629.1.

***N*-(3-*oxo*-8-Phenyloctanoyl)-L-homoserine Lactone (8b).** RP-HPLC: t_r = 19.46 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.554 (d, 1H, NH, J = 7.7 Hz), 7.269−7.129 (m, 5H, Ar−H), 4.565−4.510 (m, 1H), 4.334 and 4.332 (dt, 1H, J = 9.0 Hz), 4.222−4.171 (m, 1H), 3.322 (d, 2H, J = 1.8 Hz), 2.541 (t, 2H, J = 7.7 Hz), 2.502 (t, 2H, J = 7.3 Hz), 2.429−2.373 (m, 1H), 2.174−2.092 (m, 1H), 1.571−1.508 (m, 2H), 1.507−1.448 (m, 2H), 1.272−1.211 (m, 2H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{18}H_{23}NNaO_4$, 340.2; found, 340.2.

***N*-(3-*oxo*-9-Phenylnonanoyl)-L-homoserine Lactone (9b).** RP-HPLC: t_r = 21.09 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.553 (d, 1H, NH, J = 7.6 Hz), 7.271−7.129 (m, 5H, Ar−H), 4.568−4.513 (m, 1H), 4.336 and 4.333 (dt, 1H, J = 8.8 Hz), 4.224−4.172 (m, 1H), 3.323 (d, 2H, J = 1.4 Hz), 2.544 (t, 2H, J = 7.7 Hz), 2.491 (t, 2H, J = 7.2 Hz), 2.433−2.377 (m, 1H), 2.177−2.098 (m, 1H), 1.570−1.506 (m, 2H), 1.469−1.411 (m, 2H), 1.283−1.218 (m, 4H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{19}H_{25}NNaO_4$, 354.2; found, 354.3; $[2M + Na]^+$ calcd for $C_{38}H_{50}N_2NaO_8$, 685.3; found, 685.0.

***N*-(3-*oxo*-10-Phenyldecanoyl)-L-homoserine Lactone (10b).** RP-HPLC: t_r = 22.73 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.552 (d, 1H, NH, J = 7.7 Hz), 7.268−7.127 (m, 5H, Ar−H), 4.564−4.510 (m, 1H), 4.334 and 4.331 (dt, 1H, J = 8.9 Hz), 4.221−4.171 (m, 1H), 3.319 (d, 2H, J = 2.0 Hz), 2.546 (t, 2H, J = 7.8 Hz), 2.506−2.475 (m, 2H and DMSO), 2.432−2.375 (m, 1H), 2.117−2.097 (m, 1H), 1.572−1.507 (m, 2H), 1.462−1.400 (m, 2H), 1.292−1.171 (m, 6H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{20}H_{27}NNaO_4$, 368.2; found, 368.4; $[2M + Na]^+$ calcd for $C_{40}H_{54}N_2NaO_8$, 713.4; found, 713.0.

***N*-(3-*oxo*-11-Phenylundecanoyl)-L-homoserine Lactone (11b).** RP-HPLC: t_r = 24.37 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.554 (d, 1H, NH, J = 7.9 Hz), 7.287−7.127 (m, 5H, Ar−H), 4.588−4.506 (m, 1H), 4.344 and 4.340 (dt, 1H, J = 9.0 Hz), 4.243−4.168 (m, 1H), 3.328 (s, 2H), 2.555 (t, 2H, J = 7.9 Hz), 2.525−2.472 (m, 2H and DMSO), 2.456−2.374 (m, 1H), 2.204−2.075 (m, 1H), 1.600−1.506 (m, 2H), 1.488−1.396 (m, 2H), 1.300−1.200 (br m, 8H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{21}H_{29}NNaO_4$, 382.2; found, 382.2.

***N*-(3-*oxo*-12-Phenyldodecanoyl)-L-homoserine Lactone (12b).** RP-HPLC: t_r = 26.01 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.587 (d, 1H, NH, J = 7.7 Hz), 7.289−7.122 (m, 5H, Ar−H), 4.592−4.506 (m, 1H), 4.375−4.307 (m, 1H), 4.242−4.160 (m, 1H), 3.329 (s, 2H), 2.552 (t, 2H, J = 7.6 Hz), 2.523−2.467 (m, 2H and DMSO), 2.452−2.362 (m, 1H), 2.205−2.068 (m, 1H), 1.597−1.501 (m, 2H), 1.488−1.383 (m, 2H), 1.322−1.171 (m, 10H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{22}H_{31}NNaO_4$, 396.2; found, 396.3.

***N*-(3-*oxo*-12-Phenyldodecanoyl)-D-homoserine Lactone (D-12b).** RP-HPLC: t_r = 26.04 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.580 (d, 1H, NH, J = 8.0 Hz), 7.285−7.128 (m, 5H, Ar−H), 4.591−4.510 (m, 1H), 4.345 and 4.340 (dt, 1H, J = 7.7 Hz), 4.241−4.164 (m, 1H), 3.329 (s, 2H), 2.553 (t, 2H, J = 7.6 Hz), 2.521−2.475 (m, 2H and DMSO), 2.451−2.371 (m, 1H), 2.200−2.074 (m, 1H), 1.591−1.504 (m, 2H), 1.474−1.393 (m, 2H), 1.310−1.177 (m, 10H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{22}H_{31}NNaO_4$, 396.2; found, 396.1.

***N*-(3-*oxo*-13-Phenyltridecanoyl)-L-homoserine Lactone (13).** RP-HPLC: t_r = 27.07 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.573−8.510 (m, 1H, NH), 7.292−7.111 (m, 5H, Ar−H), 4.576−4.483 (m, 1H), 4.363−4.297 (m, 1H), 4.234−4.153 (m, 1H), 3.313 (s, 2H), 2.507−2.335 (m, 5H), 2.192−2.066 (m, 1H), 1.537−1.326 (m, 4H), 1.268−0.956 (m, 12H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{23}H_{33}NNaO_4$, 410.2; found, 410.4.

***N*-(3-*oxo*-14-Phenyltetradecanoyl)-L-homoserine Lactone (14).** RP-HPLC: t_r = 29.16 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.559 (d, 1H, NH, J = 7.4 Hz), 7.280−7.136 (m, 5H, Ar−H), 4.576−4.517 (m, 1H), 4.344 (t, 1H, J = 8.9 Hz), 4.236−4.176 (m, 1H), 3.330 (s, 2H), 2.574−2.535 (m, 4H), 2.444−2.375 (m, 1H), 2.187−2.107 (m, 1H), 1.580−1.520 (m, 2H), 1.498−1.412 (m, 2H), 1.295−1.203 (m, 14H). ESI-MS m/z: $[M + Na]^+$ calcd for $C_{24}H_{35}NNaO_4$, 424.3; found, 424.3. $[2M + H]^+$ calcd for $C_{48}H_{71}N_2O_8$, 803.5; found, 803.5.

Biological Evaluation: Cell Culture. Human colorectal carcinoma cell lines, H630 (parental) and H630-1 (5-fluorouracil resistant), and human prostate carcinoma cell line, PC3, were cultured in RPMI-1640 phenol-red free (PRF) media supplemented with 10% dialyzed fetal bovine serum (dFBS), without antibiotics. H630-1 cells were grown in media containing 1 μM 5-fluorouracil (5-FU) until 1 week prior to experimentation. All cells were maintained in a humidified incubator at 37 °C and 5% CO_2.

Growth Inhibition of Cancer Cell Lines. Growth inhibition was measured by the sulforhodamine B (SRB) assay in 96-well plate format adapted from methods previously described.[16] Briefly, the compounds were added to the wells of 96-well plates at 10× final concentration in EtOH (20 μL) in duplicate or quadruplicate. After overnight drying, H630, H630-1 and PC3 cells were seeded at 2×10^3, 4×10^3, and 2×10^3 cells/well, respectively, in RPMI-1640PRF supplemented with 10% dFBS. After 5 days, media was removed and cells were fixed with 10% trichloroacetic acid for ≥1 h at 4 °C. Plates were washed with shaking (dH_2O, 3 × 300 μL/well) and allowed to dry. After 15 min incubation with 0.4% SRB solution (100 μL/well), plates were washed with shaking (1% acetic acid, 4 × 300 μL/well) and allowed to dry. Protein-bound SRB dye was dissolved in 100 mM TRIS (100 μL/well) with shaking for 5 min and quantified by measurement of optical density at 570 nm (OD_{570}). IC_{50} values represent the average of three independent experiments and were determined from growth curves generated using Sigmaplot software.

Activation of Quorum Sensing. To assess the ability of PHLAs to activate quorum sensing, we used the strain *Pseudomonas aeruginosa* MW1 (*lasI*-, *rhlI*-) carrying pUM15, which contains an *rsaL::gfp* transcriptional fusion. The bacteria were grown in LB with 150 μg/mL of carbenicillin. Assays were similar to those previously described.[15] The reporter culture was inoculated from an overnight starter culture to an OD_{600} = 0.05 with shaking at 37 °C for 1−2 h. Bacterial cell cultures (0.5 mL) in midlogarithmic

growth were then added to test tubes containing OdDHL or PHLA to achieve a final concentration of 0.3 μM and 33 μM, respectively. OdDHL at 0.3 μM achieves half-maximal induction of *rsaL::gfp* fusion reporter. Tubes were incubated at 37 °C with shaking. After 5 h, fluorescence from 100 μL of sample was measured with an excitation wavelength of 485 nm and an emission wavelength of 535 nm using a Tecan Genios Pro Platereader. Results represent the average of three independent experiments.

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Supporting Information Available: HPLC and NMR spectra for PHLA library. This material is available free of charge via the Internet at http://pubs.acs.org.

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