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Discovery and characterization of pure RhlR antagonists against *Pseudomonas aeruginosa* infections

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ABSTRACT

Pseudomonas aeruginosa (P. aeruginosa) is an opportunistic human pathogen that forms biofilms and produces virulence factors *via* quorum sensing (QS). Blocking the QS system in *P. aeruginosa* is an excellent strategy to reduce biofilm formation and the production of virulence factors. RhlR plays an essential role in the QS system of *P. aeruginosa*. We synthesized 55 analogs based on the chemical structure of 4-gingerol and evaluated their RhlR inhibitory activities using cell-based reporter strain assay. Comprehensive structure-activity relationship studies identified the alkynyl ketone **30** as the most potent RhlR antagonist. This compound displayed selective RhlR antagonism over LasR and PqsR, strong inhibition of biofilm formation, and reduced production of virulence factors in *P. aeruginosa*. Furthermore, the survival rate of *Tenebrio molitor* larvae treated with **30** *in vivo* greatly improved. Therefore, compound **30**, a pure RhlR antagonist, can be utilized for developing QS-modulating molecules in the control of *P. aeruginosa* infections.

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a causative human pathogen that induces chronic disease in immune-compromised patients.[1] It induces infections in burn wound, cystic fibrosis, acute leukemia, organ transplantation, and intravenous-drug addiction patients.[2] The World Health Organization (WHO) categorizes it as a critical priority pathogen that requires considerable attention.[3] *P. aeruginosa* is resistant to conventional antibiotic treatments mostly due to the formation of thick biofilm.[4] It can colonize on various surfaces by forming biofilms in which bacterial cells embedded within self-produced exopolysaccharides.[5] Biofilms are widely found in medical, dental, agricultural, industrial, and environmental settings.[6] In particular, biofilms in medical settings are associated with about 80% of bacterial infections in humans and increased antibiotic resistance.[7]

Quorum sensing (QS) is a bacterial cell-to-cell communication process that occurs *via* chemical signal molecules and allows bacteria to share information in response to environmental changes.[8] Once bacterial density reaches a certain threshold, signaling molecules (autoinducers) bind to their cognate receptor proteins and alter gene expression to regulate collective behaviors.[9] *P. aeruginosa* possesses three major QS systems (*las*, *rhl*, and *pqs*) that are tightly interconnected with each other. Gram-negative bacteria including *P. aeruginosa* use *N*-acyl-L-homoserine lactones (AHLs) as autoinducers of QS.[10] Typically, AHLs are produced by LuxI-type synthases (e.g., LasI and RhlI) and recognized by cytoplasmic LuxR-type receptors (e.g., LasR and RhlR). LasI and RhlI produce *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (OdDHL, **1a**) and *N*-butyryl-L-homoserine lactone (BHL, **1b**) as autoinducers, respectively (Figure 1).[11] In addition to these systems, *pqs* circuit is the third system regulated via *Pseudomonas* quinolone signal (PQS) recognized by PqsR. These three systems are hierarchically controlled. The *las* system activates *rhl* and *pqs* systems,

and the *rhl* system represses the *pqs* system. This interactive signal-network regulation of *P. aeruginosa* leads to biofilm formation, the production of virulence factors, and the modulation of host immune response.[12] Therefore, small molecules that modulate the recognition of autoinducers toward their cognate receptors have the potential to control virulence factors and biofilm formation of *P. aeruginosa*.[13]

Most QS inhibitors of *P. aeruginosa* focus on targeting LasR because it is located at the top of the *P. aeruginosa* QS network hierarchy.[14-16] However, even though RhlR also plays an important role in the QS process of *P. aeruginosa*, RhlR modulators have rarely been reported.[17-20] Up to date, few modulators for the interaction between BHL and RhlR as agonists or antagonists have been reported.[21-24] Blackwell and co-workers reported that BHL analogs with RhlR agonism activity reduced the production of *P. aeruginosa* virulence factors such as pyocyanin.[25, 26] They also reported mixed LasR/RhlR antagonists which increased pyocyanin production.[26] Bassler and co-workers recently conducted an experiment with BHL-independent and active *rhlR* mutants, which showed that RhlR drives biofilm formation and production of virulence factors in *P. aeruginosa*.[27]

Previously, we reported that (*S*)-6-gingerol (**1c**, Figure 1) from ginger extracts is a moderate LasR antagonist of *P. aeruginosa*.[28] It reduced biofilm formation, production of virulence factors, and expression of QS-related genes in *P. aeruginosa*. We performed comprehensive structure-activity relationship (SAR) studies of gingerol analogs and identified the LasR antagonist that was more potent than (*S*)-6-gingerol.[29] Based on our previous studies, we hypothesized that gingerols with alkyl chain shorter than **1c** might bind to RhlR because of their structural similarity to BHL. We describe the design, synthesis, and biochemical characterization of gingerol analogs as pure RhlR antagonists. We have established SAR of gingerol analogs by extensively modifying their chemical structures. The

pure and potent RhlR antagonist which we discovered in this study can be utilized in the discovery of novel agents that inhibit biofilm formation and production of virulence factors, as well as in the elucidation of *rhl*-RhlR mechanism in QS network of *P. aeruginosa*.

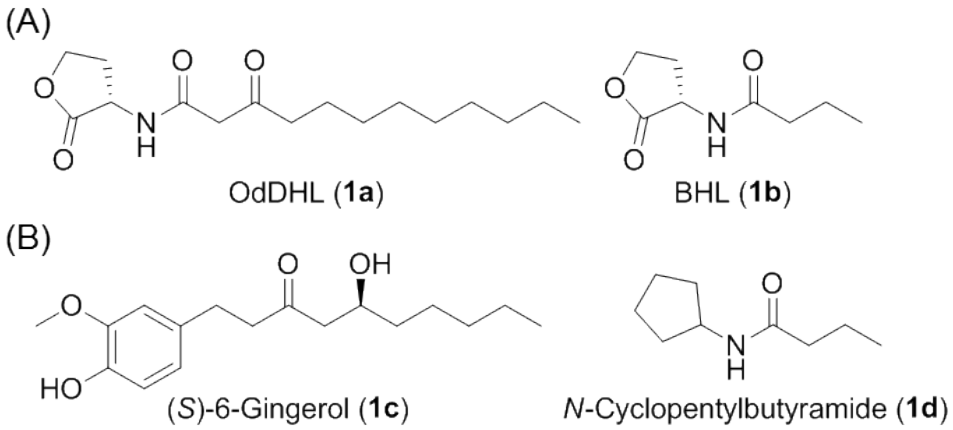


Figure 1. Structures of endogenous and synthetic molecules interacting with the QS receptor of *Pseudomonas aeruginosa.*

RESULTS AND DISCUSSION

As an attempt to discover pure RhlR antagonists, we first screened the relative RhlR activities of in-house gingerol analogs with various alkyl chain lengths, from 4-gingerol to 10-gingerol (See Supporting Scheme S1), which were previously reported.[29] We used DMSO as a negative control and (*S*)-6-gingerol (**1c**) and *N*-cyclopentylbutyramide (**1d**) (Figure 1) as positive controls in *in vitro* biological evaluation studies. As shown in Table 1, the more potent RhlR antagonism activities increased with shorter alkyl chain length of gingerols, suggesting that gingerols with the shorter alkyl chain have a higher affinity for RhlR. Interestingly, all tested gingerols showed very low RhlR agonistic activity (<4%). Compound **2** (4-gingerol) with the shortest alkyl chain in the series was the most potent with a relative RhlR activity of 54%. As the chemical structure of **2** is more similar to that of BHL than the others, it was assumed that it competed against BHL for binding to RhlR. Based on preliminary results, we used 4-gingerol as an initial hit compound for further structural modification.

Table 1. Relative RhlR activity (%) of the synthesized compounds.

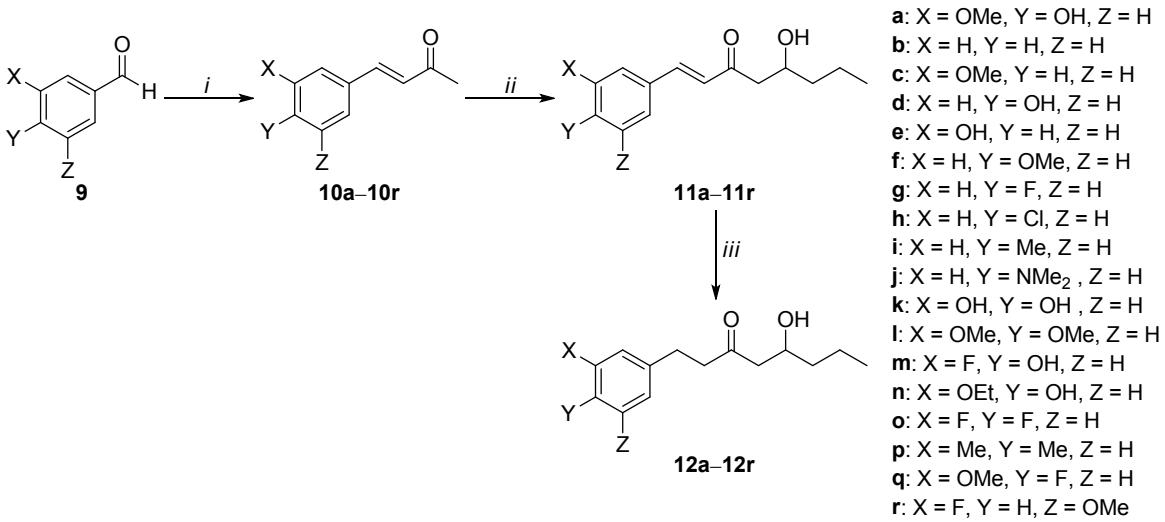
Compound[a]	Relative RhlR activity		Compound	Relative RhlR activity		Compound	Relative RhlR activity	
	Antagonsim[b]	Agonism[c]		Antagonsim	Agonism		Antagonsim	Agonism
1c	75**	3**	**11p**	72**	2**	**14b**	49**	2**
1d	54**	4**	**11q**	43**	1**	**15a**	55**	5**
2	54**	3**	**11r**	74**	3**	**15b**	41**	2**
3	62**	2**	**12a**	69**	4**	**16a**	61**	5**
4	76**	4**	**12b**	88*	4**	**21**	29**	3**
5	83**	3**	**12c**	90*	2**	**23a**	32**	3**
6	90*	2**	**12d**	64**	3**	**23b**	27**	3**
7	41**	2**	**12e**	81**	2**	**23c**	34**	3**
8	36**	2**	**12f**	71**	2**	**25**	79**	3**
11a	65**	4**	**12g**	51**	2**	**27**	45**	2**
11b	93*	4**	**12h**	69**	2**	**30**	12**	4**
11c	94	2**	**12i**	76**	1**	**31**	16**	4**

11d	51**	1**	**12j**	74**	1**	**32**	17**	3**
11e	78**	2**	**12k**	47**	2**	**33a**	32**	3**
11f	55**	1**	**12l**	94	1**	**33b**	25**	3**
11g	33**	1**	**12m**	73**	1**	**33c**	38**	3**
11h	64**	1**	**12n**	98	1**	**34a**	71**	4**
11i	65**	2**	**12o**	49**	1**	**34b**	67**	3**
11j	53**	2**	**12p**	82**	3**	**34c**	64**	2**
11k	43**	3**	**12q**	79**	1**	**35a**	74**	3**
11l	69**	1**	**12r**	85**	3**	**35b**	72**	3**
11m	60**	2**	**13a**	51**	4**	**35c**	67**	4**
11n	91	1**	**13b**	41**	2**			
11o	20**	2**	**14a**	55**	4**			

[a] DMSO (negative control) and **1c** and **1d** (positive controls) were used. [b] RhlR antagonism activity of the compound (100 μM) in the presence of **1b** (10 μM). [c] RhlR agonism activity of the compound (100 μM). (**) $P<0.005$ and (*) $P<0.05$ as compared with the control.

To establish SAR of 4-gingerol for RhlR antagonism, we performed structural modification as follows: 1) variation of the substituents in the phenyl ring, 2) introduction of double or triple bond between the phenyl ring and the carbonyl group to increase rotational rigidity, 3) removal of β-hydroxyl group, and 4) change of absolute configuration. First, we synthesized 34 derivatives to determine the effect of the substituents in the phenyl ring on RhlR antagonism. 3'-OMe and 4'-OH groups of the phenyl ring in 4-gingerol were replaced with diverse functional groups (-F, -Cl, -OH, -OMe, -OEt, -Me, and $-N(CH_3)_2$). Scheme 1 describes the synthesis of 4-gingerol derivatives with various substituents of the phenyl ring. Compounds **10a**–**10r** were synthesized from commercial benzaldehydes by treating 10% NaOH in acetone and ethanol (or water) at 25 ℃. Reaction of **10a**–**10r** with diisopropylamide (LDA) at –78 ℃, followed by addition of *n*-butanal afforded compounds **11a**–**11r** in 24–60% yield. Low-to-moderate yield in this step might be because of side reactions such as elimination or dimer

formation. Compounds **12a**–**12r** were obtained in 40–93% yield by reducing **11a**–**11r** with hydrogen gas in the presence of 10% Pd/C.



Scheme 1. Synthesis of 4-gingerol derivatives with the variation of phenyl ring. Reagents and conditions: (*i*) Acetone, EtOH (or water), 10% NaOH, rt, 2 to 24 h, 32%–quantitative yield; (*ii*) LDA 1.0 M in THF/hexanes, *n*-butanal, THF, –78 ℃, 2 to 12 h, 24–60% yield; (*iii*) H_2 gas, 10% Pd/C, MeOH, rt, 2 h, 43–93% yield.

As summarized in Table 1, compound **11b** without any substituent in the phenyl region, completely lost its RhlR antagonistic property with a relative RhlR activity of 93%, indicating that a polar functional group in the phenyl ring is required for binding to RhlR. To assess the necessity of 4'-OH and 3'-OMe groups in the phenyl ring for RhlR antagonism, we removed the -OH group at 4'-position (**11c**) and the -OMe group at 3'-position (**11d**). As shown in Table 1, compound **11c** displayed a reduced ability to inhibit RhlR (relative 94% RhlR activity). However, compound **11d** showed slightly stronger RhlR antagonism than **11a** (51% vs. 65%). However, compound **11f** with an -OMe group at 4'-position was less potent than **11d**, implying that the presence of the polar group at 4'-position is favorable for RhlR-binding affinity. In the

case of the 3'-position, compound **11e** with an -OH group only showed stronger RhlR antagonism than compound **11c** with an -OMe group only (78% vs. 94%).

To expand the initial SAR of 4-gingerol, we synthesized a variety of mono-substituted (**11g**–**11j**) and di-substituted (**11k**–**11r**) analogs. Among the mono-substituted compounds, **11g** with 4'-F was the most potent with a relative RhlR activity of 33%. Interestingly, compound **11j** (53%) with a bulky 4'-$N(CH_3)_2$ was more potent than **11a**, while **11h** (64%) with 4'-Cl and **11i** (65%) with 4'-Me were comparable to **11a**. A similar trend was observed with the di-substituted compounds. Compound **11o** with 3',4'-di-F was the most potent in this series (relative RhlR activity, 20%). Notably, these trends indicated that the F-substitution in the phenyl ring is preferred for the structural modification of the phenyl ring. Compound **11q** (43%) with 3'-OMe and 4'-F was more active than **11a** with 3'-OMe and 4'-OH, suggesting that the F group at 4'-position can replace the OH group of 4-gingerol. However, compound **11r** (74%) with 3'-F and 5'-OMe was less potent than **11a**, confirming the necessity of the small and polar group (i.e., -F and -OH) at 4'-position. As expected, the hydrophobic -Me group (**11p**, 72%) at both 3'- and 4'-position showed decreased RhlR inhibitory activity. Compound **11n** with 3'-OEt and 4'-OH dramatically decreased the antagonistic activity of RhlR (91%), implying that the region of RhlR which interacts with the 3'-position of 4-gingerol was highly sensitive to the substituent size. SAR results indicated that the 4'-position is related to hydrogen-bonding interaction with RhlR, while the 3'-position is sensitive to the bulkiness of the substituent.

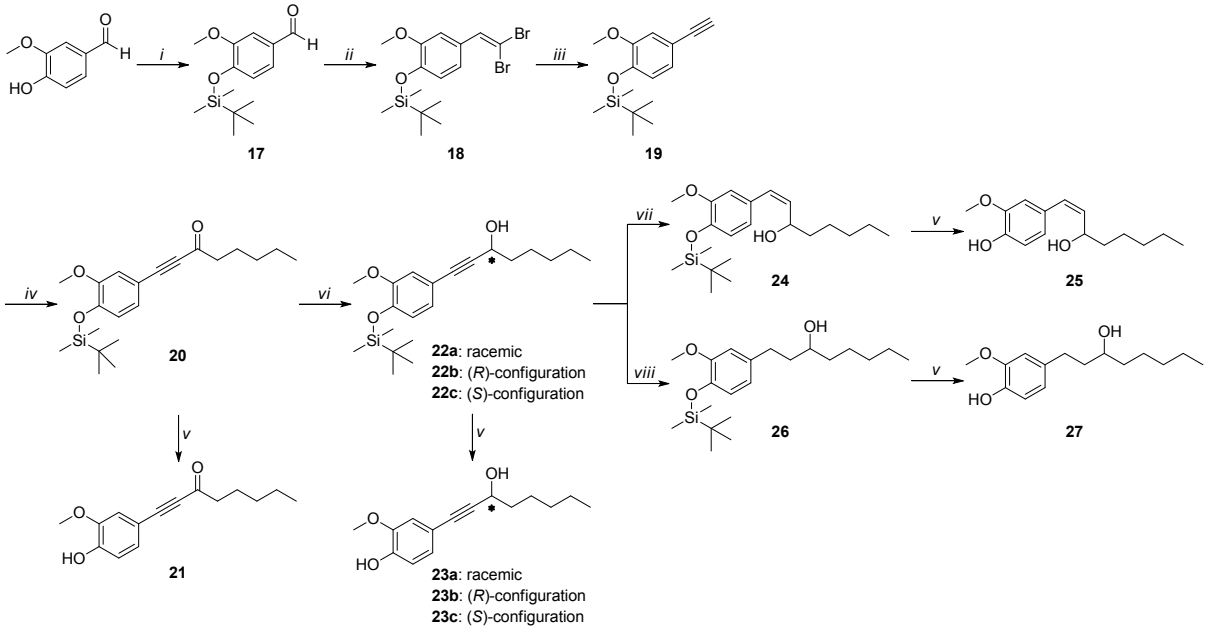
The effect of rotational rigidity on RhlR antagonism was evaluated by comparing the compounds (**11a**–**11r**) with a double bond between the phenyl ring and the carbonyl group with the corresponding compounds (**12a**–**12r**) with a single bond. As shown in Table 1, the compounds with a double bond (**11a**–**11r**) displayed stronger RhlR antagonism than those with a single bond (**12a**–**12r**), irrespective of the substituents in the phenyl ring. This result implied

that the restricted rotational flexibility between the phenyl ring and the carbonyl group significantly increased binding affinity for RhlR. Overall, the double-bond compound **11o**, substituted with the F group at both 3'- and 4'-position, showed the most potent RhlR antagonism with low RhlR agonist activity (2%) among the compounds with variations in the phenyl ring.

The synthesis of 4-gingerol derivatives with variations in the middle section is outlined in Scheme S1 (See supporting information). Compounds (**13a**–**13b**) without β-hydroxyl group were synthesized by reacting 2-heptanone with vanillin or 3,4-difluorobenzaldehyde under basic conditions at 25 ℃ for 72 h in 30–50% yield. The α,β-unsaturated carbonyl group of **13a**–**13b** was reduced by two different reagents: 1) H_2 and Pd/C, and 2) $NaBH_4$. The double bond was reduced to the single bond via catalytic hydrogenation, providing compounds **14a**–**14b** in ~76% yield. The carbonyl group was reduced to the secondary alcohol by treatment with $NaBH_4$ to afford compounds **15a** and **15b** in 94% and 90% yield, respectively. Compound **15a** was reduced to the single-bond compound **16a** in 60% yield. As shown in Table 1, compound **13b** (41%) without the β-hydroxyl group was more potent than **11a** (65%), indicating that the β-hydroxyl group was not essential for binding to RhlR. The α,β-unsaturated carbonyl analog (**13b**) was more potent than the corresponding α,β-saturated one (**14b**). The reduction of the α,β-unsaturated carbonyl group to alcohol (**15b**, 41%) maintained the RhlR-binding affinity. However, the RhlR antagonistic activities of 3,4-difluorophenyl ring analogs (**13a**–**15a**) were almost similar.

Gingerol derivatives with a triple bond in the middle section was prepared by applying synthetic route described in Scheme 2. The phenolic OH of vanillin was protected with the *tert*-butyldimethylsilyl (TBDMS) group, affording compound **17**. Reaction of **17** with CBr_4 and PPh_3 in dichloromethane provided the dibromo alkene **18** in 98% yield. Elimination and

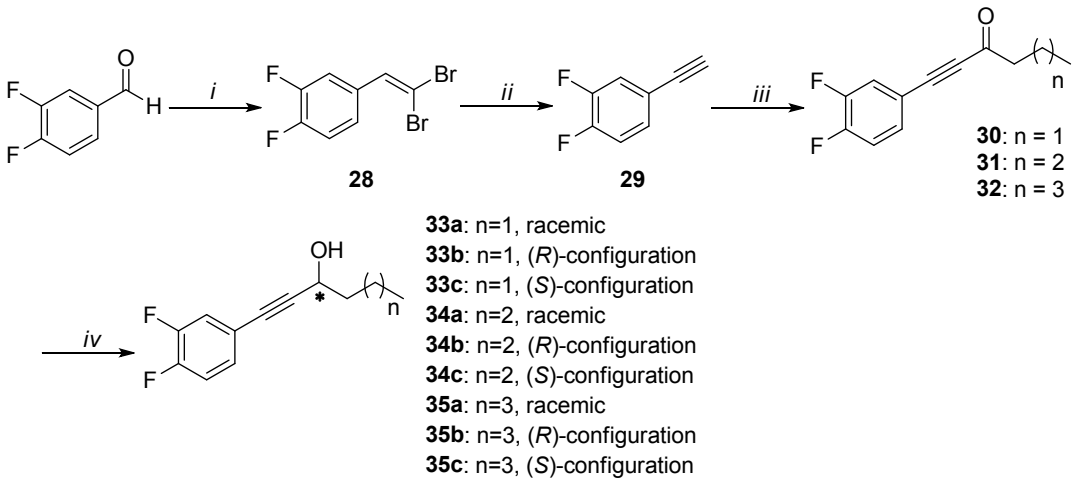
lithiation of **18** in the presence of 3 equivalents of *n*-BuLi afforded the terminal alkyne **19** in 96% yield.[30] Compound **20** was obtained by reacting **19** with Weinreb amide. The alkynyl ketone **21** was prepared from **20** by using tetrabutylammoniumfluoride (TBAF) in 94% yield. Enantiomerically-enriched alkynyl alcohol analogs (**23b**–**23c**) were prepared from **20** by using chiral catalysts such as RuCl[(*R*,*R*)-TsDPEN(mesitylene)] or RuCl[(*S*,*S*)-TsDPEN(mesitylene)][31], followed by desilylation with TBAF. Compound **23a**, a racemic mixture, was obtained from **20** by treatment of $NaBH_4$ and subsequent desilylation. The alkynyl alcohols (**23a**–**23c**) were analyzed by chiral reversed-phase (RP)-HPLC. As compound **23a** was a racemic mixture, two distinct peaks were observed at 9.91 min and 12.58 min in chiral HPLC with a 1:1 ratio (See supporting information). Compound **23b** with (*R*)-configuration was eluted at 12.54 min while compound **23c** with (*S*)-configuration was eluted at 9.88 min with *ee* values >99% (Supporting Figure S1). Z-selective reduction of the alkyne (**22a**) to *cis*-isomer (**25**) was accomplished by the treatment of Lindlar catalyst and 1,4-benzoquinone under an atmosphere of hydrogen, followed by desilylation with TBAF.[32] The alkanyl alcohol **27** was obtained from **22a** in 94% yield via catalytic hydrogenation and subsequent desilylation.



Scheme 2. Synthesis of 4-gingerol derivatives with the variation of rotational rigidity and absolute configuration. Reagents and conditions: (*i*) *tert*-Butyldimethylsilyl chloride, imidazole, CH_2Cl_2, rt, 18 h, 94% yield; (*ii*) PPh_3, CBr_4, CH_2Cl_2, rt, 3 h, 98% yield; (*iii*) *n*-BuLi 1.6 M in hexanes (3.0 eq.), THF, rt, 2 h, 96% yield; (*iv*) *n*-BuLi 1.6 M in hexanes (1.1 eq.), *N*-methoxy-*N*-methylhexanamide, THF, –78 ℃ to rt, 16 h, 75% yield; (*v*) *n*-Bu_4NF, 0.1 M in THF, rt, 1 h, 94% yield (for **21**), 94% yield (for **23a**), 94% yield (for **23b**), 93% yield (for **23c**), 70% yield (for **25**), and 94% yield (for **27**); (*vi*) $NaBH_4$, MeOH, rt, 1 h, 77% yield (for **22a**), RuCl[(*R*,*R*)-TsDPEN(mesitylene)], KOH, 2-propanol, 4 h, 88% yield (for **22b**), and RuCl[(*S*,*S*)-TsDPEN(mesitylene)], KOH, 2-propanol, 4 h, 68% yield (for **22c**); (*vii*) Lindlar cat., H_2 gas, 1,4-benzoquinone, MeOH, 0 ℃, 1 h (from **22a**); (*viii*) H_2 gas, 10% Pd/C, MeOH, rt, 2 h, 94% yield (from **22a**).

The alkynyl ketone **21** displayed strong relative RhlR activity (29%) than the corresponding alkenyl ketone **13b** (41%) and alkanyl ketone **14b** (49%) (Table 1). Similarly, the alkynyl alcohol **23a** (32%) was also more potent than the alkene **15b** (41%) and the alkane **27** (45%). The relative RhlR activities of the alkynyl ketone (**21**) or the alkyny alcohol compounds (**23a**–**23c**) were <34%. They were more potent than the reported RhlR antagonist (**1d**, 54%). Regarding the effect of absolute configuration on RhlR affinity, the (*R*)-isomer **23b** (27%) was stronger than the corresponding (*S*)-isomer **23c** (34%) and the racemate **23a** (32%). The (*Z*)-alkenyl alcohol **25** with a relative RhlR activity of 79% was much weaker than the (*E*)-alkenyl alcohol **15b** (41%), implying that the *cis*-isomer may not be properly located in the RhlR active site. Overall, SAR data indicated that the carbonyl group or the hydroxyl group at the γ-position from the phenyl group is important for binding to RhlR.

Based on the SAR studies of 4-gingerol analogs, we synthesized 3,4-difluorophenyl derivatives which showed the most potent RhlR-binding activity in the variation of the phenyl ring. As described in Scheme 3, the alkynyl ketones (**30**–**32**) and the alkynyl alcohols (**33a**–**33c**, **34a**–**34c**, and **35a**–**35c**) were prepared from commercial 3,4-difluorobenzaldehyde by applying the same synthetic strategy used for 4-gingerol derivatives. The purity and *ee* ratio of the final compounds were analyzed using chiral HPLC (See Supporting Figure S2). The alkynyl ketones (**30**–**32)** were more potent than the corresponding alkene and alkane compounds, with relative RhlR activities <17% at 100 μM. In addition, the (*R*)-stereoisomers (**33b**–**35b**) were also more active than the corresponding (*S*)-stereoisomers (**33c**–**35c**) or the racemates (**33a**–**35a**). Furthermore, the compounds with the shorter alkyl chain length (**30** and **33a**–**33c**) were stronger than the corresponding ones with the longer alkyl chain lengths (**31**, **32**, **34a**–**34c**, and **35a**–**35c**). The alkynyl ketone with the *n*-propyl group (**30**) displayed the most potent activity in this series, with a relative RhlR activity of 12%, which is a much stronger activity profile than the reported antagonist **1d** (54%).



Scheme 3. Synthesis of 3,4-difluorophenyl derivatives. Reagents and conditions: (*i*) PPh_3, CBr_4, CH_2Cl_2, rt, 3 h, 97% yield; (*ii*) *n*-BuLi 1.6 M in hexanes (3.0 eq.), THF, rt, 2 h; (*iii*) *n*-BuLi 1.6 M in hexanes (1.1 eq.), appropriate Weinreb amides, THF, –78 ℃ to rt, 16 h, 72%

yield (for **30**), 88% yield (for **31**), and 72% yield (for **32**); (*iv*) $NaBH_4$, MeOH, rt, 1 h (for **33a**–**35a**), 88% yield (for **33a**), 80% yield (for **34a**), and 86% yield (for **35a**), RuCl[(*R*,*R*)-TsDPEN(mesitylene)], KOH, 2-propanol, 4 h (for **33b**–**35b**), 86% yield (for **33b**), 82% yield (for **34b**), and 80% yield (for **35b**), and RuCl[(*S*,*S*)-TsDPEN(mesitylene)], KOH, 2-propanol, 4 h (for **33c**-**35c**), 86% yield (for **33c**), 82% yield (for **34c**), and 82% yield (for **35c**).

To elucidate the binding mode of compound **30** in RhlR, we performed *in silico* molecular docking studies of **30** and **1d** with a RhlR homology model. Compound **30** interacted with RhlR via a π-π stacking interaction with Tyr72 and a hydrogen-bond with Trp 68, while **1d** made hydrogen-bonding interactions with Asp81, Thr121, and Ser135 (Figure 2). Trp 68 was reported as one of key amino acids in a BHL-independent and active *rhlR* mutants.[26] As the alkyl chain length gets longer (**30** vs. **31** and **32**), the phenyl moiety made weaker π-π stacking interactions with Tyr72 (See Supporting Figure S3), elucidating the importance of the π-π stacking interaction in 3,4-difluorophenyl analogs.

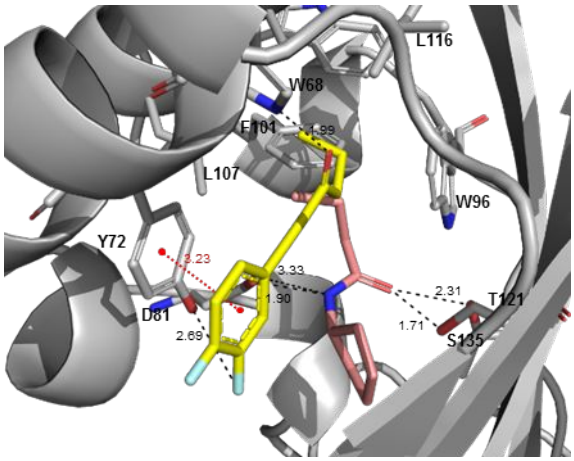
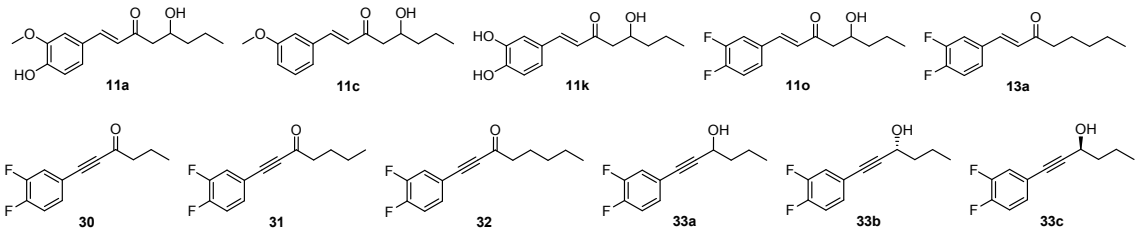


Figure 2. The docked poses of **30** (yellow) and **1d** (pink) with the RhlR homology model. Red and black dotted lines represent π-π stacking and hydrogen-bond interactions, respectively.

Table 2. *In vitro* biological data of the representative compounds.



Compound[a]	Relative RhlR activity (%)				Relative biological activity (%)					
	Antagonism[b]		Agonism[c]		Biofilm formation[d]		Bacterial growth[e]		Rhamnolipid production[f]	
	10	100	100	1000	10	100	10	100	10	100
1c	86*	75**	3**	5**	86	71**	96*	101	76*	68*
1d	74*	54**	4**	37**	84	69**	95*	98	58**	45**
11a	73**	65**	4**	5**	82	65**	102	95	73*	57*
11c	89	94	2**	4**	88	73**	96	95*	91	83
11k	60**	43**	3**	5**	84*	72**	99	104*	56*	50*
11o	60**	20**	2**	5**	72*	53**	101	105*	63*	50**
13a	53**	51**	4**	3**	80*	61**	103	103	64**	62*
30	31**	12**	4**	4**	68*	47**	103	99	57**	42*
31	29**	16**	4**	6**	75*	55**	100	89**	59**	49**
32	39**	17**	3**	5**	71*	56**	100	88**	60**	58**
33a	45**	32**	3**	5**	73**	60**	100	104*	73*	49**
33b	45**	25**	3**	5**	74*	60**	98	102	60*	48**
33c	59**	38**	3**	5**	79*	64**	98	99	59*	52**

[a] DMSO (negative control) and **1c** and **1d** (positive controls) were used. [b] RhlR antagonism activity of the compound (10 or 100 μM) in the presence of **1b** (10 μM). [c] RhlR agonism activity of the compound (100 or 1000 μM). [d] Biofilm formed by *P. aeruginosa* at 10 or 100 μM compound in static conditions. [e] Growth of *P. aeruginosa* at 10 or 100 μM compound for 24 h. [f] Rhamnolipid produced by *P. aeruginosa* at 10 or 100 μM compound. (**) $P<0.005$ and (*) $P<0.05$ as compared with the control.

To validate the SAR results and compare the activities under the same experimental conditions, we selected 11 representative compounds (**11a**, **11c**, **11k**, **11o**, **13a**, **30**, **31**, **32**, **33a**,

33b, and **33c**) and determined RhlR antagonism, RhlR agonism, static biofilm formation, bacterial growth, and rhamnolipid production (Table 2). The removal of the OH group at the 4'-position of the phenyl ring (**11c**) significantly reduced the RhlR antagonism activity profile. In contrast, replacement of -OMe at the 3'-position of the phenyl ring with -OH (**11k**) increased RhlR antagonism. Comparison of **11o** and **13a** confirmed that the β-hydroxyl group in the middle section is not essential for RhlR activity. The alkynyl ketone compounds (**30**–**32**) with restricted rotational flexibility exhibited increased RhlR activity. We also confirmed that the (*R*)-alkynol (**33b**) is a better RhlR antagonist than the racemate (**33a**) and the (*S*)-alkynol (**33c**). Next, we determined the RhlR agonism activity of the selected compounds. As summarized in Table 2, none of the synthesized compounds showed RhlR agonism activity even at 1000 μM. However, the reported RhlR antagonist **1d** exhibited RhlR agonism with a relative RhlR agonistic activity of 37% at 1000 μM. Compared with the control, compounds **11o**, **13a**, **30**, **31**, and **32** significantly inhibited biofilm formation at 100 μM. In particular, compound **30** showed approximately 47% relative biofilm formation of *P. aeruginosa*, which is much more potent than (*S*)-6-gingerol (**1c**) and the known RhlR antagonist (**1d**). Next, we measured the effect of bacterial growth inhibition by the compounds at 10 and 100 μM concentrations. Compounds **31** and **32** slightly inhibited bacterial growth at 100 μM. However, compound **30** had no effect on bacterial growth at the same concentration. Furthermore, we examined the production of rhamnolipid, a representative virulence factor in *P. aeruginosa*.[33] RhlR is known to directly regulate the expression of rhamnolipid production enzymes. As shown in Table 2, the most potent RhlR antagonist **30** (13% of relative RhlR activity) displayed the lowest rhamnolipid production (42%) among the selected compounds. Moreover, compound **30** showed stronger inhibition activities on biofilm formation and rhamnolipid production without inhibiting bacterial growth than the other compounds (**7**, **8**, **11d**, **11f**, **21**, **23a**, **23b**, and **23c**)

with high RhlR activity but not included in the representative set (See Supporting Information Table S1).

Next, we performed the comprehensive biological assays of the most potent RhlR antagonist (**30**) against *P. aeruginosa*, using **1c**, **1d**, and 4-gingerol (**12a**) as positive controls. First, the RhlR IC_{50} values of all four compounds were measured at different concentrations (0–100 μM). The IC_{50} of **1c**, **1d**, **12a**, and **30** were 945, 86, 218, and 26 μM, respectively (Figure 3A), confirming that compound **30** was the most potent RhlR antagonist. We also determined the agonist and antagonist activities of the compounds against LasR and PqsR as *las* and *pqs* systems are also involved in the QS processes of *P. aeruginosa.*[12] All four compounds showed no LasR- and PqsR-agonistic activity nor PqsR-antagonism activity at 0.1 and 10 μM, respectively (Figures 3B and 3C). However, (*S*)-6-gingerol (**1c**) displayed moderate LasR antagonism as previously reported.[28] 4-Gingerol (**12a**) exhibited no LasR antagonism, confirming the result of our previous study.[29] The most potent RhlR antagonist **30** showed high selectivity for RhlR over LasR and PqsR, indicating that it is a pure RhlR antagonist.

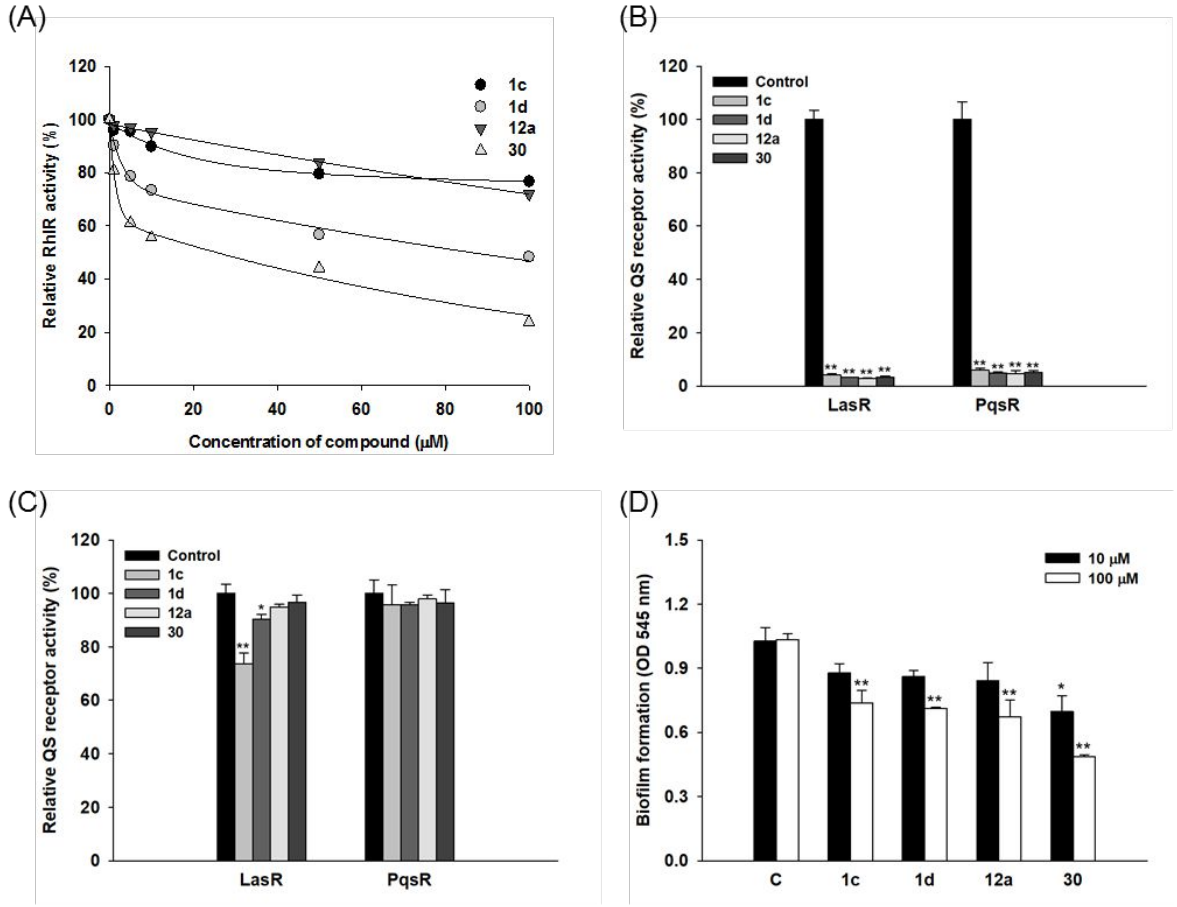


Figure 3. *In vitro* biological activities of compounds **1c**, **1d**, **12a**, and **30**. DMSO (negative control) and **1c**, **1d**, and **12a** (positive controls) were used. (A) Relative RhlR activity dose-response curves. (B) Relative QS receptor (LasR and PqsR) agonism activity at 0.1 and 10 μM of compound, respectively. (C) Relative QS receptor (LasR and PqsR) antagonism activity at 0.1 and 10 μM of compound, respectively. (D) Static biofilm formation of *P. aeruginosa* at 10 or 100 μM of compound. (**) $P<0.005$ and (*) $P<0.05$ as compared with the control.

As shown in Figure 3D, all four compounds strongly inhibited *P. aeruginosa* biofilm formation by 14–32% (10 μM) and 29–53% (100 μM), without affecting bacterial growth (See Supporting Figure S4). Dynamic biofilm inhibition by using a drip-flow reactor was analyzed at 48 h of the reactor operation. As shown in Figure 4A, control condition displayed typical

mushroom morphology with 44.15 $\mu m^3/\mu m^2$ volume and 53.90 μm thickness. However, treatment with the other compounds (**1c**, **1d**, and **30**) showed thinner and sparser formation of biofilm, as well as smaller volume and thickness, than that with the control. In particular, compound **30** inhibited biofilm formation by 74% and decreased the amount of carbohydrate and protein by 39 and 72%, respectively, as compared with the control (Figures 4B and 4C).

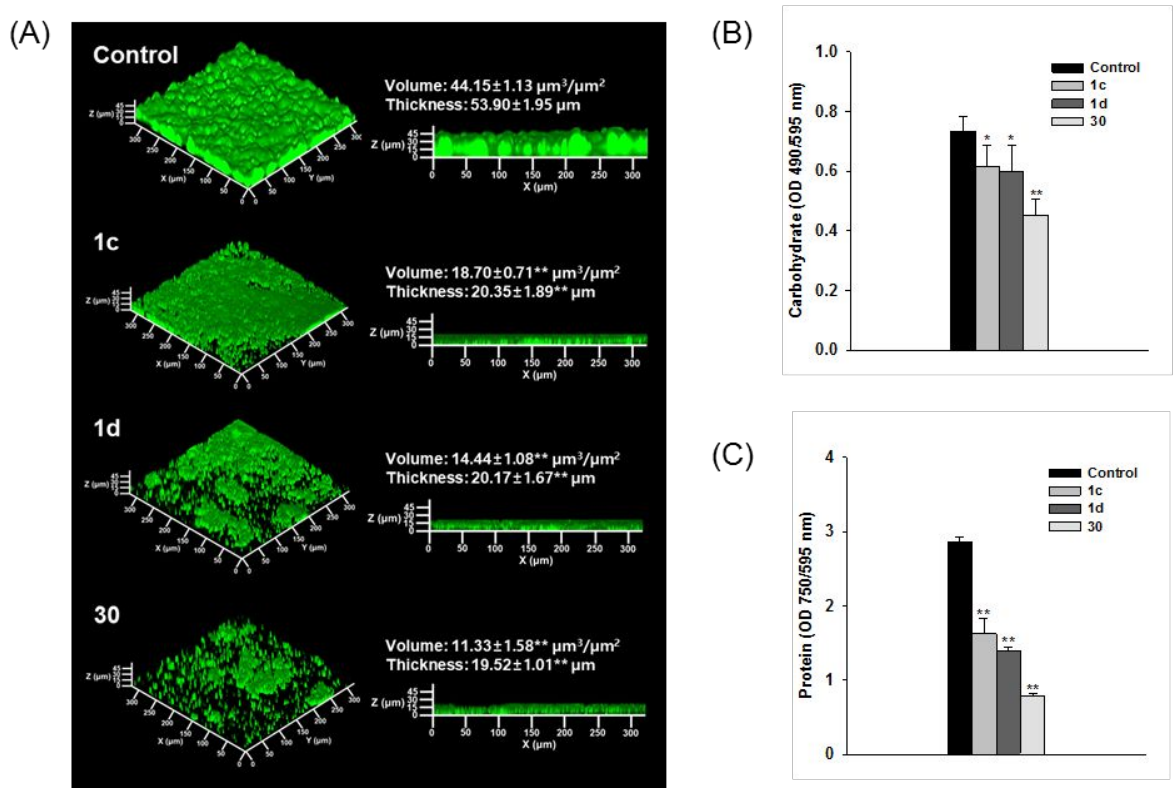


Figure 4. Biofilm formation by *P. aeruginosa* with compounds **1c**, **1d**, and **30** at 10 μM in flow conditions. DMSO (negative control) and **1c**, **1d**, and **12a** (positive controls) were used. (A) CLSM images with biofilm volume and thickness. (B) The amount of carbohydrate in EPS. (C) The amount of protein in EPS. (**) $P<0.005$ and (*) $P<0.05$ as compared with the control.

In the next virulence production experiment, compound **30** reduced significantly rhamnolipid production by *P. aeruginosa* at 10 and 100 μM concentrations (Figure 5A), indicating that RhlR antagonists can regulate rhamnolipid production and play an important role in biofilm development stages.[34] In general, rhamnolipid increases the hydrophobicity of cells and bacterial twitching motility, affecting attachment and maturation in biofilm formation stages.[35] *P. aeruginosa rhlA* mutants which are down-regulated for the synthesis of rhamnolipid did not form mushroom-like biofilms,[36] suggesting that RhlR antagonist-induced reduction of rhamnolipid production may control biofilm formation in *P. aeruginosa*.

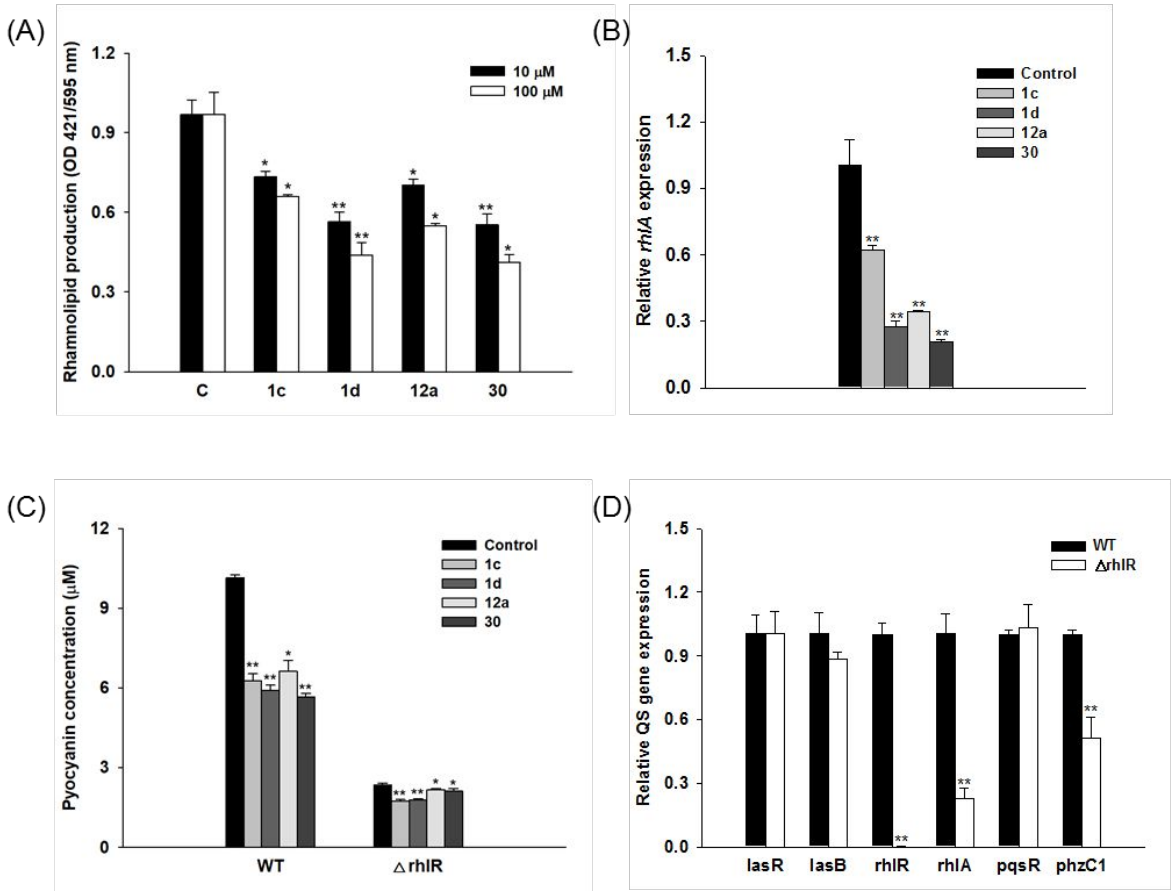


Figure 5. The production of virulence factors and expression of QS-induced genes by *P. aeruginosa* with compounds **1c**, **1d**, **12a,** and **30**. DMSO (C, negative control) and **1c**, **1d**, and

12a (positive controls) were used. (A) Rhamnolipid production by wild-type *P. aeruginosa* at 10 or 100 μM compound. (B) Relative *rhlA* expression of biofilm cells at 10 μM compound. (C) Pyocyanin production by wild-type *P. aeruginosa* and *rhlR* mutants at 10 μM compound. (D) Relative QS-related gene expression of wild-type *P. aeruginosa* and *rhlR* mutants. (**) $P<0.005$ and (*) $P<0.05$ as compared with the control.

We hypothesized that compound **30**-induced inhibition of biofilm formation and virulence factor production are closely associated with the *rhl* system. To verify this hypothesis, we measured rhamnolipid synthesis gene (*rhlA)* expression in compound **30**-treated biofilm cells using RT-qPCR (Figure 5B). The RhlR antagonists (**1d**, **12a**, and **30**) down-regulated *rhlA* expression of biofilm cells more significantly (65–79%) than compound **1c** (38%), indicating that *rhlA* expression can be controlled by RhlR antagonists. These RT-qPCR results demonstrated that compound **30** could inhibit *P. aeruginosa* biofilm formation and virulence factor production by down-regulating the *rhlA* expression of *P. aeruginosa.* However, the *rhl* system was reported to negatively modulate the *pqs* system, leading to increased pyocyanin production.[12] Considering the relationship between the *rhl* and *pqs* systems, Blackwell and co-workers developed an RhlR agonist and used it as an anti-virulence strategy against pathogens.[26] The RhlR agonist strongly inhibited pyocyanin production by suppressing *pqs* signaling. Based on their results, we expected that RhlR antagonists would increase pyocyanin production, thus negatively affecting biofilm formation and virulence production. Surprisingly, *rhlR* mutants produced less pyocyanin than wild-type *P. aeruginosa* as shown in Figure 5C. In addition, compound **30** reduced pyocyanin production by 44% as compared with the control. However, there was no difference of virulence factor production between compound **30** and the control in *rhlR* mutants (Figure 5C), indicating that compound **30** reduced pyocyanin

production by blocking RhlR. This difference might be related to the stage when pyocyanin is produced in *P. aeruginosa*. RhlR agonists are suggested to suppress the *pqs* system only before basal pyocyanin is produced.[26] According to the microarray experiment by Givskov and co-workers, *rhlR* mutants repressed *rhl* system-related genes, especially rhamnolipid production-related genes (*rhlA* and *rhlB*), but insignificantly affect *las*- or *pqs*-related system genes.[37] Pyocyanin production-related genes (*phzA-D*) were also down-regulated in *rhlR* mutants. Bassler and co-workers reported the down-regulation of pyocyanin biosynthetic genes (*phzA1-G1*) and decreased pyocyanin production in *rhlR* mutants.[38] Similarly, according to our RT-qPCR experiment results, *rhlR* mutants decreased *rhlA* and *phzC1* expression ($P<0.005$) without affecting the expression of other QS-related genes (*lasR*, *lasB*, and *pqsR*) (Figure 5D).

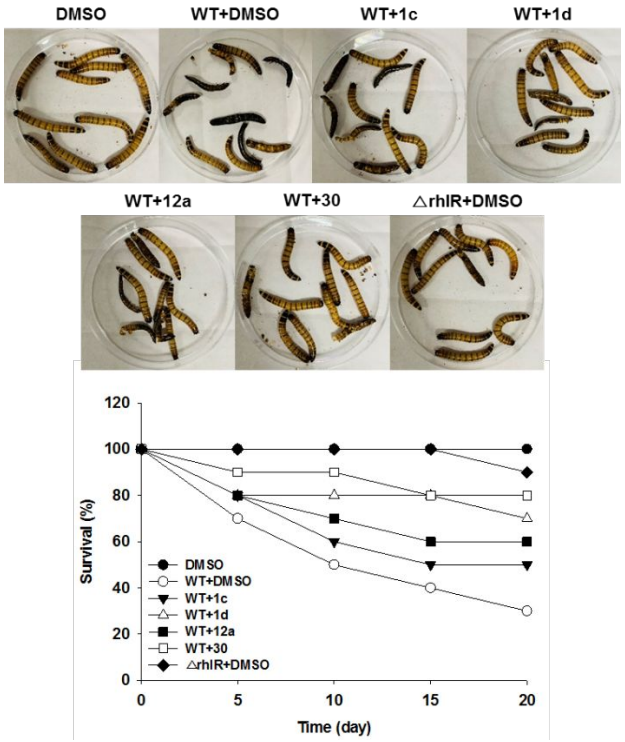


Figure 6. Mortality of *T. molitor* larvae injected *P. aeruginosa* with compounds **1c**, **1d**, **12a**, and **30** at 10 μM. DMSO (negative control) and **1c**, **1d**, and **12a** (positive controls) were used.

Encouraged by *in vitro* assay results, we determined the effect of compound **30** on *Tenebrio molitor (T. molitor)* larvae mortality. Larvae injected with *P. aeruginosa* started to die in initial incubation time and 70% of them died at the end of the 20-day incubation period (Figure 6). The survival rate of compound **30**-treated larvae was greatly improved, with approximately 80% larvae surviving at the end of the incubation period. In addition, larvae injected with *rhlR* mutants of *P. aeruginosa* showed a 90% survival rate after 20 days. Moreover, when treated *rhlR* mutants with different concentrations of rhamnolipid (0.01–10 μM) in larvae, their mortality was increased depending on the rhamnolipid concentration, emphasizing the key role of RhlR in the production of virulence factor (Figure S5). Based on *in vitro* and *in vivo* results, we propose that the pure RhlR antagonist **30** inhibits rhamnolipid and pyocyanin production by inactivating of virulence factor production gene expression (e.g., *rhl* and *phz* genes) as described in a working model (Figure 7).

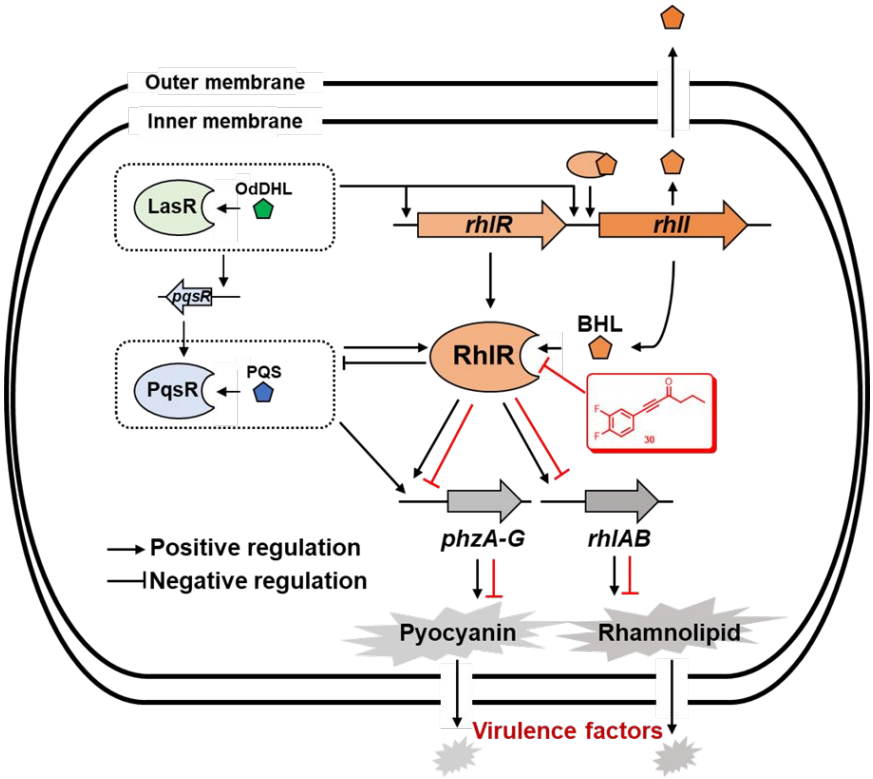


Figure 7. A working model to control QS system of *P. aeruginosa* by compound **30**.

CONCLUSIONS

RhlR is one of the key LuxR-type receptors in the QS network of *P. aeruginosa* and considered as an attractive target protein for the discovery of biofilm inhibitors. We systemically synthesized 55 gingerol analogues and evaluated their relative RhlR activities using QS reporter strain assay. Comprehensive SAR studies identified the alkynyl ketone **30** as the most potent RhlR antagonist. Compound **30** displayed high RhlR antagonism (no RhlR agonism at 1000 μM), strong inhibition of biofilm formation in static and dynamic settings, and reduced production of virulence factors such as rhamnolipid and pyocyanin in *P. aeruginosa*. It also displayed selective affinity for RhlR over LasR and PqsR. Furthermore, compound **30** significantly increased the *in vivo* survival rate of *T. molitor* larvae as compared with the control. In conclusion, the pure and potent RhlR antagonist **30** which we discovered for the first time can be utilized for investigating *rhl*-related QS mechanism and developing a novel anti-virulence strategy to control *P. aeruginosa* infections.

EXPERIMENTAL SECTION

General

All chemicals and solvents used in the reaction were purchased from Sigma-Aldrich, TCI, and Acros and were used without further purification. Reaction progress was monitored by TLC on pre-coated silica gel plates with silica gel $60F_{254}$ (Merck; Darmstadt, Germany) and visualized by UV254 light and/or $KMnO_4$ staining for detection purposes. Column chromatography was performed on silica gel (Silica gel 60; 230–400 mesh ASTM, Merck, Darmstadt, Germany). Nuclear magnetic resonance (NMR) spectra were recorded at room temperature on either a Bruker BioSpin Avance 300 MHz NMR (^{1}H, 300 MHz; ^{13}C, 75 MHz) or a Bruker Ultrashield 600 MHz Plus (^{1}H, 600 MHz; ^{13}C, 150 MHz) spectrometer. All chemical shifts are reported in parts per million (ppm) from tetramethylsilane ($\delta = 0$) and were measured relative to the solvent in which the sample was analyzed ($CDCl_3$: δ 7.26 for ^{1}H NMR, δ 77.0 for ^{13}C NMR; MeOH-d_4: δ 3.31 for ^{1}H NMR, δ 49.0 for ^{13}C NMR). The ^{1}H NMR shift values are reported as chemical shift (δ), the corresponding integral, multiplicity (s = singlet, br = broad, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, td = triplet of doublets, qd = quartet of doublets), coupling constant (J in Hz) and assignments. High-resolution mass spectra (HRMS) were recorded on an Agilent 6530 Accurate Mass Q-TOF LC/MS spectrometer. The purity of all final compounds was measured by analytical reverse-phase HPLC on an Agilent 1260 Infinity (Agilent) with a C18 column (Phenomenex, 150 mm×4.6 mm, 3 μm, 110Å). RP-HPLC was performed using the following isocratic conditions: for method A, mobile phase was acetonitrile and water (30:70, v/v); for method B, mobile phase was acetonitrile and water with 0.1% TFA (30:70, v/v); for method C, mobile phase was acetonitrile and water (40:60, v/v); for method D, mobile phase was acetonitrile and water (50:50, v/v); for method E, mobile phase was acetonitrile and water (55:45, v/v); for method F, mobile phase was acetonitrile and water

(60:40, v/v); for method G, mobile phase was methanol and water (50:50, v/v); for method H, mobile phase was methanol and water (60:40, v/v); for method I, mobile phase was methanol and water (70:30, v/v). All compounds were eluted with a flow rate of 1 mL/min and monitored at UV detector (220 nm). The purity of the tested compounds was >95%.

Chemical Synthesis

Compounds **2**−**8** were reported by our previous study.[29] The chemical structure and name for compounds **2**−**8** can be found in the Supporting Information (Scheme S1).

General procedure A for compounds **11a**–**11r**

To a stirred solution of benzylideneacetone compound in THF (20 mL) was added LDA (1.2–3.6 eq.) at –78 ℃. The solution was stirred under argon for 1 h at the same temperature then *n*-butanal (10.0 eq.) was added dropwise. The reaction mixture was vigorously stirred at the same temperature until TLC analysis indicated complete conversion (typically 3–24 h), quenched with aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 × 25 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel to furnish compounds.

(E)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)oct-1-en-3-one (**11a**)

Compound **11a** was prepared in 60% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one* (300 mg, 1.56 mmol), LDA (4.10 mL, 2.4 eq.) and stirring for 3 h. The crude residue was purified by column chromatography on silica gel (Toluene/EtOAc = 6:1 to 3:1, v/v). R_f = 0.40

(Toluene/EtOAc = 2:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 16.1 Hz, 1H), 7.13 (dd, J = 1.8 and 8.2 Hz, 1H), 7.08 (d, J = 1.7 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 6.61 (d, J = 16.1 Hz, 1H), 5.94 (s, 1H), 4.23–4.09 (m, 1H), 3.96 (s, 3H), 3.30 (d, J = 2.9 Hz, 1H), 2.90 (dd, J = 2.7 and 17.1 Hz, 1H), 2.75 (q, J = 9.0 Hz, 1H), 1.59–1.36 (m, 4H), 0.97 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.03, 148.50, 146.89, 143.87, 126.72, 124.15, 123.76, 114.87, 109.51, 67.65, 55.98, 46.52, 38.68, 18.75, 14.05, 13.97. HRMS m/z calculated for $C_{15}H_{20}O_4$ $[M–H]^-$: 263.1289; found: 263.1304. >95% purity (as determined by RP-HPLC, method C, t_R = 6.34 min, method G, t_R = 7.33 min).

(E)-5-Hydroxy-1-phenyloct-1-en-3-one (**11b**)

Compound **11b** was prepared in 36% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-phenylbut-3-en-2-one* (300 mg, 2.05 mmol), LDA (2.70 mL, 1.2 eq.) and stirring for 2 h. The crude residue was purified by column chromatography on silica gel (Toluene/EtOAc = 6:1 to 3:1, v/v). R_f = 0.40 (Toluene/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (d, J = 16.2 Hz, 1H), 7.56–7.54 (m, 2H), 7.43–7.39 (m, 3H), 6.74 (d, J = 16.2 Hz, 1H), 4.19–4.13 (m, 1H), 3.17 (d, J = 3.30 Hz, 1H), 2.88 (dd, J = 2.6 and 17.3 Hz, 1H), 2.77 (q, J = 9.1 Hz, 1H), 1.62–1.36 (m, 4H), 0.96 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.24, 140.75, 128.56, 128.29, 126.22, 67.36, 49.32, 45.06, 38.59, 29.53, 18.65, 13.98. HRMS m/z calculated for $C_{14}H_{18}O_2$ $[M–H]^-$: 217.1234; found: 217.1252. >95% purity (as determined by RP-HPLC, method C, t_R = 12.91 min, method G, t_R = 15.79 min).

(E)-5-Hydroxy-1-(3-methoxyphenyl)oct-1-en-3-one (**11c**)

Compound **11c** was prepared in 41% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(3-methoxyphenyl)but-3-en-2-one* (300 mg, 1.70 mmol), LDA (2.23 mL, 1.2 eq.) and stirring for 4 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 6:1, v/v). R_f = 0.48 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (d, J = 16.1 Hz, 1H), 7.33 (t, J = 7.9 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 7.08 (s, 1H), 6.98 (d, J = 8.2 Hz, 1H), 6.73 (d, J = 16.2 Hz, 1H), 4.17 (brs, 1H), 3.86 (s, 3H), 3.22 (brs, 1H), 2.91–2.88 (m, 1H), 2.80–2.76 (m, 1H), 1.61–1.40 (m, 4H), 0.97 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.04, 159.98, 143.48, 135.60, 130.02, 126.65, 121.15, 116.66, 113.20, 67.61, 55.35, 46.86, 38.69, 18.75, 14.04. HRMS *m*/*z* calculated for $C_{15}H_{20}O_3$ $[M–H]^-$: 247.1339; found: 247.1344. >95% purity (as determined by RP-HPLC, method C, t_R = 14.25 min, method G, t_R = 19.69 min).

(E)-5-Hydroxy-1-(4-hydroxyphenyl)oct-1-en-3-one (**11d**)

Compound **11d** was prepared in 43% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(4-hydroxyphenyl)but-3-en-2-one* (250 mg, 1.54 mmol), LDA (4.10 mL, 2.4 eq.) and stirring for 6 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 3:1, v/v). R_f = 0.42 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, MeOD) δ 7.61 (d, J = 16.1 Hz, 1H), 7.53 (d, J = 8.6 Hz, 1H), 6.84 (d, J = 8.6 Hz, 1H), 6.72 (d, J = 16.1 Hz, 1H), 4.14–4.12 (m, 1H), 2.88–2.84 (m, 1H), 2.78–2.75 (m, 1H), 1.55–1.41 (m, 4H), 0.97 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, MeOD) δ 200.62, 160.22, 144.09, 130.21, 125.86, 123.16, 115.54, 67.81, 39.25, 18.45, 12.98. HRMS *m*/*z* calculated for $C_{14}H_{18}O_3$ $[M–H]^-$: 233.1183; found: 233.1213. >95% purity (as determined by RP-HPLC, method C, t_R = 6.18 min, method G, t_R = 8.08 min).

(E)-5-Hydroxy-1-(3-hydroxyphenyl)oct-1-en-3-one (**11e**)

Compound **11e** was prepared in 34% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(3-hydroxyphenyl)but-3-en-2-one* (250 mg, 1.54 mmol), LDA (4.10 mL, 2.4 eq.) and stirring for 6 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 3:1, v/v). R_f = 0.30 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, MeOD) δ 7.58 (d, J = 16.1 Hz, 1H), 7.24 (t, J = 7.9 Hz, 1H), 7.11 (d, J = 7.6 Hz, 1H), 7.06 (s, 1H), 6.87 (dd, J = 8.1 and 1.8 Hz, 1H), 6.80 (d, J = 16.2 Hz, 1H), 4.16–4.11 (m, 1H), 2.90–2.86 (m, 1H), 2.79–2.77 (m, 1H), 1.54–1.40 (m, 4H), 0.97 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, MeOD) δ 200.49, 157.73, 143.65, 135.85, 129.65, 126.18, 119.78, 117.53, 114.14, 67.66, 39.25, 18.45, 12.96. HRMS *m*/*z* calculated for $C_{14}H_{18}O_3$ $[M–H]^-$: 233.1183; found: 233.1200. >95% purity (as determined by RP-HPLC, method C, t_R = 5.68 min, method G, t_R = 8.17 min).

(E)-5-Hydroxy-1-(4-methoxyphenyl)oct-1-en-3-one (**11f**)

Compound **11f** was prepared in 48% yield as white oil, following the same procedure as described in the general procedure A with *(E)-4-(4-methoxyphenyl)but-3-en-2-one* (300 mg, 1.70 mmol), LDA (2.30 mL, 1.2 eq.) and stirring for 2 h. The crude residue was purified by column chromatography on silica gel (Toluene/EtOAc = 10:1 to 3:1, v/v). R_f = 0.55 (Toluene/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55–7.50 (m, 3H), 6.93 (d, J = 8.7, 2H), 6.62 (d, J = 16.2 Hz, 1H), 4.14 (brs, 1H), 3.85 (s, 3H), 3.27 (brs, 1H), 2.88–2.85 (m, 1H), 2.75–2.71 (m, 1H), 1.62–1.42 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.01, 161.83, 143.39, 130.19, 126.87, 124.18, 114.49, 67.73, 55.41, 46.71, 38.75, 18.75, 14.04. HRMS *m*/*z* calculated for $C_{15}H_{20}O_3$ $[M–H]^-$: 247.1339; found: 247.1357. >95% purity (as determined by RP-HPLC, method C, t_R = 12.88 min, method G, t_R = 18.64 min).

(E)-1-(4-Fluorophenyl)-5-hydroxyoct-1-en-3-one (**11g**)

Compound **11g** was prepared in 38% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(4-fluorophenyl)but-3-en-2-one* (200 mg, 1.22 mmol), LDA (1.60 mL, 1.2 eq.) and stirring for 7 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 6:1, v/v). R_f = 0.40 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56–7.53 (m, 3H), 7.10 (t, J = 8.6 Hz, 2H), 6.66 (d, J = 16.2 Hz), 4.17 (brs, 1H), 3.13 (s, 1H), 2.88–2.85 (m, 1H), 2.77–2.73 (m, 1H), 1.59–1.40 (m, 4H), 0.96 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.74, 165.03, 163.36, 142.15, 130.50, 130.48, 130.35, 130.30, 126.08, 126.07, 116.30, 116.16, 67.61, 47.04, 38.70, 18.74, 14.02. HRMS *m*/*z* calculated for $C_{14}H_{17}FO_2$ $[M–H]^–$: 235.1140; found: 235.1155. >95% purity (as determined by RP-HPLC, method C, t_R = 14.12 min, method G, t_R = 17.64 min).

(E)-1-(4-Chlorophenyl)-5-hydroxyoct-1-en-3-one (**11h**)

Compound **11h** was prepared in 41% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(4-(dimethylamino)phenyl)but-3-en-2-one* (200 mg, 1.11 mmol), LDA (1.40 mL, 1.2 eq.) and stirring for 6 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 12:1 to 6:1, v/v). R_f = 0.35 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 (d, J = 16.2 Hz, 1H), 7.48 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.5 Hz, 2H), 6.70 (d, J = 16.2 Hz, 1H), 4.15 (brs, 1H), 3.13 (brs, 1H), 2.87–2.84 (m, 1H), 2.78–2.74 (m, 1H), 1.59–1.39 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.64, 141.91, 136.66, 132.75, 129.54, 129.28, 126.72, 67.58, 47.21, 38.75, 18.75, 14.02. HRMS *m*/*z* calculated for $C_{14}H_{17}ClO_2$ $[M–H]^–$: 251.0844; found:

251.0862. >95% purity (as determined by RP-HPLC, method C, t_R = 22.36 min, method G, t_R = 34.88 min).

(E)-5-Hydroxy-1-(p-tolyl)oct-1-en-3-one (**11i**)

Compound **11i** was prepared in 29% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(p-tolyl)but-3-en-2-one* (300 mg, 1.87 mmol), LDA (2.48 mL, 1.2 eq.) and stirring for 4 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 8:1 to 4:1, v/v). R_f = 0.45 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (d, J = 16.2 Hz, 1H), 7.45 (d, J = 8.2 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 6.69 (d, J = 16.2 Hz, 1H), 4.16–4.14 (m, 1H), 3.25 (d, J = 3.2 Hz, 1H), 2.88–2.85 (m, 1H), 2.77–2.72 (m, 1H), 2.38 (s,1H), 1.59–1.39 (m, 4H), 0.95 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.10, 143.63, 141.37, 131.48, 129.77, 128.44, 125.46, 46.77, 38.72, 21.53, 18.75, 14.04. HRMS *m/z* calculated for $C_{15}H_{20}O_2$ $[M–H]^-$: 231.1390; found: 231.1404. >95% purity (as determined by RP-HPLC, method C, t_R = 19.34 min, method G, t_R = 30.62 min).

(E)-1-(4-(Dimethylamino)phenyl)-5-hydroxyoct-1-en-3-one (**11j**)

Compound **11j** was prepared in 60% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(4-(dimethylamino)phenyl)but-3-en-2-one* (200 mg, 1.06 mmol), LDA (1.40 mL, 1.2 eq.) and stirring for 8 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 3:1, v/v). R_f = 0.31 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 (d, J = 16.0 Hz, 1H), 7.45 (d, J = 8.9 Hz, 2H), 6.67 (d, J = 8.9 Hz, 2H), 6.54 (d, J = 16.0 Hz, 1H), 4.13–4.11 (m, 1H), 3.50–

3.49 (m, 1H), 3.04 (s, 6H), 2.88–2.84 (m, 1H), 2.71–2.67 (m, 1H), 1.60–1.39 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.06, 152.15, 144.52, 130.34, 121.75, 121.40, 111.84, 67.90, 46.22, 40.10, 38.78, 18.77, 14.08. HRMS m/z calculated for $C_{16}H_{23}NO_2$ $[M–H]^–$: 260.1656; found: 260.1674. >95% purity (as determined by RP-HPLC, method C, t_R = 17.13 min, method G, t_R = 30.68 min).

(E)-1-(3,4-Dihydroxyphenyl)-5-hydroxyoct-1-en-3-one (**11k**)

Compound **11k** was prepared in 30% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(3,4-dihydroxyphenyl)but-3-en-2-one* (150 mg, 0.84 mmol), LDA (3.30 mL, 3.6 eq.) and stirring for 6 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 3:1 to 1:1, v/v). R_f = 0.50 (Hexane/EtOAc = 1:3, v/v). ^{1}H NMR (600 MHz, MeOD) δ 7.53 (d, J = 16.1 Hz, 1H), 7.10 (d, J = 1.8 Hz, 1H), 7.01 (dd, J = 1.8 and 8.2 Hz, 1H), 6.81 (d, J = 8.2 Hz, 1H), 6.64 (d, J = 16.1 Hz, 1H), 4.18–4.05 (m, 1H), 2.84 (q, J = 8.2 Hz, 1H), 2.74 (dd, J = 4.4 and 15.4 Hz, 1H), 1.57–1.35 (m, 4H), 0.96 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, MeOD) δ 200.69, 148.58, 145.48, 144.61, 126.45, 123.18, 122.21, 115.20, 114.03, 67.86, 39.23, 18.47, 13.00. HRMS m/z calculated for $C_{14}H_{18}O_4$ $[M–H]^–$: 249.1132; found: 249.1232. >95% purity (as determined by RP-HPLC, method B, t_R = 5.21 min, method G, t_R = 5.60 min).

(E)-1-(3,4-Dimethoxyphenyl)-5-hydroxyoct-1-en-3-one (**11l**)

Compound **11l** was prepared in 54% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(3,4-dimethoxyphenyl)but-3-en-2-one* (200 mg, 0.97 mmol), LDA (1.30 mL, 1.2 eq.) and stirring for 1 h. The crude residue was purified by

column chromatography on silica gel (Toluene/EtOAc = 10:1 to 3:1, v/v). R_f = 0.50 (Toluene/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (d, J = 16.1 Hz, 1H), 7.14 (d, J = 8.3 Hz, 1H), 7.07 (s, 1H), 6.89 (d, J = 8.3 Hz, 1H), 6.61 (d, J = 16.1 Hz, 1H), 4.15 (brs, 1H), 3.93 (s, 6H), 3.25 (s, 1H), 2.89–2.86 (m, 1H), 2.77–2.72 (m, 1H), 1.61–1.40 (m, 4H), 0.96 (t, J = 7.1 Hz, 3H) ; ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.95, 151.60, 149.30, 143.66, 127.13, 124.41, 123.33, 111.10, 109.74, 67.72, 56.00, 55.91, 46.63, 38.73, 18.74, 14.04. HRMS *m*/*z* calculated for $C_{16}H_{22}O_4$ $[M–H]^-$: 277.1445; found: 277.1478. >95% purity (as determined by RP-HPLC, method C, t_R = 8.92 min, method G, t_R = 11.13 min).

(E)-1-(3-Fluoro-4-hydroxyphenyl)-5-hydroxyoct-1-en-3-one (**11m**)

Compound **11m** was prepared in 45% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(3-fluoro-4-hydroxyphenyl)but-3-en-2-one* (100 mg, 0.55 mmol), LDA (1.50 mL, 2.4 eq.) and stirring for 4 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 3:1, v/v). R_f = 0.25 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, MeOD) δ 7.56 (d, J = 16.1 Hz, 1H), 7.42 (d, J = 12.1 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 6.95 (t, J = 8.6 Hz, 3H), 6.73 (d, J = 16.1 Hz, 1H), 4.14 (brs, 1H), 3.26 (s, 1H), 2.88–2.83 (m, 1H), 2.78–2.75 (m, 1H), 1.53–1.40 (m, 4H), 0.96 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, MeOD) δ 200.36, 152.43, 150.82, 147.75, 147.66, 142.75, 142.74, 126.70, 126.66, 125.76, 125.74, 124.49, 117.67, 117.65, 115.12, 114.99, 67.73, 39.25, 18.46, 12.99. HRMS *m*/*z* calculated for $C_{14}H_{17}FO_3$ $[M–H]^-$: 251.1089; found: 251.1117. >95% purity (as determined by RP-HPLC, method C, t_R = 6.49 min, method G, t_R = 8.91 min).

(E)-1-(3-Ethoxy-4-hydroxyphenyl)-5-hydroxyoct-1-en-3-one (**11n**)

Compound **11n** was prepared in 39% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(3-ethoxy-4-hydroxyphenyl)but-3-en-2-one* (200 mg, 0.97 mmol), LDA (2.60 mL, 2.4 eq.) and stirring for 3 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 3:1, v/v). R_f = 0.24 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 (d, J = 16.1 Hz, 1H), 7.10 (d, J = 8.1 Hz, 1H), 7.04 (s, 1H), 6.94 (d, J = 8.2 Hz, 1H), 6.58 (d, J = 16.1 Hz, 1H), 5.94 (s, 1H), 4.18–4.15 (q, 7.0 Hz, 2H), 3.26 (s, 1H), 2.88–2.85 (m, 1H), 2.75–2.71 (m, 1H), 1.50–1.47 (m, 4H), 0.95 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.02, 148.69, 146.22, 143.98, 126.64, 124.05, 123.59, 114.89, 110.44, 67.76, 64.64, 46.52, 38.70, 18.75, 14.79, 14.05. HRMS m/z calculated for $C_{16}H_{22}O_4$ $[M–H]^-$: 277.1445; found: 277.1472. >95% purity (as determined by RP-HPLC, method C, t_R = 8.42 min, method G, t_R = 10.65 min).

(E)-1-(3,4-Difluorophenyl)-5-hydroxyoct-1-en-3-one (**11o**)

Compound **11o** was prepared in 38% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(3,4-difluorophenyl)but-3-en-2-one* (200 mg, 1.10 mmol), LDA (1.60 mL, 1.2 eq.) and stirring for 18 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 2:1, v/v). R_f = 0.38 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, J = 16.1 Hz, 1H), 7.38 (t, J = 9.2 Hz, 1H), 7.30–7.28 (m, 1H), 7.20 (q, J = 8.7 Hz, 1H), 6.64 (d, J = 16.1 Hz, 1H), 4.16 (brs, 1H), 3.09 (s, 1H), 2.86–2.83 (m, 1H), 2.78–2.74 (m, 1H), 1.60–1.36 (m, 4H), 0.96 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.32, 152.61, 152.52, 151.52, 151.43, 150.92, 150.84, 149.87, 149.78, 140.88, 131.56, 131.52, 131.49, 127.09, 127.08, 125.26, 125.23, 125.21, 125.19, 118.01, 117.89, 116.58, 116.47. HRMS m/z calculated for $C_{14}H_{16}F_2O_2$ $[M–H]^-$

: 253.1045; found: 253.1062. >95% purity (as determined by RP-HPLC, method C, t_R = 17.12 min, method G, t_R = 22.40 min).

(E)-1-(3,4-Dimethylphenyl)-5-hydroxyoct-1-en-3-one (**11p**)

Compound **11p** was prepared in 37% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(3,4-dimethylphenyl)but-3-en-2-one* (200 mg, 1.15 mmol), LDA (1.50 mL, 1.2 eq.) and stirring for 8 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 8:1 to 4:1, v/v). R_f = 0.55 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (d, J = 16.1 Hz, 1H), 7.32 (s, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.16 (d, J = 7.7 Hz, 1H), 6.68 (d, J = 16.2 Hz, 1H), 4.16–4.12 (m, 1H), 3.29 (brs, 1H), 2.88–2.85 (m, 1H), 2.76–2.72 (m, 1H), 2.28 (s, 6H), 1.60–1.39 (m, 4H), 0.95 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.12, 143.86, 140.14, 137.30, 131.87, 130.30, 129.63, 126.10, 125.31, 67.67, 46.73, 38.73, 19.88, 19.75, 18.76, 14.05. HRMS m/z calculated for $C_{16}H_{22}O_2$ $[M–H]^-$: 245.1547; found: 245.1558. >95% purity (as determined by RP-HPLC, method C, t_R = 28.39 min, method H, t_R = 19.67 min).

(E)-1-(4-Fluoro-3-methoxyphenyl)-5-hydroxyoct-1-en-3-one (**11q**)

Compound **11q** was prepared in 46% yield as white oil, following the same procedure as described in the general procedure A with *(E)-4-(4-fluoro-3-methoxyphenyl)but-3-en-2-one* (120 mg, 0.62 mmol), LDA (0.70mL, 1.2 eq.) and stirring for 2 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 3:1, v/v). R_f = 0.48 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 (d, J = 16.1 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.12–7.08 (m, 2H), 6.64 (d, J = 16.1 Hz, 1H), 4.17–4.14 (m, 1H), 3.93 (s, 3H),

3.15 (brs, 1H), 2.88–2.85 (m, 1H), 2.78–2.74 (m, 1H), 1.59–1.40 (m, 4H), 0.96 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.74, 154.87, 153.19, 148.18, 148.10, 142.57, 130.89, 130.87, 126.18, 126.16, 122.10, 122.06, 116.67, 116.55, 112.46, 67.62, 56.27, 46.95, 38.71, 18.75, 14.03. HRMS *m*/*z* calculated for $C_{15}H_{19}FO_3$ $[M–H]^-$: 265.1245; found: 265.1496. >95% purity (as determined by RP-HPLC, method C, t_R = 15.14 min, method G, t_R = 19.00 min).

(E)-1-(3-Fluoro-5-methoxyphenyl)-5-hydroxyoct-1-en-3-one (**11r**)

Compound **11r** was prepared in 24% yield as yellow oil, following the same procedure as described in the general procedure A with *(E)-4-(3-fluoro-5-methoxyphenyl)but-3-en-2-one* (130 mg, 0.67 mmol), LDA (0.88 mL, 1.2 eq.) and stirring for 6 h. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1 to 6:1, v/v). R_f = 0.40 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (d, J = 16.2 Hz, 1H), 6.86–6.85 (m, 2H), 6.70–6.65 (m, 2H), 4.17–4.14 (m, 1H), 3.83 (s, 3H), 3.13 (brs, 1H), 2.87–2.84 (m, 1H), 2.79–2.74 (m, 1H), 1.59–1.40 (m, 4H), 0.95 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.68, 164.57, 162.93, 161.26, 161.18, 142.14, 142.12, 136.79, 136.72, 127.55, 110.12, 110.11, 107.09, 106.94, 103.86, 103.70, 67.56, 55.69, 47.21, 38.70, 18.74, 14.01. HRMS *m*/*z* calculated for $C_{15}H_{19}FO_3$ $[M–H]^-$: 265.1245; found: 265.1262. >95% purity (as determined by RP-HPLC, method C, t_R = 19.07 min, method H, t_R = 11.43 min).

General procedure B for compound **12a**–**12r**

To a stirred solution of **11a**–**11r** in MeOH (8 mL) was added 10% Pd/C (0.03 eq.). The solution was then stirred in an atmosphere of H_2 gas for 2 h. The reaction mixture was filtered through a Celite pad and concentrated under reduced pressure. The crude residue was purified by

column chromatography on silica gel (Hexane/EtOAc = 4:1, v/v) to furnish compound **12a**–**12r**.

5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)octan-3-one (**12a**)

Compound **12a** was prepared in 45% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11a** (109 mg, 0.412 mmol). R_f = 0.38 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82 (d, J = 8.2 Hz, 1H), 6.73–6.60 (m, 2H), 5.71 (s, 1H), 4.04 (brs, 1H), 3.86 (s, 3H), 3.06 (brs, 1H), 2.90–2.65 (m, 4H), 2.62–2.41 (m, 2H), 1.56–1.21 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.53, 146.45, 143.93, 132.63, 120.74, 114.37, 111.02, 67.38, 67.27, 55.88, 49.34, 45.44, 38.54, 29.28, 18.65, 13.98. HRMS *m*/*z* calculated for $C_{15}H_{22}O_3$ $[M–H]^-$: 265.1445; found: 265.1455. >95% purity (as determined by RP-HPLC, method C, t_R = 6.09 min, method G, t_R = 5.49 min).

5-Hydroxy-1-phenyloctan-3-one (**12b**)

Compound **12b** was prepared in 52% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11b** (50 mg, 0.229 mmol). R_f = 0.65 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29–7.26 (m, 2H), 7.20–7.17 (m, 3H), 4.05–4.03 (m, 1H), 2.94 (s, 1H), 2.90 (t, J = 7.6 Hz ,2H), 2.76 (t, J = 7.6 Hz, 2H), 2.57–2.47 (m, 2H), 1.49–1.25 (m, 4H), 0.91 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.24, 140.75, 128.56, 128.29, 126.22, 67.36, 49.32, 45.06, 38.59, 29.53, 18.65, 13.98. HRMS *m*/*z* calculated for $C_{14}H_{20}O_2$ $[M–H]^-$: 219.1390; found: 219.1414. >95% purity (as determined by RP-HPLC, method C, t_R = 14.38 min, method G, t_R = 16.36 min).

5-Hydroxy-1-(3-methoxyphenyl)octan-3-one (**12c**)

Compound **12c** was prepared in 72% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11c** (50 mg, 0.201 mmol). R_f = 0.51 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.20 (t, J = 7.9 Hz, 1H), 6.77–6.72 (m, 3H), 4.05–4.03 (m, 1H), 3.79 (s, 3H), 2.90 (d, J = 3.4 Hz, 1H), 2.88 (t, J = 7.6 Hz, 2H), 2.76 (t, J = 7.6 Hz, 2H), 2.58–2.55 (m, 1H), 2.52–2.47 (m, 1H), 1.50–1.31 (m, 4H), 0.92 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.15, 159.75, 142.38, 129.54, 120.61, 114.13, 111.46, 67.36, 55.16, 49.33, 44.96, 38.61, 29.55, 18.65, 13.98. HRMS *m*/*z* calculated for $C_{15}H_{22}O_3$ $[M–H]^-$: 250.1569; found: 249.1518. >95% purity (as determined by RP-HPLC, method C, t_R = 13.80 min, method G, t_R = 15.38 min).

5-Hydroxy-1-(4-hydroxyphenyl)octan-3-one (**12d**)

Compound **12d** was prepared in 72% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11d** (88 mg, 0.376 mmol). R_f = 0.45 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, MeOD) δ 7.02 (d, J = 8.5 Hz, 1H), 6.70 (d, J = 8.5 Hz, 1H), 4.04–4.01 (m, 1H), 2.77 (s, 4H), 2.58–2.54 (m, 1H), 2.52–2.48 (m, 1H), 1.46–1.33 (m, 4H), 0.93 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, MeOD) δ 210.62, 155.21, 131.87, 128.88, 114.78, 67.24, 49.89, 45.03, 39.19, 28.39, 18.37, 12.94. HRMS *m*/*z* calculated for $C_{14}H_{20}O_3$ $[M–H]^-$: 235.1339; found: 235.1360. >95% purity (as determined by RP-HPLC, method C, t_R = 6.02 min, method G, t_R = 6.14 min).

5-Hydroxy-1-(3-hydroxyphenyl)octan-3-one (**12e**)

Compound **12e** was prepared in 68% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11e** (50 mg, 0.213 mmol). R_f = 0.35 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, MeOD) δ 7.07 (t, J = 7.8 Hz, 1H), 6.67 (d, J = 7.6 Hz, 1H), 6.64 (s, 1H), 6.61–6.60 (m, 1H), 4.05–4.02 (m, 1H), 2.80 (s, 4H), 2.59–2.55 (m, 1H), 2.53–2.50 (m, 1H), 1.48–1.31 (m, 4H), 0.94 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, MeOD) δ 210.35, 157.09, 142.67, 129.03, 119.16, 114.81, 112.55, 67.26, 49.87, 44.58, 39.20, 29.14, 18.37, 12.93. HRMS *m*/*z* calculated for $C_{14}H_{20}O_3$ $[M–H]^-$: 235.1339; found: 235.1360. >95% purity (as determined by RP-HPLC, method C, t_R = 5.57 min, method G, t_R = 5.23 min).

5-Hydroxy-1-(4-methoxyphenyl)octan-3-one (**12f**)

Compound **12f** was prepared in 80% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11f** (40 mg, 0.161 mmol). R_f = 0.40 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.09 (d, J = 8.5 Hz, 2H), 6.82 (d, J = 8.4 Hz, 2H), 4.03 (brs, 1H), 3.78 (s, 3H), 2.93 (s, 1H), 2.84 (t, J =7.5 Hz, 2H), 2.73 (t, J = 7.6 Hz, 2H), 2.57–2.54 (m, 1H), 2.50–2.46 (m, 1H), 1.49–1.29 (m, 4H), 0.91 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.46, 158.03, 132.75, 129.40, 129.22, 113.96, 67.35, 55.27, 49.32, 45.34, 38.57, 28.70, 18.65, 13.98. HRMS *m*/*z* calculated for $C_{15}H_{22}O_3$ $[M–H]^-$: 249.1496; found: 249.1527. >95% purity (as determined by RP-HPLC, method C, t_R = 13.20 min, method G, t_R = 14.78 min).

1-(4-Fluorophenyl)-5-hydroxyoctan-3-one (**12g**)

Compound **12g** was prepared in 65% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11g** (84 mg, 0.356 mmol). R_f = 0.41

(Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.14–7.12 (m, 3H), 6.96 (t, J = 8.7 Hz, 2H), 5.56 (s, 1H), 4.04–4.03 (brs, 1H), 4.05 (brs, 1H), 2.90 (s, 1H), 2.87 (t, J =7.6 Hz, 2H), 2.74 (t, J = 7.5 Hz, 2H), 2.57–2.54 (m, 1H), 2.51–2.47 (m, 1H), 1.49–1.32 (m, 4H), 0.92 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.91, 162.22, 160.60, 136.41, 136.40, 129.73, 129.68, 115.35, 115.20, 67.36, 49.38, 45.09, 38.63, 28.64, 18.64, 13.96. HRMS *m*/*z* calculated for $C_{14}H_{19}FO_2$ $[M–H]^–$: 237.1296; found: 237.1309. >95% purity (as determined by RP-HPLC, method C, t_R = 12.21 min, method G, t_R = 18.56 min).

1-(4-Chlorophenyl)-5-hydroxyoctan-3-one (**12h**)

Compound **12h** was prepared in 40% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11h** (48 mg, 0.190 mmol). R_f = 0.45 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30–7.26 (m, 2H), 7.21–7.17 (m, 2H), 4.05–4.03 (m, 1H), 2.91 (t, J = 7.6 Hz, 2H), 2.76 (t, J = 7.6 Hz, 2H), 2.58–2.55 (m, 1H), 2.51–2.47 (m, 1H), 1.51–1.31 (m, 4H), 0.92 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.24, 140.75, 128.56, 128.29, 126.23, 67.36, 49.31, 45.07, 38.59, 29.53, 18.65, 13.98. HRMS *m*/*z* calculated for $C_{14}H_{19}ClO_2$ $[M–H]^–$: 253.1001; found: 253.1430. >95% purity (as determined by RP-HPLC, method C, t_R = 11.95 min, method G, t_R = 16.89 min).

5-Hydroxy-1-(p-tolyl)octan-3-one (**12i**)

Compound **12i** was prepared in 70% yield as a yellow oil, by following the same procedure as described in the general procedure B with **11i** (107 mg, 0.461 mmol). R_f = 0.48 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.07 (dd, J = 8.0 and 18.2 Hz, 4H), 4.05–4.02 (m, 1H), 2.92 (d, J = 3.5 Hz, 1H), 2.86 (t, J = 7.6 Hz, 2H), 2.74 (t, J = 7.6 Hz, 2H), 2.57–2.54 (m,

1H), 2.50–2.46 (m, 1H), 2.31 (s, 3H), 1.49–1.31 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.32, 137.66, 135.69, 129.23, 128.17, 67.36, 49.36, 45.21, 38.65, 29.13, 21.00, 18.67, 14.00. HRMS m/z calculated for $C_{15}H_{22}O_2$ $[M–H]^-$: 233.1547; found: 233.1575. >95% purity (as determined by RP-HPLC, method C, t_R = 21.76 min, method G, t_R = 32.04 min).

1-(4-(Dimethylamino)phenyl)-5-hydroxyoctan-3-one (**12j**)

Compound **12j** was prepared in 63% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11j** (80 mg, 0.306 mmol). R_f = 0.60 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.04 (d, J = 8.1 Hz, 2H), 6.68 (d, J = 8.1 Hz, 2H), 4.02 (brs, 1H), 2.98 (s, 1H), 2.90 (s, 6H), 2.80 (t, J = 7.3 Hz, 2H), 2.70 (t, J = 7.5 Hz, 2H), 2.57–2.54 (m, 1H), 2.50–2.45 (m, 1H), 1.47–1.33 (m, 4H), 0.91 (t, J = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.83, 149.28, 128.88, 128.66, 113.04, 67.35, 49.30, 45.53, 40.83, 38.59, 28.68, 18.67, 14.00. HRMS m/z calculated for $C_{16}H_{25}NO_2$ $[M–H]^-$: 262.1812; found: 262.1828. >95% purity (as determined by RP-HPLC, method D, t_R = 6.20 min, method G, t_R = 23.83 min).

1-(3,4-Dihydroxyphenyl)-5-hydroxyoctan-3-one (**12k**)

Compound **12k** was prepared in 93% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11k** (36 mg, 0.144 mmol). R_f = 0.55 (Hexane/EtOAc = 1:3, v/v). ^{1}H NMR (600 MHz, MeOD) δ 6.67 (d, J = 8.2 Hz, 1H), 6.64 (d, J = 1.8 Hz, 1H), 6.52 (dd, J = 1.8 and 8.2 Hz, 1H), 4.07–3.98 (m, 1H), 2.80–2.69 (m, 4H), 2.57 (q, J = 8.2 Hz, 1H), 2.50 (dd, J = 4.4 and 15.4 Hz, 1H), 1.53–1.28 (m, 4H), 0.93 (t, J = 7.2 Hz,

3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.85, 144.78, 143.07, 132.68, 119.13, 115.09, 114.95, 67.25, 49.93, 45.01, 39.18, 28.65, 18.36, 12.91. HRMS *m*/*z* calculated for $C_{16}H_{25}NO_2$ $[M–H]^-$: 251.1289; found: 251.1387. >95% purity (as determined by RP-HPLC, method A, t_R = 4.86 min, method G, t_R = 3.93 min).

1-(3,4-Dimethoxyphenyl)-5-hydroxyoctan-3-one (**12l**)

Compound **12l** was prepared in 63% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11l** (59 mg, 0.212 mmol). R_f = 0.33 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.78 (d, J = 8.4 Hz, 1H), 6.71–6.70 (m, 2H), 4.04 (brs, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 2.92 (s, 1H), 2.85 (t, J =7.5 Hz, 2H), 2.74 (t, J = 7.5 Hz, 2H), 2.58–2.55 (m, 1H), 2.52–2.47 (m, 1H), 1.48–1.32 (m, 4H), 0.92 (t, J = 7.1 Hz, 3H) ; ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.40, 148.93, 147.45, 133.35, 120.07, 111.67, 111.34, 67.38, 55.93, 55.85, 49.37, 45.34, 38.60, 29.20, 18.65, 13.98. HRMS *m*/*z* calculated for $C_{16}H_{24}O_4$ $[M–H]^-$: 279.1602; found: 279.1620. >95% purity (as determined by RP-HPLC, method C, t_R = 8.52 min, method G, t_R = 8.61 min).

1-(3-Fluoro-4-hydroxyphenyl)-5-hydroxyoctan-3-one (**12m**)

Compound **12m** was prepared in 63% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11m** (55 mg, 0.218 mmol). R_f = 0.66 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, MeOD) δ 6.91 (d, J = 12.2 Hz ,1H), 6.82–6.81 (m, 2H), 4.04 (brs, 1H), 2.79 (s, 4H), 2.59–2.55 (m, 1H), 2.53–2.49 (m, 1H), 1.46–1.31 (m, 4H), 0.94 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, MeOD) δ 210.17, 152.10, 150.51, 142.74, 142.66, 133.11, 133.07, 123.85, 123.83, 117.20, 117.19, 115.41, 115.29, 67.24, 49.85,

44.62, 39.21, 28.11, 18.36, 12.91. HRMS *m*/*z* calculated for $C_{14}H_{19}FO_3$ $[M–H]^-$: 253.1245; found: 253.1275. >95% purity (as determined by RP-HPLC, method C, t_R = 6.38 min, method G, t_R = 6.04 min).

1-(3-Ethoxy-4-hydroxyphenyl)-5-hydroxyoctan-3-one (**12n**)

Compound **12n** was prepared in 45% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11n** (60 mg, 0.216 mmol). R_f = 0.35 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.82 (d, J = 8.0 Hz, 1H), 6.67 (s, 1H), 6.65 (d, J = 8.0 Hz, 1H), 5.56 (s, 1H), 4.10–4.07 (q, J = 7.0 Hz, 1H), 4.05 (brs, 1H), 2.92 (s, 1H), 2.82 (t, J =7.5 Hz, 2H), 2.72 (t, J = 7.5 Hz, 2H), 2.57–2.54 (m, 1H), 2.50–2.46 (m, 1H), 1.48–1.31 (m, 7H), 1.25 (s,1H), 0.91 (t, J = 7.1 Hz, 3H) ; ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.49, 145.73, 144.12, 132.56, 120.65, 114.34, 111.93, 67.38, 64.44, 49.37, 45.46, 38.59, 29.29, 18.65, 14.91, 13.97. HRMS *m*/*z* calculated for $C_{16}H_{24}O_4$ $[M–H]^-$: 279.1602; found: 279.1646. >95% purity (as determined by RP-HPLC, method C, t_R = 8.06 min, method G, t_R = 7.73 min).

1-(3,4-Difluorophenyl)-5-hydroxyoctan-3-one (**12o**)

Compound **12o** was prepared in 81% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11o** (49 mg, 0.193 mmol). R_f = 0.40 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.05 (q, J = 9.0 Hz ,1H), 6.98(t, J = 9.5 Hz, 1H), 6.89–6.87 (m, 1H), 4.07–4.04 (m, 1H), 2.86 (t, J = 7.4 Hz, 2H), 2.80 (s, 1H), 2.74 (t, J = 7.4 Hz, 2H), 2.58–2.54 (m, 1H), 2.52–2.48 (m, 1H), 1.49–1.33 (m, 4H), 0.92 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.35, 151.03, 150.95, 149.77, 149.69, 149.39,

149.31, 148.14, 148.05, 137.78, 137.75, 137.74, 137.72, 124.21, 124.18, 124.16, 124.14, 117.21, 117.19, 117.10, 117.08, 67.38, 49.38, 44.66, 38.66, 28.51, 18.64, 13.94. HRMS *m*/*z* calculated for $C_{14}H_{18}F_2O_2$ $[M–H]^-$: 255.1202; found: 255.1235. >95% purity (as determined by RP-HPLC, method C, t_R = 18.49 min, method G, t_R = 21.95 min).

1-(3,4-Dimethylphenyl)-5-hydroxyoctan-3-one (**12p**)

Compound **12p** was prepared in 72% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11p** (83 mg, 0.337 mmol). R_f = 0.60 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.04 (d, J = 7.6 Hz, 1H), 6.94 (s, 1H), 6.90 (d, J = 7.6 Hz, 1H), 4.05–4.01 (m, 1H), 2.94 (d, J = 3.5 Hz, 1H), 2.83 (t, J = 7.6 Hz, 2H), 2.73 (t, J = 7.6 Hz, 2H), 2.58–2.55 (m, 1H), 2.51–2.46 (m, 1H), 2.23 (s, 3H), 2.22 (s, 3H), 1.49–1.31 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.43, 138.14, 136.67, 134.35, 129.80, 129.64, 125.57, 67.37, 49.32, 45.29, 38.63, 29.12, 19.75, 19.31, 18.67, 14.00. HRMS *m*/*z* calculated for $C_{16}H_{24}O_2$ $[M–H]^-$: 247.1703; found: 247.1725. >95% purity (as determined by RP-HPLC, method C, t_R = 31.01 min, method G, t_R = 21.80 min).

1-(4-Fluoro-3-methoxyphenyl)-5-hydroxyoctan-3-one (**12q**)

Compound **12q** was prepared in 62% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11q** (48 mg, 0.180 mmol). R_f = 0.50 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.97 (dd, J = 8.2 Hz, 11.3 Hz, 1H), 6.78 (dd, J = 8.1 and 1.9 Hz, 1H), 6.69–6.67 (m, 1H), 4.06–4.03 (m, 1H), 3.87 (s, 3H), 2.88–2.84 (m, 3H), 2.75 (t, J = 7.4 Hz, 2H), 2.58–2.55 (m, 1H), 2.52–2.48 (m, 1H), 1.50–1.31 (m, 4H), 0.92 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.91, 151.86, 150.24, 147.46,

147.39, 137.10, 137.08, 120.30, 120.25, 115.95, 115.83, 113.67, 67.38, 56.23, 49.40, 45.10, 38.63, 29.15, 18.64, 13.96. HRMS *m*/*z* calculated for $C_{15}H_{21}FO_3$ $[M–H]^-$: 267.1402; found: 267.1426. >95% purity (as determined by RP-HPLC, method C, t_R = 14.58 min, method G, t_R = 14.25 min).

1-(3-Fluoro-5-methoxyphenyl)-5-hydroxyoctan-3-one (**12r**)

Compound **12r** was prepared in 40% yield as a colorless oil, by following the same procedure as described in the general procedure B with **11r** (43 mg, 0.162 mmol). R_f = 0.45 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.51 (s, 1H), 6.49–6.44 (m, 2H), 4.06–4.04 (m, 1H), 3.77 (s, 3H), 2.86–2.84 (m, 3H), 2.75 (t, *J* = 7.6 Hz, 2H), 2.59–2.55 (m, 1H), 2.52–2.48 (m, 1H), 1.50–1.32 (m, 4H), 0.92 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.64, 164.48, 162.85, 160.97, 160.90, 143.85, 143.79, 110.03, 110.01, 107.45, 107.31, 99.37, 99.20, 67.37, 55.47, 49.34, 44.53, 38.62, 29.38, 29.36, 18.65, 13.96. HRMS *m*/*z* calculated for $C_{15}H_{21}FO_3$ $[M–H]^-$: 267.1402; found: 267.1419. >95% purity (as determined by RP-HPLC, method C, t_R = 17.95 min, method G, t_R = 22.68 min).

(E)-1-(3,4-Difluorophenyl)oct-1-en-3-one (**13a**)

To a solution of 3,4-difluorobenzaldehyde (500 mg, 3.52 mmol) in MeOH (8 mL) was added (L)-proline (61.0 mg, 0.53 mmol) and heptan-2-one (0.49 mL, 3.52 mmol) at 25 ℃ under argon. After 30 min, trimethylamine (0.12 mL, 0.88 mmol) was introduced. The reaction mixture was stirred at 25 ℃ for 72 h and then quenched with water and extracted with EtOAc (3 × 25 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel

(Hexane/EtOAc = 12:1 to 6:1, v/v) to furnish compound **13a** in 50% yield as colorless oil. R_f = 0.75 (Hexane/EtOAc = 4:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (d, J = 16.1 Hz, 1H), 7.40–7.35 (m, 1H), 7.30–7.26 (m, 1H), 7.21–7.15 (m, 1H), 6.65 (d, J = 16.1 Hz, 1H), 2.64 (t, J = 7.4 Hz, 2H), 1.72–1.63 (m, 2H), 1.40–1.29 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.05, 152.38, 152.29, 151.48, 151.40, 150.69, 150.61, 149.83, 149.74, 139.76, 131.93, 131.90, 131.86, 126.95, 126.93, 125.05, 125.02, 125.00, 124.98, 117.90, 117.78, 116.41, 116.30, 41.27, 31.46, 23.91, 22.48, 13.92. HRMS m/z calculated for $C_{14}H_{16}F_2O$ $[M–H]^-$: 237.1096; found: 237.1126. >95% purity (as determined by RP-HPLC, method E, t_R = 16.72 min, method I, t_R = 11.93 min).

(E)-1-(4-Hydroxy-3-methoxyphenyl)oct-1-en-3-one (**13b**)

Compound **13b** was prepared in 30% yield as a yellow oil, by following the same procedure as described for the synthesis **13a** but with vanillin (500 mg, 3.29 mmol) instead of 3,4-difluorobenaldehyde. R_f = 0.44 (Hexane/EtOAc = 5:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 (d, J = 16.0 Hz, 1H), 7.10 (dd, J = 1.6 and 8.2 Hz, 1H), 7.05 (d, J = 1.7 Hz, 1H), 6.93 (d, J = 8.2 Hz, 1H), 6.60 (d, J = 15.6 Hz, 1H), 6.00 (brs, 1H), 3.93 (s, 3H), 2.64 (t, J = 7.2 Hz, 2H), 1.81–1.69 (m, 2H), 1.49–1.21 (m, 4H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.93, 148.23, 146.94, 142.74, 127.05, 124.06, 123.38, 114.88, 109.52, 55.95, 40.64, 31.56, 24.26, 22.52, 13.97. HRMS m/z calculated for $C_{15}H_{20}O_3$ $[M–H]^-$: 247.1339; found: 247.1406. >95% purity (as determined by RP-HPLC, method C, t_R = 18.10 min, method H, t_R = 13.32 min).

1-(3,4-Difluorophenyl)octan-3-one (**14a**)

Compound **14a** was prepared in 76% yield as a colorless oil, by following the same procedure as described in the general procedure B with **13a** (30 mg, 0.126 mmol). R_f = 0.77 (Hexane/EtOAc = 4:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.10–7.01 (m, 1H), 7.01–6.94 (m, 1H), 6.92–6.82 (m, 1H), 2.85 (t, J = 7.4 Hz, 2H), 2.70 (t, J = 7.4 Hz, 2H), 2.37 (t, J = 7.4 Hz, 2H), 1.59–1.51 (m, 2H), 1.34–1.19 (m, 4H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 209.73, 151.00, 150.92, 149.70, 149.62, 149.36, 149.28, 148.07, 147.99, 138.20, 138.17, 138.14, 124.24, 124.22, 124.21, 124.19, 117.21, 117.12, 117.10, 117.01, 43.80, 43.05, 31.36, 28.80, 23.47, 22.43, 13.89. HRMS *m*/*z* calculated for $C_{14}H_{18}F_2O$ $[M–H]^-$: 239.1253; found: 239.1288. >95% purity (as determined by RP-HPLC, method H, t_R = 10.08 min, method I, t_R = 11.68 min).

1-(4-Hydroxy-3-methoxyphenyl)octan-3-one (**14b**)

Compound **14b** was prepared in 75% yield as a colorless oil, by following the same procedure as described in the general procedure B with **13b** (21 mg, 0.085 mmol). R_f = 0.51 (Hexane/EtOAc = 5:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.81 (d, J = 7.8 Hz, 1H), 6.69 (s, 1H), 6.67 (d, J = 7.8 Hz, 1H), 5.47 (brs, 1H), 3.87 (s, 3H), 2.82 (t, J = 7.8 Hz, 2H), 2.69 (t, J = 7.8 Hz, 2H), 2.37 (t, J = 7.8 Hz, 2H), 1.68–1.51 (m, 2H), 1.38–1.21 (m, 6H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.65, 146.38, 143.87, 133.15, 120.78, 114.31, 111.06, 55.88, 44.63, 43.12, 31.40, 30.33, 29.71, 29.56, 23.50, 22.46, 13.92. HRMS *m*/*z* calculated for $C_{15}H22O_3$ $[M–H]^-$: 249.1496; found: 249.1531. >95% purity (as determined by RP-HPLC, method C, t_R = 17.50 min, method H, t_R = 9.64 min).

(E)-1-(3,4-Difluorophenyl)oct-1-en-3-ol (**15a**)

To a stirred solution of **13a** (55.0 mg, 0.231 mmol) in MeOH (8 mL) was added $NaBH_4$ (12.2 mg, 0.315 mmol) at 0 ℃. The reaction mixture was stirred for 1 h at the room temperature. The reaction mixture was concentrated, then extracted with EtOAc (3 × 25 mL). The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 8:1, v/v) to furnish Compound **15a** in 94% yield as colorless oil. R_f = 0.51 (Hexane/EtOAc = 4:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.23–7.16 (m, 1H), 7.14–7.06 (m, 2H), 6.50 (d, J = 15.9 Hz, 1H), 6.16 (dd, J = 6.5 and 13.1 Hz, 1H), 4.29 (q, J = 6.5 Hz, 1H), 1.78 (brs, 1H), 1.71–1.55 (m, 2H), 1.51–1.25 (m, 6H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.25, 150.65, 150.57, 149.70, 149.61, 149.00, 148.92, 134.12, 134.08, 134.05, 133.76, 133.75, 128.02, 122.66, 122.64, 122.62, 122.60, 117.34, 117.23, 114.79, 114.68, 72.73, 37.35, 31.76, 25.10, 22.60, 14.03. HRMS m/z calculated for $C_{14}H_{18}F_2O$ $[M–H]^-$: 239.1253; found: 239.1290. >95% purity (as determined by RP-HPLC, method E, t_R = 10.05 min, method I, t_R = 9.67 min).

(E)-4-(3-Hydroxyoct-1-en-1-yl)-2-methoxyphenol (**15b**)

Compound **15b** was prepared in 90% yield as a brown oil, by following the same procedure as described for the synthesis **15a** but with **13b** (31 mg, 0.125 mmol) instead of **13a**. R_f = 0.36 (Hexane/EtOAc = 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.94 (d, J = 1.6 Hz, 1H), 6.92–6.88 (m, 2H), 6.50 (d, J = 15.8 Hz, 1H), 6.08 (q, J = 7.1 Hz, 1H), 5.67 (s, 1H), 4.27 (dd, J = 6.5 and 13.3 Hz, 1H), 3.93 (s, 3H), 1.72–1.58 (m, 4H), 1.37–1.30 (m, 4H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.63, 145.52, 130.33, 130.31, 129.33, 120.29, 114.4, 108.27, 73.33, 55.90, 37.42, 31.81, 25.21, 22.63, 14.07. HRMS m/z calculated for $C_{15}H_{22}O_3$ $[M–H]^-$: 249.1496; found: 249.1525. >95% purity (as determined by RP-HPLC, method C, t_R = 11.86 min, method H, t_R = 9.02 min).

1-(3,4-Difluorophenyl)octan-3-ol (**16a**)

Compound **16a** was prepared in 60% yield as a colorless oil, by following the same procedure as described in the general procedure B with **15a** (30 mg, 0.125 mmol). R_f = 0.58 (Hexane/EtOAc = 4:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.09–7.02 (m, 1H), 7.02–6.96 (m, 1H), 6.93–6.86 (m, 1H), 3.64–3.55 (m, 1H), 2.81–2.72 (m, 1H), 2.67–2.59 (m, 1H), 1.80–1.64 (m, 2H), 1.53–1.23 (m, 9H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.02, 150.94, 149.54, 149.45, 149.38, 149.30, 147.91, 147.83, 139.23, 139.20, 139.17, 124.18, 124.16, 124.14, 124.12, 117.14, 117.03, 117.01, 116.90, 71.09, 38.80, 37.68, 31.84, 31.24, 25.28, 22.62, 14.02. HRMS *m*/*z* calculated for $C_{14}H_{20}F_2O$ $[M–H]^-$: 241.1409; found: 241.1452. >95% purity (as determined by RP-HPLC, method E, t_R = 12.84 min, method I, t_R = 14.86 min).

4-((tert-Butyldimethylsilyl)oxy)-3-methoxybenzaldehyde (**17**)

To a stirred solution of vanillin (1.00 g, 6.57 mmol) was dissolved in dry CH_2Cl_2 (50 mL) was added imidazole (1.29 g, 18.96 mmol) and *tert*-butyldimethylsilyl chloride (1.42 g, 9.39 mmol) at 0 ℃. The reaction mixture was stirred under argon for 16 h at room temperature, quenched with water, and extracted with CH_2Cl_2 (3 × 25 mL). The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 4:1, v/v) to furnish compound **17** in 94% yield as a colorless oil. R_f = 0.89 (Hexane/EtOAc = 1:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.85 (s, 1H), 7.40 (d, J = 1.9 Hz, 1H), 7.37 (dd, J = 4.0 and 8.0 Hz, 1H),

6.97 (d, J = 8.0 Hz, 1H), 3.87 (s, 3H), 1.00 (s, 9H), 0.20 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.19, 151.80, 151.51, 131.09, 126.40, 120.87, 110.25, 55.61, 25.74, 18.66, –4.41.

tert-Butyl(4-(2,2-dibromovinyl)-2-methoxyphenoxy)dimethylsilane (**18**)

To a stirred solution of CBr_4 (3.37 g, 10.14 mmol) was dissolved in dry CH_2Cl_2 (30 mL) was slowly added PPh_3 (5.32 g, 20.28 mmol) at 0 ℃. After stirring under argon for 1 h at the same temperature, a solution of compound **17** (1.35 g, 5.07 mmol) in dry CH_2Cl_2 (10 mL) was slowly added over 10 min. The reaction mixture was stirred under argon for 2 h and extracted with CH_2Cl_2 (3 × 25 mL). The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 15:1, v/v) to furnish compound **18** in 98 % yield as a colorless oil. R_f = 0.87 (Hexane/EtOAc = 8:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (s, 1H), 7.19 (d, J = 2.0 Hz, 1H), 7.03 (dd, J = 4.1 and 8.3 Hz, 1H), 6.84 (d, J = 8.2 Hz, 1H), 3.83 (s, 3H), 1.02 (s, 9H), 0.19 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.62, 145.64, 136.59, 128.77, 121.96, 120.68, 112.03, 87.10, 55.51, 25.69, 25.66, 18.47, –4.58.

4-(2,2-Dibromovinyl)-1,2-difluorobenzene (**28**)

Compound **28** was prepared in 97% yield as a colorless oil, by following the same procedure as described for the synthesis **18** but with 3,4-difluorobenzaldehyde (1.35 g, 9.50 mmol) instead of **17**. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 10:1, v/v). R_f = 0.85 (Hexane/EtOAc = 4:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (q, J = 9.6 Hz, 1H), 7.39 (s, 1H), 7.23–7.20 (m, 1H), 7.15 (q, J = 9.1 Hz, 1H); ^{13}C NMR (150 MHz,

$CDCl_3$) δ 151.01, 150.94, 150.86, 149.35, 149.29, 149.27, 149.21, 134.73, 132.19, 132.15, 125.18, 125.15, 125.13, 125.11, 117.42, 117.30, 117.26, 117.14, 116.60, 91.07, 91.06.

tert-Butyl(4-ethynyl-2-methoxyphenoxy)dimethylsilane (**19**)

To a stirred solution of compound **18** (1.41 g, 3.34 mmol) in dry THF (20 mL) was added *n*-BuLi (1.6 M in hexanes, 5.30 mL, 8.35 mmol) at –78 ℃. The reaction mixture was stirred under argon for 2 h at the same temperature, quenched with aqueous NH_4Cl (10 mL) and extracted with EtOAc (3 × 25 mL). The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 16:1 to 10:1, v/v) to furnish compound **19** in 96% yield as a colorless oil. R_f = 0.80 (Hexane/EtOAc = 8:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.00 (dd, J = 4.0 and 8.1 Hz, 1H), 6.97 (d, J = 1.9 Hz, 1H), 3.80 (s, 3H), 2.99 (s, 1H), 0.99 (s, 9H), 0.15 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.66, 146.23, 125.46, 120.90, 115.66, 115.04, 83.96, 75.57, 55.45, 25.67, 18.47, –4.64.

4-Ethynyl-1,2-difluorobenzene (**29**)

To a stirred solution of compound **28** (800 mg, 2.69 mmol) in dry THF (40 mL) was added *n*-BuLi (1.6 M in hexanes, 4.30 mL, 6.71 mmol) at –78 ℃. The reaction mixture was stirred under argon for 2 h at the same temperature, quenched with aqueous NH_4Cl (10 mL) and extracted with hexane (3 × 25 mL). The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure at low temperature (20 ℃) to provide compound **29** which was used in the next step without further purification.

General procedure C for compounds **20**, and **30**–**32**

To a stirred solution of appropriate ethynylbenzene (400 mg) in THF (40 mL) was added *n*-BuLi (1.1 eq.) at –78 ℃. The solution was stirred under argon for 1 h at the same temperature then appropriate Weinreb amides (1.5 eq.) was added dropwise. The reaction mixture was stirred under argon at the same temperature until TLC analysis indicated complete conversion (typically 10–12 h), quenched with aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 × 25 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (Hexane/ether = 120:1 to 80:1, v/v) to furnish compounds **20**, and **30**–**32**.

1-(4-((tert-Butyldimethylsilyl)oxy)-3-methoxyphenyl)oct-1-yn-3-one (**20**)

Compound **20** was prepared in 75 % yield as a colorless oil, by following the same procedure as described in the general procedure C with **19** (400 mg, 1.52 mmol) and *N*-methoxy-*N*-methylhexanamide (364 mg, 2.28 mmol). R_f = 0.75 (Hexane/EtOAc = 8:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.12 (d, J = 8.1 Hz, 1H), 7.07 (s, 1H), 6.84 (d, J = 8.2 Hz, 1H), 3.82 (s, 3H), 2.65 (t, J = 7.4 Hz, 2H), 1.77–1.75 (m, 2H), 1.38–1.37 (m, 4H), 1.00 (s, 9H), 0.93 (t, J = 6.5 Hz, 3H), 0.18 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.36, 150.90, 148.19, 127.18, 121.14, 116.48, 112.74, 91.88, 87.48, 55.51, 45.41, 31.21, 25.62, 23.99, 22.43, 18.48, 13.92, –4.59.

1-(3,4-Difluorophenyl)hex-1-yn-3-one (**30**)

Compound **30** was prepared in 72% yield as a colorless oil, by following the same procedure as described in the general procedure C with **29** (400 mg, 2.90 mmol) and *N*-methoxy-*N*-methylbutyramide (577 mg, 4.40 mmol). R_f = 0.69 (Hexane/Ether = 6:1, v/v). ^{1}H NMR (600

MHz, $CDCl_3$) δ 7.39 (t, J = 7.9 Hz, 1H), 7.36–7.32 (m, 1H), 7.19 (q, J = 8.4 Hz, 1H), 2.64 (t, J = 7.4 Hz, 2H), 1.82–1.72 (m, 2H), 1.00 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.63, 152.92, 152.84, 151.23, 151.14, 150.93, 150.84, 149.26, 149.18, 130.06, 130.04, 130.02, 130.00, 121.97, 121.85, 118.06, 117.94, 116.94, 116.91, 116.89, 116.86, 87.83, 87.53, 47.29, 17.55, 13.48. HRMS *m*/*z* calculated for $C_{12}H_{10}F_2O$ $[M–H]^-$: 207.0627; found: 207.0630. >95% purity (as determined by RP-HPLC, method E, t_R = 8.91 min, method I, t_R = 5.56 min).

1-(3,4-Difluorophenyl)hept-1-yn-3-one (**31**)

Compound **31** was prepared in 88% yield as a colorless oil, by following the same procedure as described in the general procedure C with **29** (400 mg, 2.90 mmol), *N*-methoxy-*N*-methylpentanamide (638 mg, 4.40 mmol). R_f = 0.72 (Hexane/Ether = 6:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (t, J = 7.9 Hz, 1H), 7.36–7.29 (m, 1H), 7.19 (q, J = 8.4 Hz, 1H), 2.66 (t, J = 7.4 Hz, 2H), 1.76–1.68 (m, 2H), 1.44–1.35 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.82, 152.96, 152.88, 151.27, 151.19, 150.97, 150.88, 149.31, 149.22, 130.07, 130.05, 130.03, 130.00, 122.03, 121.90, 118.09, 117.97, 116.88, 87.89, 87.60, 45.21, 26.12, 22.14, 13.87. HRMS *m*/*z* calculated for $C_{13}H_{12}F_2O$ $[M–H]^-$: 221.0783; found: 221.0813. >95% purity (as determined by RP-HPLC, method E, t_R = 12.70 min, method I, t_R = 7.93 min).

1-(3,4-Difluorophenyl)oct-1-yn-3-one (**32**)

Compound **32** was prepared in 72% yield as a colorless oil, by following the same procedure as described in the general procedure C with **29** (400 mg, 2.90 mmol), *N*-methoxy-*N*-methylhexanamide (694 mg, 4.40 mmol). R_f = 0.79 (Hexane/EtOAc = 6:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (t, J = 7.9 Hz, 1H), 7.36–7.29 (m, 1H), 7.19 (q, J = 8.4 Hz, 1H), 2.65 (t,

J = 7.4 Hz, 2H), 1.77–1.69 (m, 2H), 1.41–1.30 (m, 4H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.87, 152.89, 151.28, 150.89, 149.23, 130.07, 130.04, 130.02, 130.00, 122.03, 121.91, 118.10, 117.97, 116.96, 87.89, 87.62, 45.46, 31.13, 23.74, 22.39, 13.89. HRMS *m*/*z* calculated for $C_{14}H_{14}F_2O$ $[M–H]^-$: 235.0940; found: 235.0971. >95% purity (as determined by RP-HPLC, method E, t_R = 18.50 min, method I, t_R = 12.86 min).

General procedure D for compounds **21**, **23a**, **23b**, **23c**, **25**, and **27**

To a stirred solution of the silyl protected compound in THF (5 mL) was added tetrabutylammonium fluoride solution (1M in THF, 2.0 eq.) at 0 ℃. The reaction mixture was stirred under argon at the same temperature until TLC analysis indicated complete conversion (typically 1 h), quenched with aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 × 25 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc) to furnish compound.

1-(4-Hydroxy-3-methoxyphenyl)oct-1-yn-3-one (**21**)

Compound **21** (27.0 mg, 0.075 mmol) was prepared in 94% yield as a white oil, by following the same procedure as described in the general procedure D with **20**. R_f = 0.21 (Hexane/EtOAc = 5:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.17 (dd, *J* = 1.8 and 7.8 Hz, 1H), 7.06 (d, *J* = 1.8 Hz, 1H), 6.91 (d, *J* = 7.8 Hz), 5.99 (brs, 1H), 3.91 (s, 3H), 2.64 (t, *J* = 7.8 Hz, 2H), 1.77–1.71 (m, 2H), 1.42–1.33 (m, 4H), 0.92 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.40, 148.53, 146.35, 128.02, 115.18, 114.87, 111.33, 91.93, 87.29, 56.10, 45.37, 31.20, 23.98, 22.43,

13.92. HRMS *m/z* calculated for $C_{15}H_{18}O_3$ $[M–H]^-$: 245.1183; found: 245.1261. >95% purity (as determined by RP-HPLC, method F, t_R = 5.67 min, method H, t_R = 19.33 min).

4-(3-Hydroxyoct-1-yn-1-yl)-2-methoxyphenol (**23a**)

Compound **23a** (28.0 mg, 0.077 mmol) was prepared in 94% yield as a white oil, by following the same procedure as described in the general procedure D with **22a**. R_f = 0.21 (Hexane/EtOAc = 5:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.98 (dd, J = 1.8 and 15.6 Hz, 1H), 6.92 (d, J = 1.8 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 5.77 (brs, 1H), 4.58 (t, J = 6.6 Hz, 1H), 3.88 (s, 3H), 1.92 (brs, 1H), 1.89–1.79 (m, 2H), 1.45–1.32 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.28, 146.13, 125.66, 114.49, 114.23, 113.95, 88.33, 84.97, 63.09, 55.98, 37.97, 31.50, 24.95, 22.59, 14.03. HRMS *m/z* calculated for $C_{15}H_{20}O_3$ $[M–H]^-$: 247.1339; found: 247.1397. >95% purity (as determined by RP-HPLC, method C, t_R = 12.46 min, method H, t_R = 10.92 min).

(R)-4-(3-Hydroxyoct-1-yn-1-yl)-2-methoxyphenol (**23b**)

Compound **23b** (28.0 mg, 0.077 mmol) was prepared in 94% yield as a white oil, by following the same procedure as described in the general procedure D with **22b**. R_f = 0.21 (Hexane/EtOAc = 5:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.98 (dd, J = 1.7 and 8.2 Hz, 1H), 6.92 (d, J = 1.7 Hz, 1H), 6.84 (d, J = 8.2 Hz, 1H), 5.72 (brs, 1H), 4.58 (q, J = 6.4 Hz, 1H), 3.89 (s, 3H), 1.87–1.73 (m, 3H), 1.58–1.47 (m, 2H), 1.39–1.30 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.29, 146.12, 125.67, 114.46, 114.23, 113.93, 88.32, 84.97, 63.10, 55.98, 37.98, 31.50, 24.94, 22.59, 14.03. HRMS *m/z* calculated for $C_{15}H_{20}O_3$ $[M–H]^-$: 247.1339;

found: 247.1397. >95% purity (as determined by RP-HPLC, method C, t_R = 7.43 min, method H, t_R = 10.95 min).

(S)-4-(3-Hydroxyoct-1-yn-1-yl)-2-methoxyphenol (**23c**)

Compound **23c** (20.0 mg, 0.055 mmol) was prepared in 93% yield as a yellow oil, by following the same procedure as described in the general procedure D with **22c**. R_f = 0.21 (Hexane/EtOAc = 5:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.97 (t, J = 6.6 Hz, 1H), 6.92 (d, J = 1.8 Hz, 1H), 6.84 (d, J = 7.8 Hz, 1H), 4.58 (t, J = 6.6 Hz, 1H), 3.88 (s, 3H), 1.89–1.76 (m, 2H), 1.63–1.55 (m, 2H), 1.43–1.31 (m, 4H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.30, 146.15, 125.65, 114.50, 114.21, 113.96, 88.34, 84.97, 63.09, 55.97, 37.97, 31.50, 24.95, 22.59, 14.03. HRMS *m*/*z* calculated for $C_{15}H_{20}O_3$ $[M–H]^-$: 247.1339; found: 247.1415. >95% purity (as determined by RP-HPLC, method C, t_R = 7.70 min, method H, t_R = 10.95 min).

1-(4-((tert-Butyldimethylsilyl)oxy)-3-methoxyphenyl)oct-1-yn-3-ol (**22a**)

To a stirred solution of **20** (210 mg, 0.582 mmol) in MeOH 15 mL was added $NaBH_4$ (33.0 mg, 0.874 mmol) at 0 ℃. The reaction mixture was stirred under argon for 1 h at the room temperature. The reaction mixture was concentrated, then extracted with EtOAc (3 × 25 mL). The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 8:1, v/v) to furnish compound **22a** in 77% yield as colorless oil. R_f = 0.59 (Hexane/EtOAc = 4:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.94–6.92 (m, 2H), 6.77 (d, J = 7.9 Hz, 1H), 4.58 (q, J = 6.4 Hz , 1H), 3.79 (s, 3H), 1.92 (d, J = 5.2 Hz, 1H), 1.81–1.76 (m, 2H), 1.53–1.49 (m, 2H), 1.35–1.34 (m, 4H), 0.98 (s, 9H), 0.91 (t, J = 7.0 Hz, 3H), 0.15 (s, 6H); ^{13}C

NMR (150 MHz, $CDCl_3$) δ 150.66, 145.84, 124.96, 120.90, 115.69, 115.31, 88.72, 85.01, 63.08, 55.44, 37.97, 31.51, 25.68, 24.95, 22.60, 18.47, 14.04, –4.65.

(Z)-4-(3-Hydroxyoct-1-en-1-yl)-2-methoxyphenol (**25**)

To a solution of **22a** (70.0 mg, 0.19 mmol) in MeOH (3 mL) was added quinoline (7 mg, 0.06 mmol) and Lindlar catalyst (7 mg, 0.02 mmol) and the mixture was stirred at 0 ℃ for 1 h under an atmosphere of hydrogen (balloon). After the complete conversion of compound **22a** (TLC, toluene/EtOAc = 10:1, v/v), the reaction mixture was filtered through a pad of Celite, and washed with MeOH (3 × 5 mL). The combined filtrates were concentrated under reduced pressure to give the crude product, which was purified by silica gel column chromatography (toluene/EtOAc = 10:1, v/v) to furnish compound **24** as colorless oil. R_f = 0.36 (toluene/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.83 (d, J = 1.8 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.77 (dd, J = 1.8 and 8.1 Hz, 1H), 6.46 (d, J = 11.4 Hz, 1H), 5.57 (dd, J = 9.0 and 12.0 Hz , 1H), 4.60 (dd, J = 7.2 and 15.3 Hz , 1H), 3.80 (s, 3H), 1.69–1.62 (m, 1H), 1.61 (brs, 1H), 1.59–1.52 (m, 1H), 1.46–1.21 (m, 6H), 1.00 (s, 9H), 0.88 (t, J = 6.6 Hz, 3H), 0.16 (s, 6H).

Compound **25** (34 mg, 0.14 mmol) was prepared in 70% yield (for two steps) as a colorless oil, by following the same procedure as described in the general procedure D with **24**. R_f = 0.21 (tolene/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.89 (d, J = 8.4 Hz, 1H), 6.88–6.86 (m, 1H), 6.85–6.81 (m, 1H), 6.48 (d, J = 11.4 Hz, 1H), 5.64 (s, 1H), 5.57 (dd, J = 9.0 and 11.7 Hz, 1H), 4.56–4.51 (m, 1H), 3.90 (s, 3H), 1.71–1.63 (m, 1H), 1.61–1.54 (m, 1H), 1.52 (brs, 1H), 1.46–1.23 (m, 6H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.36, 145.14, 133.27, 131.25, 129.15, 122.29, 114.34, 111.50, 68.20, 56.01, 37.94, 31.94, 25.31, 22.74, 14.13. HRMS m/z calculated for $C_{15}H_{22}O_3$ $[M–H]^-$: 249.1496; found: 249.1523. >95% purity (as determined by RP-HPLC, method C, t_R = 11.09 min, method H, t_R = 8.78 min).

1-(4-((tert-Butyldimethylsilyl)oxy)-3-methoxyphenyl)octan-3-ol (**26**)

Compound **26** was prepared in 94% yield as a colorless oil, by following the same procedure as described in the general procedure B with **22a** (33.0 mg, 0.091 mmol). R_f = 0.60 (Hexane/EtOAc = 5:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.75 (d, J = 8.0 Hz, 1H), 6.69 (d, J = 2.0 Hz, 1H), 6.64 (dd, J = 2.0 and 8.0 Hz, 1H), 3.79 (s, 3H), 3.65–3.57 (m, 1H), 2.75–2.66 (m, 1H), 2.64–2.55 (m, 1H), 1.81–1.66 (m, 2H), 1.54–1.38 (m, 3H), 1.38–1.22 (m, 6H), 0.99 (s, 9H), 0.87 (t, J = 7.2 Hz, 3H), 0.14 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ150.75, 143.04, 135.66, 120.72, 120.41, 112.50, 71.50, 55.50, 39.22, 37.59, 31.89, 31.81, 25.76, 25.32, 22.65, 18.45, 14.06, –4.63.

1-(4-Hydroxy-3-methoxyphenyl)octan-3-one (**27**)

Compound **27** was prepared in 94% yield as a white oil, by following the same procedure as described in the general procedure D with **26** (32.0 mg, 0.087 mmol). R_f = 0.21 (Hexane/EtOAc = 5:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.83 (d, J = 7.9 Hz, 1H), 6.72–6.67 (m, 2H), 5.50 (brs, 1H), 3.87 (s, 3H), 3.65–3.59 (m, 1H), 2.77–2.69 (m, 1H), 2.65–2.55 (m, 1H), 1.82–1.66 (m, 2H), 1.53–1.37 (m, 4H), 1.37–1.22 (m, 6H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.41, 143.68, 134.13, 120.90, 114.26, 110.99, 71.52, 55.88, 39.37, 37.59, 31.89, 31.80, 25.30, 22.64, 14.05. HRMS m/z calculated for $C_{15}H_{24}O_3$ $[M–H]^-$: 251.1652; found: 251.1683. >95% purity (as determined by RP-HPLC, method C, t_R = 5.37 min, method G, t_R = 29.83 min).

1-(3,4-Difluorophenyl)hex-1-yn-3-ol (**33a**)

Compound **33a** was prepared in 88% yield as a colorless oil, by following the same procedure as described for the synthesis **22a** but with **30** (50.0 mg, 0.240 mmol) instead of **20**. R_f = 0.25 (Hexane/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26–7.20 (m, 1H), 7.18–7.13 (m, 1H), 7.12–7.05 (m, 1H), 4.59 (t, J = 6.7 Hz, 1H), 2.11 (brs, 1H), 1.83–1.72 (m, 2H), 1.59–1.49 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.46, 151.37, 150.78, 150.69, 149.79, 149.71, 149.13, 149.04, 135.49, 128.34, 128.31, 128.30, 128.27, 120.69, 120.56, 119.56, 119.53, 119.51, 119.48, 117.48, 117.36, 90.77, 90.75, 82.73, 62.63, 39.83, 18.48, 13.74. HRMS *m*/*z* calculated for $C_{12}H_{12}F_2O$ $[M–H]^–$: 209.0783; found: 209.0820. >95% purity (as determined by RP-HPLC, method E, t_R = 5.43 min, method I, t_R = 4.90 min).

1-(3,4-Difluorophenyl)hept-1-yn-3-ol (**34a**)

Compound **34a** was prepared in 80% yield as a colorless oil, by following the same procedure as described for the synthesis **22a** but with **31** (17.0 mg, 0.076 mmol) instead of **20**. R_f = 0.29 (Hexane/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26–7.21 (m, 1H), 7.18–7.14 (m, 1H), 7.10 (q, J = 8.4 Hz, 1H), 4.57 (dd, J = 6.4 and 12.3 Hz, 1H), 1.88 (d, J = 5.3 Hz, 1H), 1.85–1.73 (m, 2H), 1.55–1.43 (m, 2H), 1.43–1.34 (m, 2H), 0.94 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.39, 150.79, 150.71, 149.81, 149.72, 149.15, 128.36, 128.33, 128.31, 128.29, 120.71, 120.59, 119.56, 119.50, 117.50, 117.38, 90.76, 82.75, 62.89, 37.50, 27.34, 22.38, 14.01. HRMS *m*/*z* calculated for $C_{13}H_{14}F_2O$ $[M–H]^–$: 223.0940; found: 223.0966. >95% purity (as determined by RP-HPLC, method E, t_R = 7.35 min, method I, t_R = 6.77 min).

1-(3,4-Difluorophenyl)oct-1-yn-3-ol (**35a**)

Compound **35a** was prepared in 86% yield as a colorless oil, by following the same procedure as described for the synthesis **22a** but with **32** (58.0 mg, 0.245 mmol) instead of **20**. R_f = 0.35 (Hexane/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26–7.20 (m, 1H), 7.18–7.13 (m, 1H), 7.09 (q, J = 8.4 Hz, 1H), 4.57 (dd, J = 6.4 and 12.3 Hz, 1H), 2.00 (d, J = 5.6 Hz, 1H), 1.86–1.71 (m, 2H), 1.57–1.43 (m, 2H), 1.40–1.21 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.38, 150.79, 150.70, 149.80, 149.71, 149.14, 149.05, 128.35, 128.32, 128.31, 128.28, 120.70, 120.57, 119.56, 119.54, 119.51, 119.49, 117.49, 117.37, 90.78, 82.74, 62.89, 37.74, 31.45, 24.87, 22.56, 14.00. HRMS *m/z* calculated for $C_{14}H_{16}F_2O$ $[M–H]^-$: 237.1096; found: 237.1138. >95% purity (as determined by RP-HPLC, method E, t_R = 10.34 min, method I, t_R = 9.96 min).

General procedure E for compounds **22b**, **22c**, **33b**, **34b**, **35b**, **33c**, **34c**, and **35c**

A 0.1 M solution of an ynone compound in 2-propanol was added KOH and catalyst with the ratio of ynone : catalyst : KOH = 200:1:1.2. The reaction mixture was stirred under argon at room temperature until TLC analysis indicated complete conversion (typically 4 h) and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel to furnish stereoselective compounds. The *ee* values were determined by the chiral HPLC analyses on a chiral column (CHIRALPAK IG, 10% ethanol in hexane)

(R)-1-(4-((tert-Butyldimethylsilyl)oxy)-3-methoxyphenyl)oct-1-yn-3-ol (**22b**)

Compound **22b** was prepared in 88% yield as colorless oil following the same procedure as described in the general procedure E with **20** (150 mg, 0.416 mmol) in 2-propanol (4.16 mL),

RuCl[(*R*,*R*)-TsDPEN(mesitylene)] (1.29 mg, 2.08 μmol) and KOH (0.138 mg, 2.50 μmol). The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 12:1, v/v) to furnish compound **22b**. R_f = 0.59 (Hexane/EtOAc = 4:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.94–6.92 (m, 2H), 6.77 (d, J = 7.9 Hz, 1H), 4.58 (q, J = 6.4 Hz , 1H), 3.79 (s, 3H), 1.90 (d, J = 5.5 Hz, 1H), 1.81–1.76 (m, 2H), 1.53–1.50 (m, 2H), 1.36–1.33 (m, 4H), 0.98 (s, 9H), 0.91 (t, J = 7.0 Hz, 3H), 0.15 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.66, 145.84, 124.95, 120.90, 115.68, 115.30, 88.71, 85.02, 63.09, 55.44, 37.97, 31.51, 25.68, 24.95, 22.60, 18.47, 14.04, –4.65.

(S)-1-(4-((tert-Butyldimethylsilyl)oxy)-3-methoxyphenyl)oct-1-yn-3-ol (**22c**)

Compound **22c** was prepared in 68% yield as colorless oil following the same procedure as described in the general procedure E with **20** (150 mg, 0.416 mmol) in 2-propanol (4.16 mL), RuCl[(*S*,*S*)-TsDPEN(mesitylene)] (1.29 mg, 2.08 μmol) and KOH (0.138 mg, 2.50 μmol). The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 12:1, v/v) to furnish compound **22c**. R_f = 0.59 (Hexane/EtOAc = 4:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.94–6.92 (m, 2H), 6.77 (d, J = 7.9 Hz, 1H), 4.58 (t, J = 6.5 Hz, 1H), 3.79 (s, 3H), 1.91 (s, 1H), 1.81–1.76 (m, 2H), 1.53–1.49 (m, 2H), 1.35–1.34 (m, 4H), 0.98 (s, 9H), 0.91 (t, J = 7.0 Hz, 3H), 0.15 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.66, 145.84, 124.95, 120.90, 115.68, 115.31, 88.71, 85.01, 63.08, 55.44, 37.97, 31.51, 25.68, 24.95, 22.60, 18.47, 14.04, –4.65.

(R)-1-(3,4-Difluorophenyl)hex-1-yn-3-ol (**33b**)

Compound **33b** was prepared in 86% yield as colorless oil following the same procedure as described in the general procedure E with **30** (100 mg, 0.48 mmol) in 2-propanol (4.8 mL), RuCl[(*R*,*R*)-TsDPEN(mesitylene)] (1.49 mg, 2.40 μmol) and KOH (0.16 mg, 2.90 μmol). The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 20:1, v/v) to furnish compound **33b**. R_f = 0.25 (Hexane/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.25–7.19 (m, 1H), 7.18–7.13 (m, 1H), 7.12–7.04 (m, 1H), 4.59 (t, J = 2.9 Hz, 1H), 2.18 (brs, 1H), 1.83–1.72 (m, 2H), 1.58–1.49 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.45, 151.37, 150.78, 150.69, 149.79, 149.70, 149.12, 149.04, 128.34, 128.31, 128.29, 128.27, 120.68, 120.56, 119.56, 119.53, 119.51, 119.48, 117.48, 117.36, 90.77, 90.76, 82.72, 62.62, 39.83, 18.49, 13.74. HRMS *m*/*z* calculated for $C_{12}H_{12}F_2O$ $[M–H]^–$: 209.0783; found: 209.0811. >95% purity (as determined by RP-HPLC, method E, t_R = 5.42 min, method I, t_R = 4.86 min).

(R)-1-(3,4-Difluorophenyl)hept-1-yn-3-ol (**34b**)

Compound **34b** was prepared in 82% yield as colorless oil following the same procedure as described in the general procedure E with **31** (100 mg, 0.45 mmol) in 2-propanol (4.5 mL), RuCl[(*R*,*R*)-TsDPEN(mesitylene)] (1.40 mg, 2.30 μmol) and KOH (0.15 mg, 2.70 μmol). The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 20:1, v/v) to furnish compound **34b**. R_f = 0.29 (Hexane/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26–7.19 (m, 1H), 7.19–7.13 (m, 1H), 7.12–7.05 (m, 1H), 4.57 (t, J = 2.9 Hz, 1H), 2.18 (brs, 1H), 1.85–1.74 (m, 2H), 1.53–1.45 (m, 2H), 1.42–1.34 (m, 2H), 0.94 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.78, 150.69, 149.79, 149.70, 149.12, 149.04, 128.34, 128.32, 128.30, 128.28, 120.69, 120.56, 119.54, 119.49, 117.48, 117.36, 90.80, 82.71, 62.85, 37.48, 27.35, 22.37, 13.99. HRMS *m*/*z* calculated for $C_{13}H_{14}F_2O$ $[M–H]^–$: 223.0940; found:

223.0973. >95% purity (as determined by RP-HPLC, method E, t_R = 7.32 min, method I, t_R = 6.64 min).

(R)-1-(3,4-Difluorophenyl)oct-1-yn-3-ol (**35b**)

Compound **35b** was prepared in 80% yield as colorless oil following the same procedure as described in the general procedure E with **32** (100 mg, 0.42 mmol) in 2-propanol (4.2 mL), RuCl[(*R*,*R*)-TsDPEN(mesitylene)] (1.31 mg, 2.10 μmol) and KOH (0.14 mg, 2.50 μmol). The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 20:1, v/v) to furnish compound **35b**. R_f = 0.35 (Hexane/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.25–7.21 (m, 1H), 7.20–7.15 (m, 1H), 7.14–7.03 (m, 1H), 4.57 (dd, J = 6.0 and 10.8 Hz, 1H), 2.28 (d, J = 4.2 Hz, 1H), 1.89–1.72 (m, 2H), 1.58–1.42 (m, 2H), 1.38–1.33 (m, 4H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.45, 151.36, 150.77, 150.69, 149.78, 149.70, 149.12, 149.04, 128.33, 128.31, 128.29, 128.27, 120.68, 120.55, 119.58, 119.55, 119.53, 119.50, 117.47, 117.35, 90.82, 82.70, 62.85, 37.72, 31.44, 24.88, 22.56, 13.98. HRMS *m*/*z* calculated for $C_{14}H_{16}F_2O$ $[M–H]^-$: 237.1096; found: 237.1139. >95% purity (as determined by RP-HPLC, method E, t_R = 10.26 min, method I, t_R = 11.03 min).

(S)-1-(3,4-Difluorophenyl)hex-1-yn-3-ol (**33c**)

Compound **33c** was prepared in 86% yield as colorless oil following the same procedure as described in the general procedure E with **30** (100 mg, 0.48 mmol) in 2-propanol (4.8 mL), RuCl[(*S*,*S*)-TsDPEN(mesitylene)] (1.49 mg, 2.40 μmol) and KOH (0.16 mg, 2.90 μmol). The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 20:1, v/v) to furnish compound **33c**. R_f = 0.25 (Hexane/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz,

$CDCl_3$) δ 7.26–7.18 (m, 1H), 7.18–7.12 (m, 1H), 7.12–7.03 (m, 1H), 4.59 (t, J = 2.9 Hz, 1H), 2.39 (brs, 1H), 1.84–1.72 (m, 2H), 1.59–1.47 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.44, 151.36, 150.76, 150.68, 149.77, 149.69, 149.12, 149.03, 128.33, 128.30, 128.28, 128.26, 120.67, 120.54, 119.57, 119.54, 119.52, 119.49, 117.46, 117.35, 90.79, 82.71, 82.69, 62.59, 39.81, 18.49, 13.72. HRMS *m*/*z* calculated for $C_{12}H_{12}F_2O$ $[M–H]^-$: 209.0783; found: 209.0816. >95% purity (as determined by RP-HPLC, method E, t_R = 5.42 min, method I, t_R = 4.88 min).

(S)-1-(3,4-Difluorophenyl)hept-1-yn-3-ol (**34c**)

Compound **29c** was prepared in 82% yield as colorless oil following the same procedure as described in the general procedure E with **31** (100 mg, 0.45 mmol) in 2-propanol (4.5 mL), RuCl[(*S*,*S*)-TsDPEN(mesitylene)] (1.4 mg, 2.30 μmol) and KOH (0.15 mg, 2.70 μmol). The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 20:1, v/v) to furnish compound **34c**. R_f = 0.29 (Hexane/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26–7.19 (m, 1H), 7.19–7.12 (m, 1H), 7.12–7.04 (m, 1H), 4.57 (t, J = 2.9 Hz, 1H), 2.37 (brs, 1H), 1.86–1.72 (m, 2H), 1.54–1.43 (m, 2H), 1.43–1.34 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.44, 151.36, 150.77, 150.68, 149.77, 149.69, 149.12, 149.03, 128.33, 128.31, 128.29, 128.27, 120.67, 120.55, 119.58, 119.55, 119.53, 119.50, 117.46, 117.34, 90.83, 90.82, 82.69, 62.82, 37.47, 27.35, 22.37, 13.97. HRMS *m*/*z* calculated for $C_{13}H_{14}F_2O$ $[M–H]^-$: 223.0940; found: 223.0971. >95% purity (as determined by RP-HPLC, method E, t_R = 7.32 min, method I, t_R = 6.72 min).

(S)-1-(3,4-Difluorophenyl)oct-1-yn-3-ol (**35c**)

Compound **35c** was prepared in 82% yield as colorless oil following the same procedure as described in the general procedure E with **32** (100 mg, 0.42 mmol) in 2-propanol (4.2 mL), RuCl[(*S*,*S*)-TsDPEN(mesitylene)] (1.31 mg, 2.10 μmol) and KOH (0.14 mg, 2.50 μmol). The crude residue was purified by column chromatography on silica gel (Hexane/EtOAc = 20:1, v/v) to furnish compound **35c**. R_f = 0.35 (Hexane/EtOAc = 10:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26–7.19 (m, 1H), 7.19–7.12 (m, 1H), 7.12–7.04 (m, 1H), 4.57 (t, J = 2.9 Hz, 1H), 2.31 (brs, 1H), 1.85–1.72 (m, 2H), 1.56–1.44 (m, 2H), 1.39–1.28 (m, 4H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.44, 151.36, 150.77, 150.68, 149.78, 149.69, 149.12, 149.04, 128.33, 128.31, 128.29, 128.26, 120.67, 120.55, 119.58, 119.55, 119.53, 119.50, 117.47, 117.35, 90.82, 82.70, 62.85, 37.72, 31.44, 24.88, 22.56, 13.97. HRMS *m*/*z* calculated for $C_{14}H_{16}F_2O$ $[M–H]^-$: 237.1096; found: 237.1137. >95% purity (as determined by RP-HPLC, method E, t_R = 10.31 min, method I, t_R = 9.79 min).

Chiral HPLC analysis

Enantiomeric excess (*ee*) of the compounds was determined by the chiral HPLC analyses using a chiral column (CHIRALPAK IG (4.6 i.d × 250 mm)). Chromatographic analysis was performed on high-performance liquid chromatography (Agilent 1260 series) by two methods (A and B). Method A was applied over 30 min at a flow rate of 1 mL/min with an isocratic of 10% ethanol in hexane (v/v). The auto-sampler and the column compartment temperature were set at 25 °C. UV detection was carried out at a wavelength of 254 nm. 5 μL of the sample was injected. Method B was applied over 30 min at a flow rate of 0.8 mL/min with an isocratic of 1% ethanol in hexane (v/v). The auto-sampler and the column compartment temperature were set at 25 °C. UV detection was carried out at a wavelength of 254 nm. 3 μL of the sample was injected.

QS reporter strain assay

Escherichia coli (*E. coli*) DH5α contain a LuxR homolog SdiA that can detect exogenous AHL synthesized by other microbial species, especially BHL.[39] To avoid this possible interference, we used a *sdiA* mutants co-transformed with two plasmids, pJN105R (RhlR expression plasmid) and pSC11 (RhlA::*lacZ* fusion plasmid), for RhlR reporter strain assay. 1% of an overnight culture of reporter strain (OD 595 nm = 1.0) with 10 μg/mL gentamicin and 50 μg/mL ampicillin was incubated in Luria−Bertani (LB) medium (Difco, Detroit, MI, USA) at 37 °C for 2 h. Incubated reporter strain (OD 595 nm = 0.3) with positive controls or the compounds (0–1000 μM), BHL (0–10 μM) (Sigma-Aldrich), and 0.4% arabinose (Sigma-Aldrich) was reacted at 37 °C for 1.5 h. β-galactosidase activity was estimated using a Tropix plus kit (Applied Biosystems, CA, USA), and OD 595 nm and luminescence were measured on a VICTOR x5 multimode plate reader (PerkinElmer, Waltham, MA, USA). Relative RhlR

activity was quantified by dividing luminescence with OD 595 nm. For LasR reporter strain assay, *E. coli* DH5α co-transformed with two plasmids, pJN105L (LasR expression plasmid) and pSC11 (lasI::*lacZ* fusion plasmid) and OdDHL (Sigma-Aldrich) were used. And for PqsR reporter strain assay, *E. coli* DH5α co-transformed with two plasmids, pJN105P (PqsR expression plasmid) and pSC11 (PqsA::*lacZ* fusion plasmid) and PQS (Sigma-Aldrich) were used.

Static biofilm formation assay

An overnight culture of *P. aeruginosa* (OD 595 nm = 1.0) was diluted with AB medium (1:20) (300 mM NaCl, 50 mM $MgSO_4$, 0.2% vitamin-free casamino acids, 10 mM potassium phosphate, 1 mM L-arginine, and 1% glucose, pH 7.5) with positive controls or the compounds (0−100 μM) in borosilicate bottles. After incubation at 37 °C for 24 h without shaking, OD 595 nm of the suspended culture was measured on the multimode plate reader to measure growth inhibition activity. The biofilm cells attached to the bottles were washed two times with phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4, pH 7.2). Then, remained biofilm cells were stained using 0.1% crystal violet for 10 min and washed with deionized water to remove unbound crystal violet. The bounded crystal violet to biofilm cells was eluted in 100% ethanol, and the OD of the eluted ethanol samples was measured at 545 nm using the multimode plate reader.

Dynamic biofilm formation assay

Glass slides were inserted into a drip-flow reactor, and AB medium containing 5% of *P. aeruginosa* (OD 595 nm = 1.0) with positive controls or the compounds (0–10 μM) was

continuously fed into the reactor using a peristaltic pump (Masterflex C/L tubing pumps, Cole-Parmer, IL, USA) at 0.3 ml/min. After the operation at 37 ℃ for 48 h, the unattached biofilm cells were removed with PBS. The remained biofilm cells were stained with DAPI (Sigma-Aldrich) for 10 min and observed using confocal laser scanning microscopy (CLSM; Carl Zeiss LSM700, Jena, Germany). CLSM images of biofilm cells were obtained under blue fluorescence light (excitation wavelength: 350 nm, emission wavelength: 470 nm) and a 20× objective lens (W NAchroplan×20/0.5W (DIC) M27) using Z-stack mode in Zen 2011 program (Carl Zeiss). Biofilm volume and thickness were measured by Comstat2 in the ImageJ program based on the CLSM images.[40]

EPS analysis

Biofilm samples were prepared after drip-flow reactor operation by scrapping cells attached to the slides. After resuspension of centrifuged biofilm cells with 0.01M KCl, the suspension was disrupted with a sonicator (VCX 750, SONICS, Newtown, CT, USA) for 4 cycles of 5 s of operation and 5 s of pause at a power level of 3.5 Hz for carbohydrate and protein analysis. The supernatant of the sonicated suspension was filtered through a 0.22 μm membrane filter (Millex filter, Carl Roth, Karlsruhe, Germany). For protein analysis, mixtures containing 40 μL filtrate and 200 μL Lowry reagent (L3540, Sigma-Aldrich) was incubated for 10 min at room temperature. Following adding 20 μL Folin-Ciocalteu reagent (Sigma-Aldrich) and incubating at room temperature for 30 min, OD 750 nm was measured using the multimode plate reader. The amount of protein was quantified by dividing OD 750 nm by OD 595 nm. For carbohydrate analysis, mixtures containing 50 μL filtrate and 150 μL 99.9% sulfuric acid was incubated for 30 min at room temperature. Following adding 5% phenol and incubating at

90 °C for 5 min, OD 490 nm was measured using the multimode plate reader. The amount of protein was quantified by dividing OD 490 nm by OD 595 nm.

Rhamnolipid production assay

An overnight culture of *P. aeruginosa* (OD 595 nm = 1.0) was inoculated in AB medium (1:100) with positive controls or the compounds (0−100 μM) and then incubated using a shaking incubator at 37 ℃ for 24 h. The culture was centrifuged at 12,000 g at 4 ℃ for 5 min. Crude rhamnolipid was initially extracted twice by mixing 200 μL of supernatant and 400 μL of 100% diethyl ether (JUNSEI, Tokyo, Japan). The ether fraction was transferred and evaporated into a new tube. The dry sample was eluted in 20 μL of deionized water, then reacted with 180 μL of Orcinal solution (0.19% Orcinal (Sigma-Aldrich) in 53% H_2SO_4). The reacted sample was boiled at 80 °C for 30 min and cooled at room temperature for 15 min. The amounts of rhamnolipid were measured OD 421 nm and normalized with OD 595 nm in bacterial culture using the multimode plate reader.

Pyocyanin production assay

Diluted overnight culture of *P. aeruginosa* (OD 595 nm = 1.0) (1:100) using AB medium with positive controls or the compounds (0−10 μM) was incubated at 37 ℃ for 24 h and centrifuged at 10,000 rpm for 10 min. To extract pyocyanin, supernatant was reacted with 50% Trifluoroacetic acid (TFA, Sigma-Aldrich) at 37 ℃ for 1 h. The reacted supernatant was centrifuged at 10,000 rpm for 10 min, then passed through a 0.22 μm filter. The filtered supernatants were analyzed by 1260 Infinity Ⅱ Prep-HPLC System (Agilent Technologies, Santa Clara, CA, USA) and EC-C18 column (4.6×150 mm, Agilent Technologies). The

detailed HPLC conditions were as follows: 99:1 water/TFA (v/v) mobile phase; 10 μL injection volume; 25 ℃ temperature; and 0.5 mL/min flow rate. The retention time of pyocyanin (Sigma-Aldrich) was 20 min. The height of analyzed peaks at the 20 min of retention time was detected for estimating the amounts of pyocyanin.

Mortality experiment of *T. molitor* larvae

The mortality of *Tenebrio molitor* (*T. molitor*) larvae with positive controls or the compounds (0−10 μM) was measured according to the previous study.[41] An overnight culture of *P. aeruginosa* (OD 595 nm = 1.0) was centrifuged at 10,000 rpm for 10 min. 10 μL of filtered supernatant through a 0.22 μm filter was injected into larvae using syringe needles. Larvae were maintained in Petri dishes at 25 °C for 20 days. The mortality of larvae was observed every 5 days.

RT-qPCR analysis

An overnight culture of *P. aeruginosa* (OD 595 nm = 1.0) was diluted (1:20) with AB medium with positive controls or the compounds (0−10 μM) in borosilicate bottles. Following incubation at 37 ℃ for 24 h, biofilm cells were collected for RNA extraction. Total RNA was extracted using the TRI REAGENT (Molecular Research Center, OH, USA) following the manufacturer's instruction. RT-qPCR was performed to quantify and compare the levels of QS-related gene expression. SYBR Premix Ex TaqTM (Takara, Shiga, Japan), CFX-96 real-time system (Bio-Rad, Hercules, CA, USA), and QS-related gene primer sets were used for RT-qPCR.[28] Thermal profiles of the RT-qPCR were as follows: initial denaturation at 95 °C for 10 s, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 10 s,

and extension at 63 °C for 34 s. Fluorescent signal intensity was collected at the end of the extension step.

In silico docking studies

A homology model of RhlR based on the crystal structure of SdiA in complex with 3-oxo-C6-homoserine lactone (PDB code 4Y15)[42] was generated. The protein sequence of RhlR was obtained from NCBI protein database (http://ncbi.nlm.nih.gov/protein) as FASTA format. The homology model of RhlR was generated using SWISS-MODEL (https://swissmodel.expasy.org). All final compounds were generated as 3D structure by *Chem3D Pro* (ver 12.0) and the group of compounds was saved as *.sdf* file. *'Sanitize'* preparation protocol in *SYBYL-X 2.1.1* (Tripos Inc., St Louis) was applied to ligand preparation and optimization process. The prepared final compounds were docked into the RhlR homology model using the *Surflex-Dock GeomX* module of *SYBYL-X 2.1.1*. The protein minimization for molecular docking was performed by the '*POWELL*' method with '*Tripos*' Force Field setting. The initial optimization option was set to *None*. '*Surflex-Dock protomol*' was used to guide docking site as defined by the '*Residues*' method with the selected amino acids (Tyr43, Val60, Tyr64, Trp68, Asp81, Ile84, Ser135; radius setting: 4.0; Those amino acids were selected based on the active site of SdiA, the template protein of RhlR homology model). Other docking parameters were kept to the default values.

Statistical analysis

P values were estimated by Student's t test (SigmaPlot version 10, Systat Software Inc., San Jose, CA, USA).

ASSOCIATED CONTENT

Supporting Information

Analytical data (^{1}H-NMR, ^{13}C-NMR and HPLC spectra) of the key intermediates and final compounds and biological data are available free of charge via the Internet at http://pubs.acs.org.

PDB coordinates of homology RhlR model in complex with compounds **1d** and **30**−**32** (PDB)

Molecular formula strings and biological data for final compounds (CSV)

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Author Contributions

S.N., S.-Y.H., H.-S.K., S.-H.S., H.-D.P., Y.B. were involved in the initial stages of the project, including the design of gingerol analogs and the development of biological experiments. S.N., H.K., S.M., T.L., S.-H.S. synthesized and analyzed gingerol analogs. S.-Y.H., H.-S.K., J.-H.L. performed biological experiments. S.N., S.-Y.H., H.-S.K., S.-H.S., H.-D.P., Y.B. analyzed the data and wrote the paper. All authors contributed to editing the final manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

ACN, acetonitrile; AHL, *N*-acyl-L-homoserine lactones; BHL, *N*-butyryl-L-homoserine lactone; CLSM, confocal laser scanning microscopy; ESI, electrospray ionization; HRMS, high-resolution mass spectra; IC50, half maximal inhibitory concentration; LDA, diisopropylamide; NMR, nuclear magnetic resonance; OdDHL, *N*-(3-oxo-dodecanoyl)-L-homoserine lactone; PBS, phosphate-buffered saline; PQS, *Pseudomonas* quinolone signal; QS, quorum sensing; RP-HPLC, reverse-phase high-performance liquid chromatography; SAR, structure-activity relationship; TBAF, tetrabutylammoniumfluoride; TBDMS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography

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TOC graphic

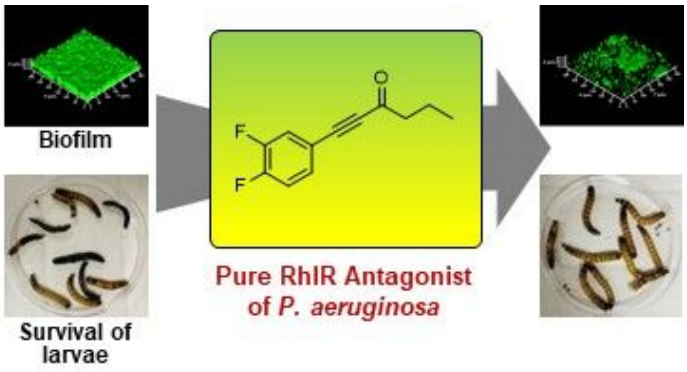

Biofilm
Survival of larvae
F
F
O
Pure RhlR Antagonist of P. aeruginosa

Figure 1

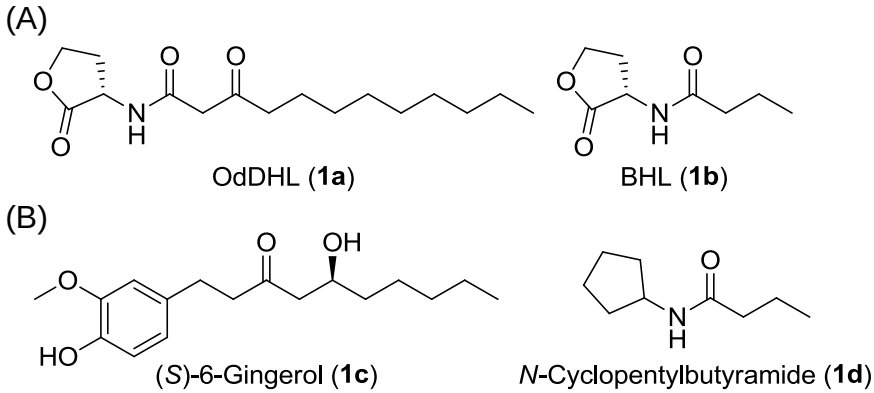

(A)
OdDHL (1a)
BHL (1b)
(B)
(S)-6-Gingerol (1c)
N-Cyclopentylbutyramide (1d)

Figure 2

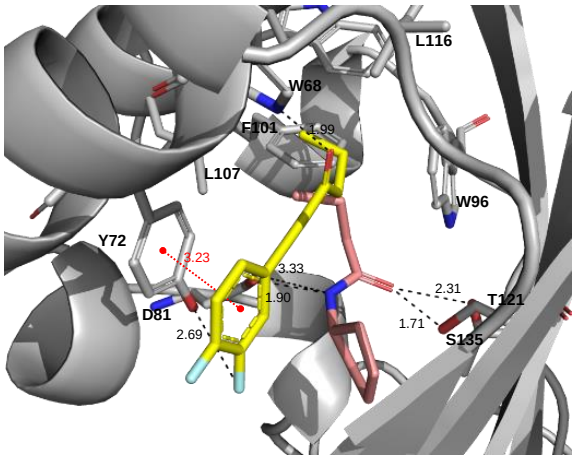

L116
W68
F101
1.99
L107
W96
Y72
3.23
3.33
2.31
T121
D81
1.90
1.71
2.69
S135

Figure 3

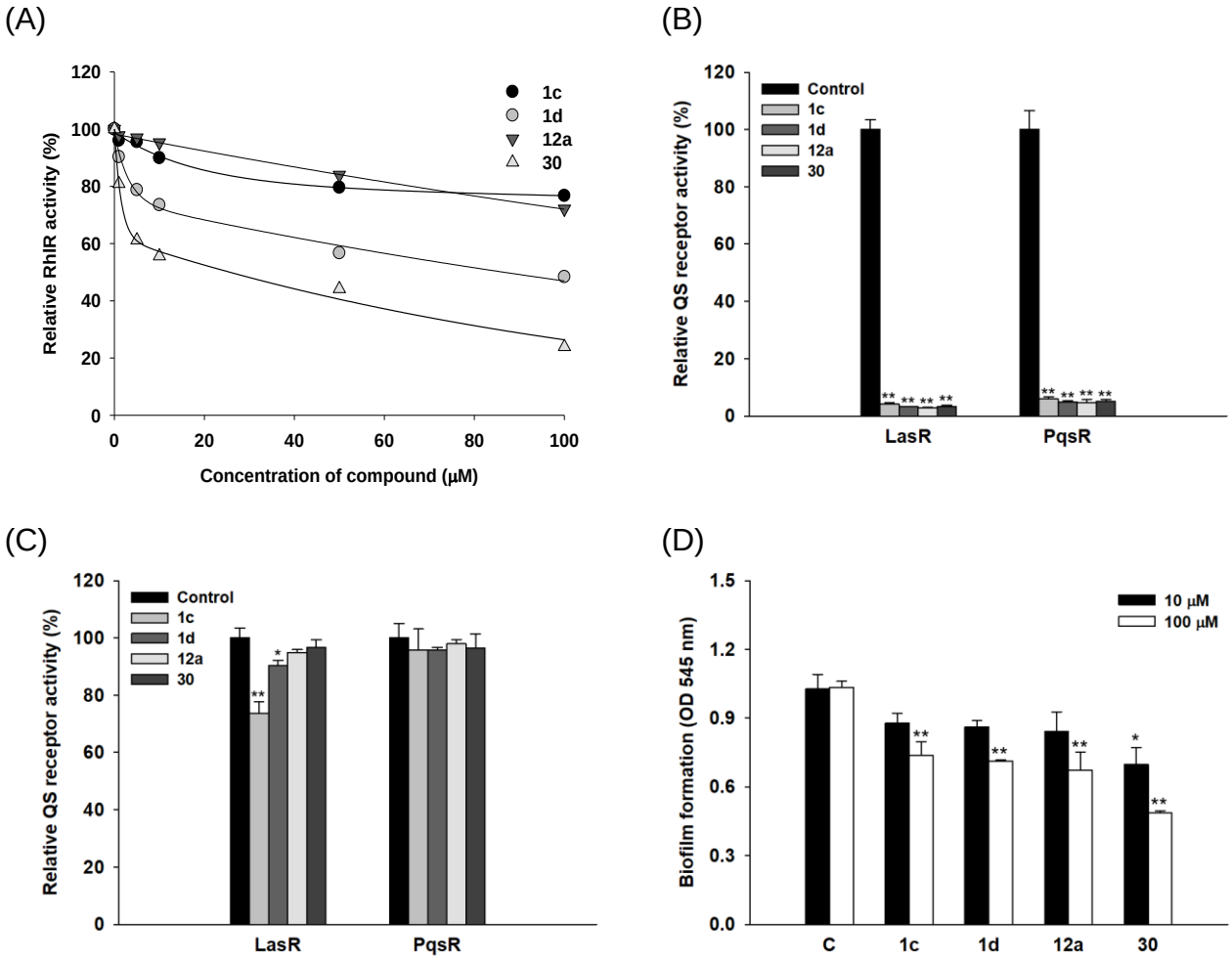

(A)
Relative RhlR activity (%)
Concentration of compound (μM)
1c
1d
12a
30
(B)
Relative QS receptor activity (%)
Control
1c
1d
12a
30
LasR
PqsR
(C)
Relative QS receptor activity (%)
Control
1c
1d
12a
30
LasR
PqsR
(D)
Biofilm formation (OD 545 nm)
10 μM
100 μM
C
1c
1d
12a
30

Figure 4

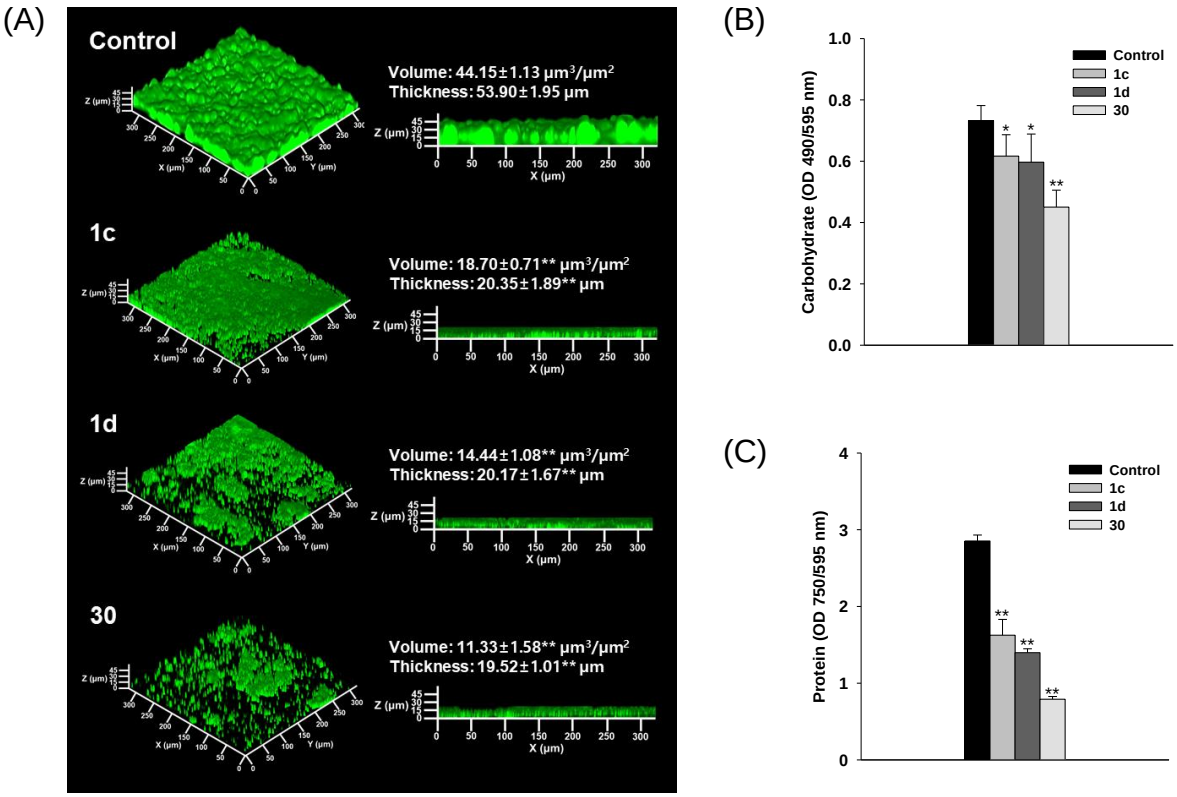

(A)
Control
Volume: 44.15±1.13 μm³/μm²
Thickness: 53.90±1.95 μm
1c
Volume: 18.70±0.71** μm³/μm²
Thickness: 20.35±1.89** μm
1d
Volume: 14.44±1.08** μm³/μm²
Thickness: 20.17±1.67** μm
30
Volume: 11.33±1.58** μm³/μm²
Thickness: 19.52±1.01** μm
Z (μm)
X (μm)
Y (μm)
(B)
Carbohydrate (OD 490/595 nm)
Control
1c
1d
30
(C)
Protein (OD 750/595 nm)
Control
1c
1d
30

Figure 5

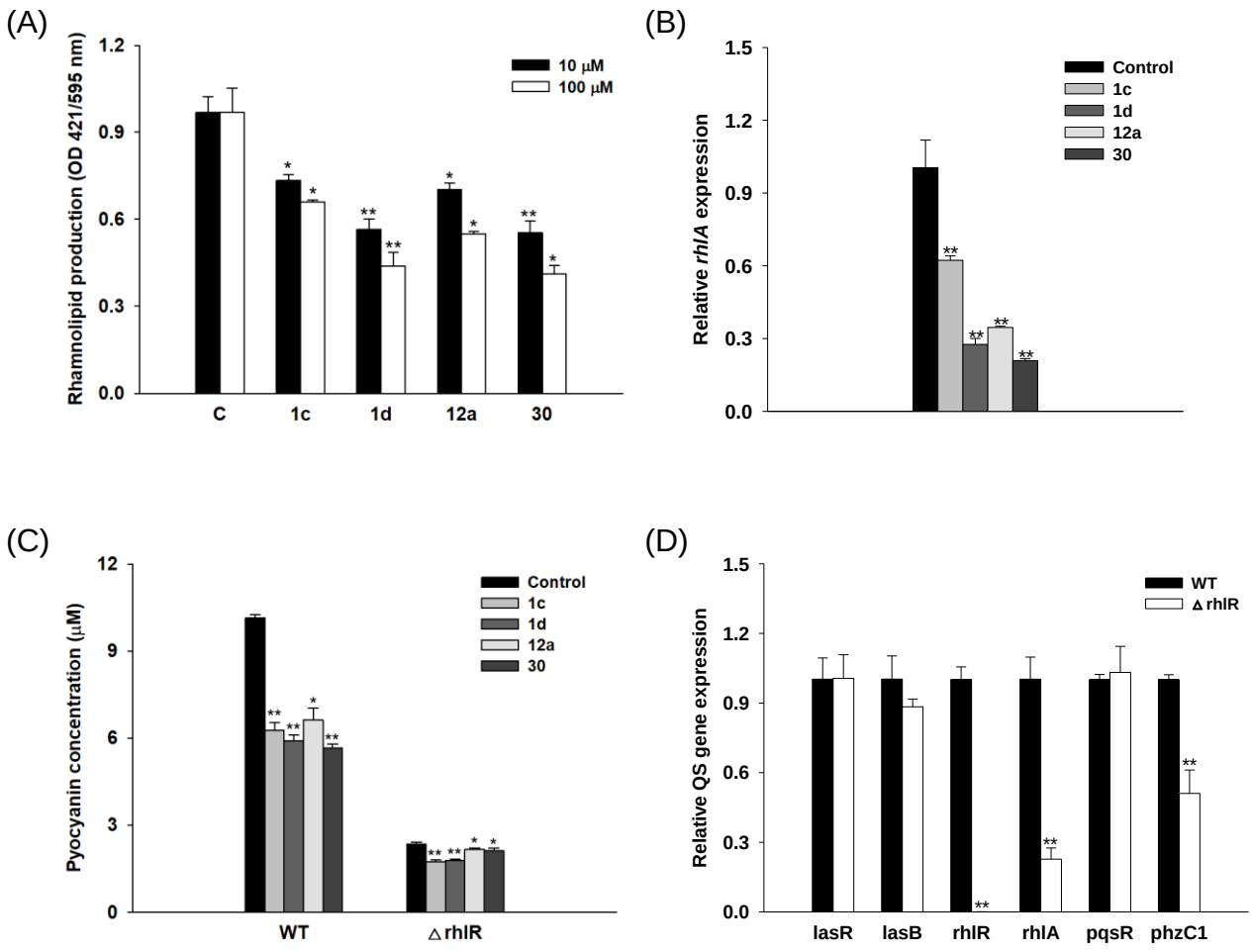

(A)
Rhamnolipid production (OD 421/595 nm)
10 μM
100 μM
C
1c
1d
12a
30
(B)
Relative rhlA expression
Control
1c
1d
12a
30
(C)
Pyocyanin concentration (μM)
Control
1c
1d
12a
30
WT
△rhlR
(D)
Relative QS gene expression
WT
△rhlR
lasR
lasB
rhlR
rhlA
pqsR
phzC1

Figure 6

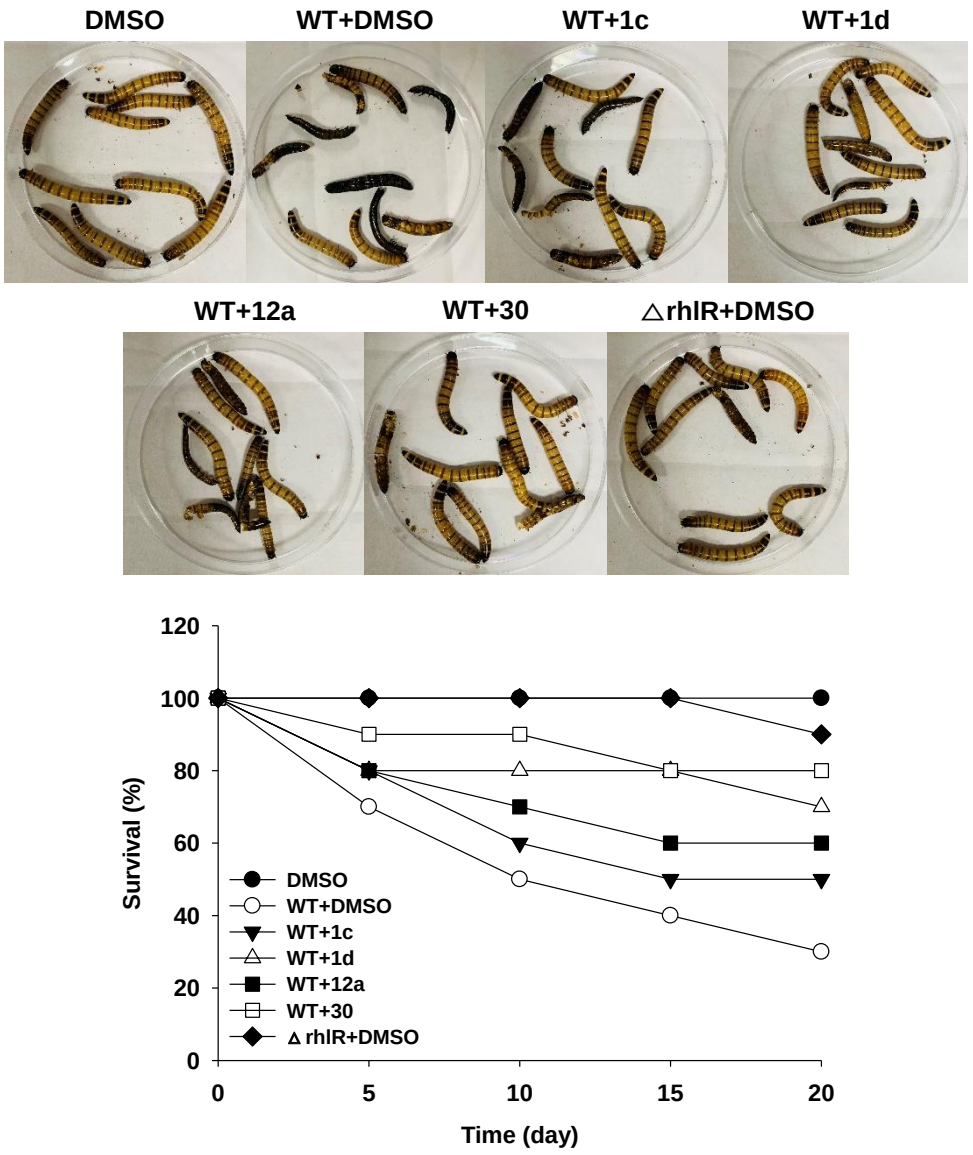
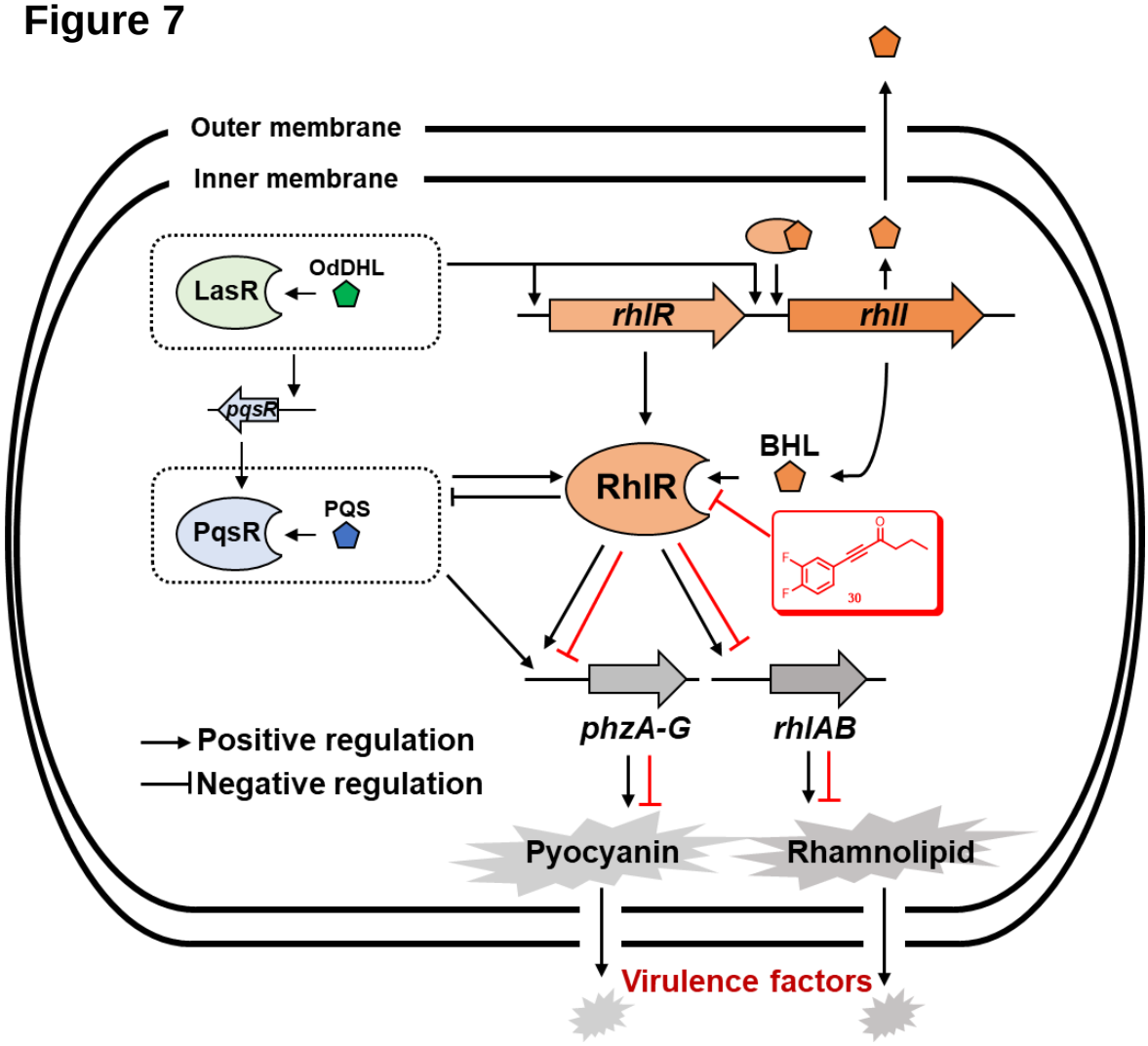
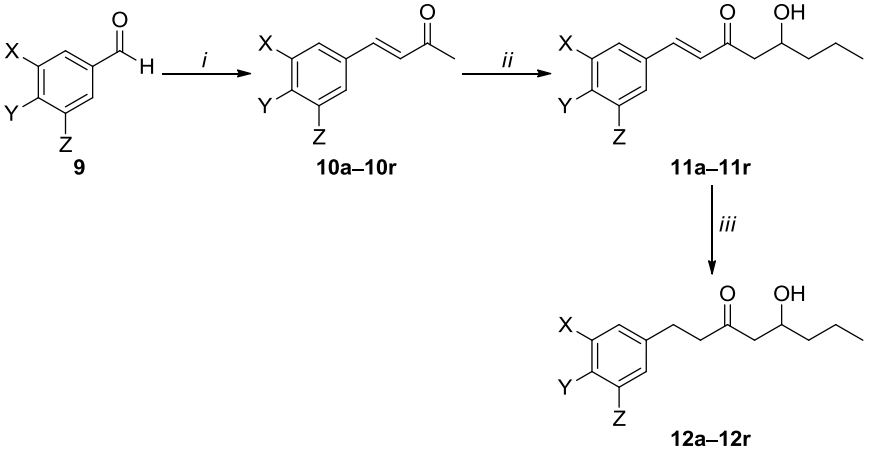

DMSO
WT+DMSO
WT+1c
WT+1d
WT+12a
WT+30
△rhlR+DMSO
Survival (%)
Time (day)
0
20
40
60
80
100
120
0
5
10
15
20
DMSO
WT+DMSO
WT+1c
WT+1d
WT+12a
WT+30
△rhlR+DMSO

Figure 7

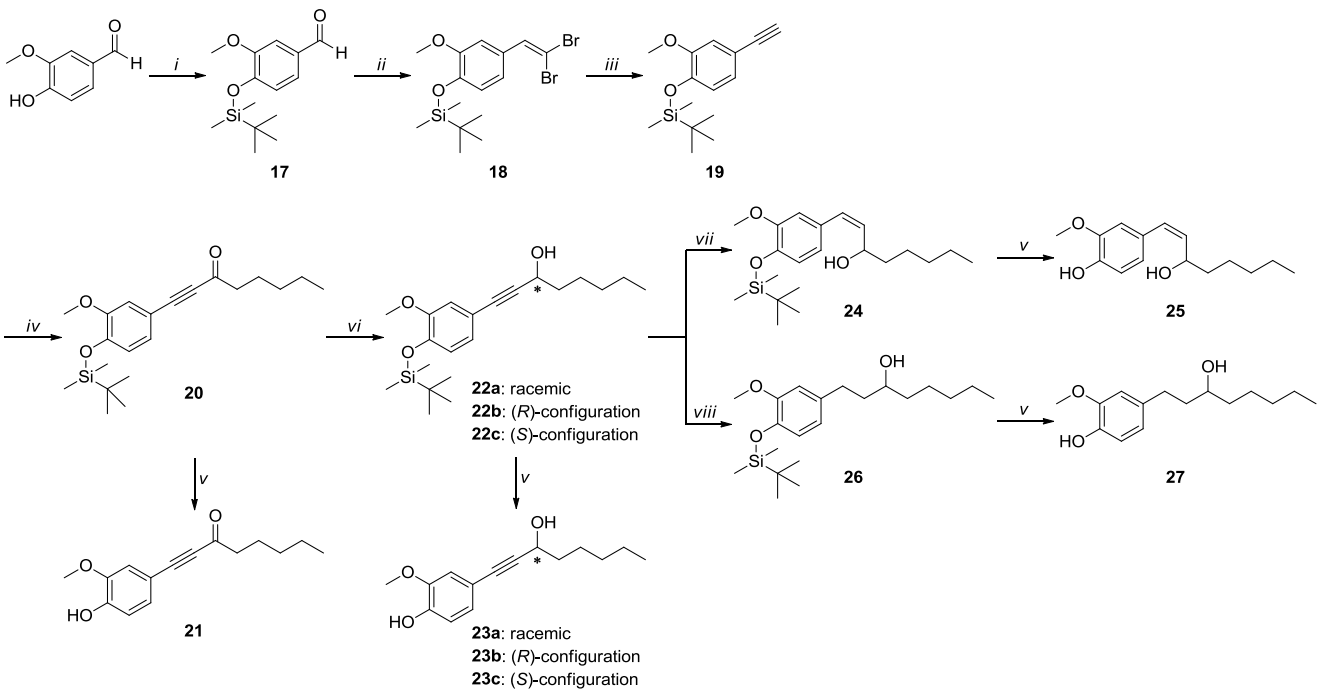

Outer membrane
Inner membrane
LasR
OdDHL
rhlR
rhlI
pqsR
PqsR
PQS
RhlR
BHL
30
phzA-G
rhlAB
Positive regulation
Negative regulation
Pyocyanin
Rhamnolipid
Virulence factors

Scheme 1

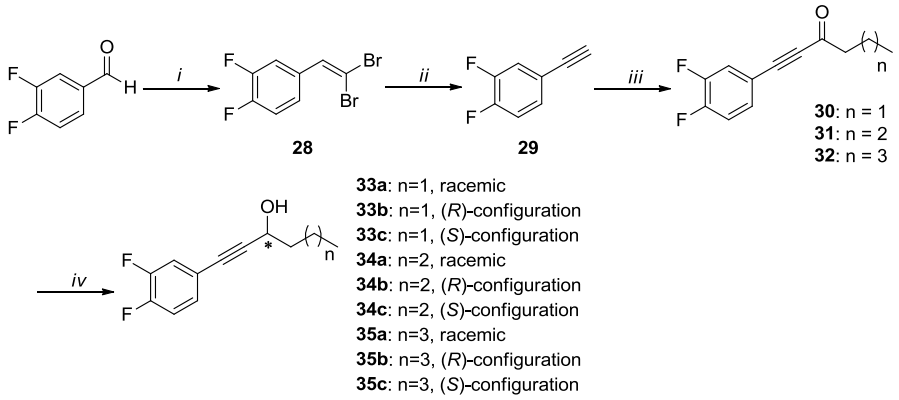


a: X = OMe, Y = OH, Z = H
b: X = H, Y = H, Z = H
c: X = OMe, Y = H, Z = H
d: X = H, Y = OH, Z = H
e: X = OH, Y = H, Z = H
f: X = H, Y = OMe, Z = H
g: X = H, Y = F, Z = H
h: X = H, Y = Cl, Z = H
i: X = H, Y = Me, Z = H
j: X = H, Y = NMe_2 , Z = H
k: X = OH, Y = OH , Z = H
l: X = OMe, Y = OMe, Z = H
m: X = F, Y = OH, Z = H
n: X = OEt, Y = OH, Z = H
o: X = F, Y = F, Z = H
p: X = Me, Y = Me, Z = H
q: X = OMe, Y = F, Z = H
r: X = F, Y = H, Z = OMe

Scheme 2


i
ii
iii
17
18
19
iv
vi
vii
v
viii
20
22a: racemic
22b: (R)-configuration
22c: (S)-configuration
24
25
26
27
21
23a: racemic
23b: (R)-configuration
23c: (S)-configuration

Scheme 3



33a: n=1, racemic
33b: n=1, (*R*)-configuration
33c: n=1, (*S*)-configuration
34a: n=2, racemic
34b: n=2, (*R*)-configuration
34c: n=2, (*S*)-configuration
35a: n=3, racemic
35b: n=3, (*R*)-configuration
35c: n=3, (*S*)-configuration