Journal of Medicinal Chemistry
CORNELL UNIVERSITY LIBRARY

Subscriber access provided by CORNELL UNIVERSITY LIBRARY

Article

Novel 2-Substituted 3-Hydroxy-1,6-dimethylpyridin-4(1H)-ones as Dual-Acting Biofilm Inhibitors of Pseudomonas aeruginosa

Jun Liu, Jin-Song Hou, Yi-Bin Li, Zhi-Ying Miao, Ping-Hua Sun, Jing Lin, and Wei-Min Chen

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.0c00763 • Publication Date (Web): 31 Aug 2020

Downloaded from pubs.acs.org on September 2, 2020

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

Novel 2-Substituted 3-Hydroxy-1,6-dimethylpyridin-4(1*H*)-ones as Dual-Acting Biofilm Inhibitors of *Pseudomonas aeruginosa*

Jun Liu, Jin-Song Hou, Yi-Bin Li, Zhi-Ying Miao, Ping-Hua Sun*, Jing Lin*, Wei-Min Chen*

College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China

*Corresponding authors. Tel.: +86 20 8522 1367(J. Lin), +86 20 8522 4497 (W.-M. Chen). Fax: +86 20 8522 4766.

E-mail address: pinghuasunny@163.com(P.-H. Sun), linjing_jnu@163.com (J. Lin), twmchen@jnu.edu.cn (W.-M. Chen).

ABSTRACT

2-Heptyl-3-hydroxy-4(1*H*)-quinolone (PQS), a compound from *P. aeruginosa*, functions as both a quorum sensing (QS) regulator and a potent iron chelator to induce expression of pyoverdine and pyochelin which are involved in high-affinity iron transport systems. A potential dual-acting anti-biofilm strategy requires molecules designed to interfere with iron uptake and the QS system of *P. aeruginosa*. A series of 2-substituted 3-hydroxy-1,6-dimethylpyridin-4-ones have been designed, synthesized and tested as biofilm inhibitors of *P. aeruginosa.* One compound, *N*-((1,3,6-trimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)hexanamide (**10d**) exhibits 68.67% biofilm inhibitory activity at 20 μM. Further mechanistic studies have confirmed that this compound not only inhibits the QS systems of *P. aeruginosa*, but also acts as an iron chelator to compete strongly with pyoverdine, causing iron

deficiency in bacteria. The pyoverdine receptor FpvA was revealed as the target of **10d** by the *Pvds* mutant strain, *fpvA*-overexpressed strain and *in silico* studies.

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a common pathogen that by the formation of biofilm often causes drug resistance in clinically acquired infections[1]. The quorum sensing (QS) systems mediated by chemical signal molecules are among the most common regulatory mechanisms in the biofilm formation of *P. aeruginosa* and the release of bacterial virulence factors[2]. *P. aeruginosa* possesses three main QS systems, *las*, *rhl* and *pqs*, which are mediated by three chemically distinct classes of signal molecules, *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL)[3], *N*-butanoyl-L-homoserine lactone (C4-HSL)[4,5], and 2-heptyl-3-hydroxy-4(1*H*) quinolone (PQS)[6,7]. In the las system for example, when the concentration of 3-oxo-C12-HSL exceeds a certain threshold, it binds to the transcriptional receptor LasR and activates the expression of genes that produce virulence factors such as elastase. Similar to 3-oxo-C12-HSL, C4-HSL and PQS can bind to their respective receptors RhlR and PqsR and activate certain target genes, including those responsible for the production of rhamnolipids and pyocyanin respectively[8,9]. Recent reports show that a fourth inter-cellular communication signal molecule, IQS (2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde), which is capable of sensing phosphate depletion stress, modulates bacteria pathogenesis by interacting with the quorum sensing network[10]. In the past decade, QS inhibitors (QSIs) have been considered to be agents that potentially can reduce pathogenicity and overcome

bacterial antibiotic tolerance. Many anti-biofilm compounds that act as specific QSIs have been described in recent years, but none of them have been successfully used clinically[11]. Consequently, new strategies for the design and development of biofilm inhibitors are necessary.

Iron is an essential element in *P. aeruginosa* and plays a crucial role in bacterial biofilm formation[12,13]. Microorganisms acquire iron from their hosts. The ferrous cation is the form involved in bacterial biochemical pathways, but under physiological conditions it is relatively unavailable and *P. aeruginosa* indirectly exploits the more readily available ferric cation as a source[14,15]. Bacteria have evolved to have a series of iron acquisition systems, including iron transport systems based on pyoverdine (Pvd) and pyochelin (Pch) which uptake iron[16,17]. Published results have shown that iron chelators can interfere with bacterial biofilm formation[18] and more interesting, as presented in **Figure 1**, PQS not only acts as a QS signal molecule in regulating the formation of bacterial biofilm but also functions as a potent iron chelator which induces expression of the Pvd and Pch iron transport systems[19]. This suggests that PQS analogs could be developed as inhibitors that interfere with both iron uptake and QS systems, thus performing as dual-acting inhibitors of *P. aeruginosa* biofilm formation.

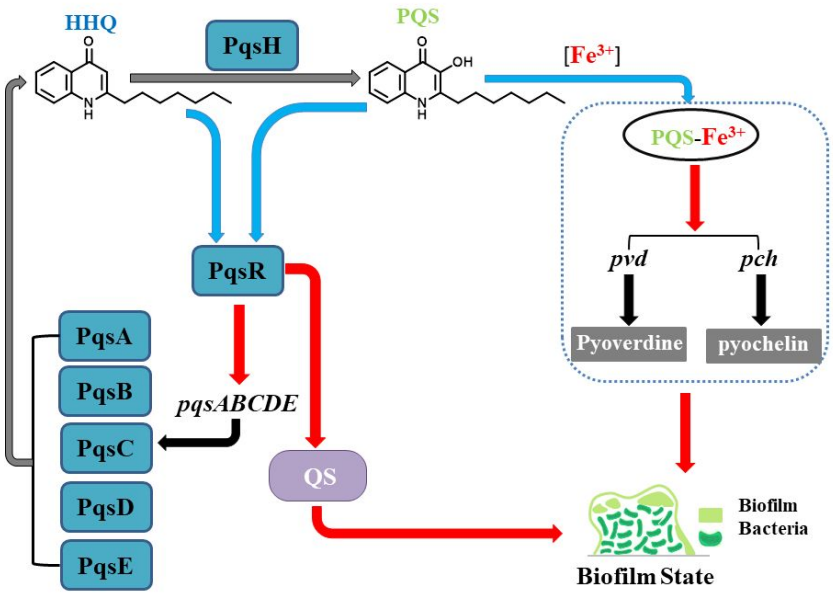


Figure 1. Diagrammatic representation of the role of PQS, and regulation of biofilm formation between PqsR and iron uptake systems in *P. aeruginosa*[19]. 2-Alkyl-4-quinolone (AQ) biosynthesis depends on the actions of PqsA, B, C and D on anthranilate (provided *via* anthranilate synthase, PhnAB) to generate HHQ. PQS is produced from HHQ *via* the *pqsH* gene product. Both PQS and HHQ are co-inducers of PqsR which drives the expression of the *pqsABCDE* operon. Unlike HHQ, PQS induces microvesicle formation and is a potent iron chelator which induces expression of the pyoverdin and pyochelin high affinity iron transport systems. Gray arrows represent PQS biosynthesis. Black arrows indicate gene expression. Blue arrows represent the combination of signal molecules to pqsR or Fe^{3+} and red arrows indicate up-regulation.

In this study, a class of 2-substituted 3-hydroxy-1,6-dimethylpyridin-4-one derivatives with the structural characteristics of both QS inhibitors and iron chelators were designed and evaluated as dual-acting biofilm inhibitors of *P. aeruginosa*. The structure–activity relationships (SAR) of 3-hydroxy-1,6-dimethylpyridine-4-one derivatives were investigated. *N*-((1,3,6-Trimethyl-4-oxo-1,4-dihydropyridin-2-yl)

methyl)hexanamide (**10d**) was found to exhibit 68.67% biofilm inhibitory activity at 20 μM. There was also a reduction in the cytotoxicity for Vero and LO2 cells caused by the acute infectivity of *P. aeruginosa*. Further experiments demonstrated that **10d** affects both iron acquisition systems and QS systems.

RESULTS AND DISCUSSION

Chemistry.

A working hypothesis is that the structures derived from the QS signal molecules and iron chelators could be combined to produce novel biofilm inhibitors which interfere with both iron chelation and bacterial QS systems. Since the PQS molecule contains a 3-hydroxypyridin-4-one fragment found in known iron chelators, 3-hydroxypyridin-4-one was selected as the core skeleton of a series of new compounds. Some of the QS signal molecules have a hydrophobic alkyl side chain and a series of side chains were therefore used to conjugate with hydroxypyridinone (**Figure 2)**. To explore the effects of different side chains, including the hydrophobic alkyl group (**10a**–**10f**) and a cyclic or phenyl ring group (**11a**–**11u**), the biofilm inhibitory activity of the compounds against *P. aeruginosa* were examined. Compounds with a sulfonamide bond replacing an amide bond (**13a**–**13g**) and those with an amino group replacing an amide bond (**16a–16j**) were designed and synthesized (**Figure 2**).

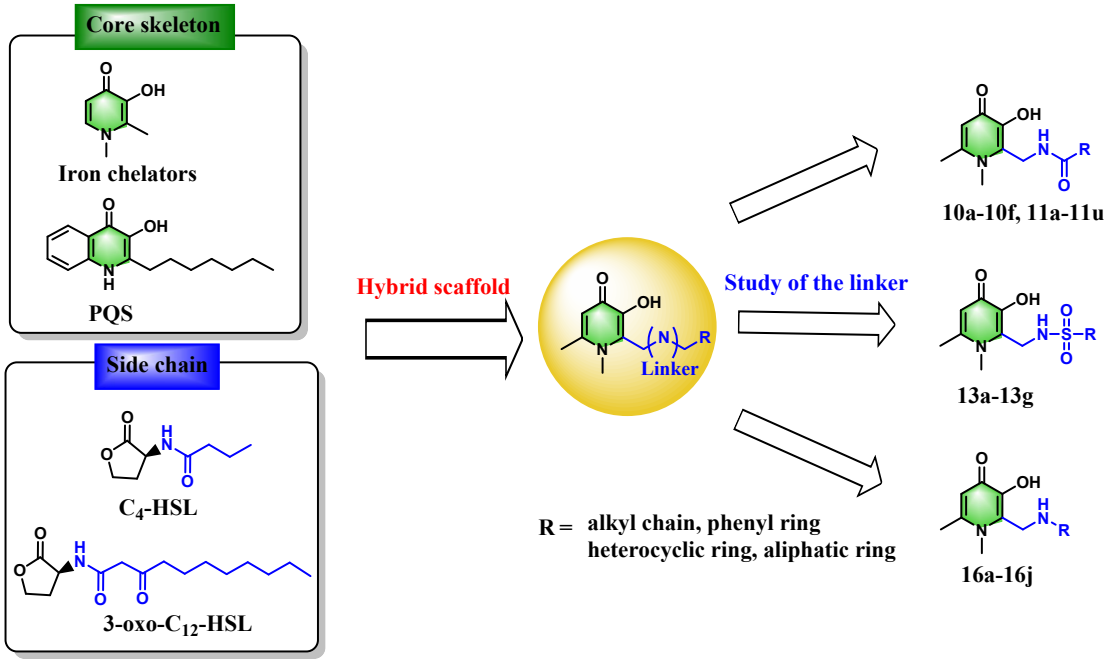
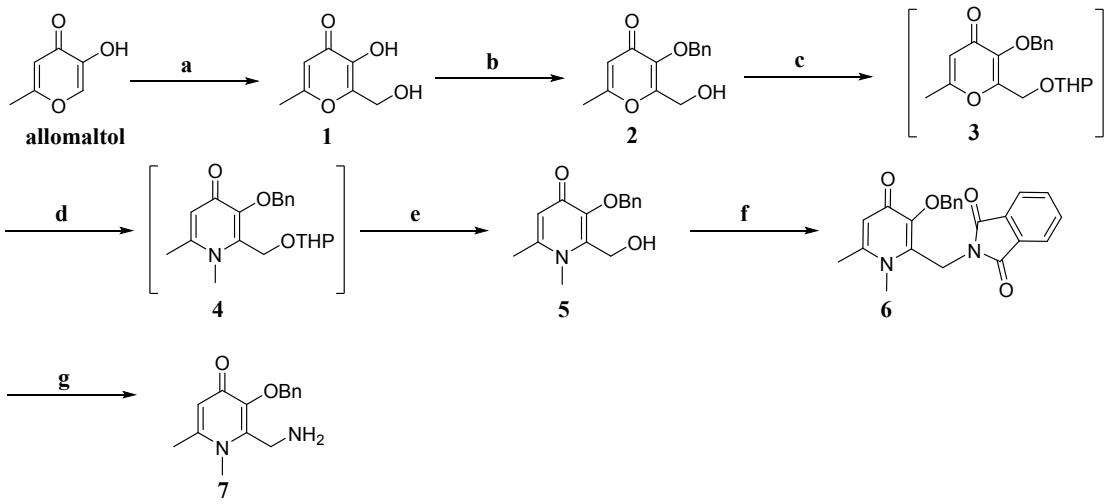


Figure 2. The design of a drug scaffold, and the structures of 3-hydroxy-1,6-dimethylpyridine-4-one derivatives with various 2-substituted side chains.

Synthesis of target compounds 10a–10f, 11a–11u, and 13a–13g. The precursor, allomaltol was prepared from the readily available kojic acid according to a published method[20]. Compound **1** was synthesized by condensation of allomaltol with formaldehyde in the presence of sodium hydroxide. The 3-hydroxyl group was then protected by a benzyl group to obtain **2**. Next, the 2-(1′-hydroxyl) group of **2** was protected using 3,4-dihydro-2*H*-pyran to obtain **3**. Compound **3** reacted with methylamine, resulting in the formation of **4**, and the protecting group was removed by heating with 2 mol/L HCl to yield **5**. The transformation of the 2-(1′-hydroxyl) group in **5** to a 2-(1′-amino) group was achieved by the Mitsunobu reaction of **5** with phthalimide, triphenylphosphine and diethyl azodicarboxylate, leading to **6** which was subsequently hydrolyzed by hydrazine hydrate, resulting in the formation of 2-(aminomethyl)-3-(benzyloxy)-1,6-dimethylpyridin-4(1*H*)-one (**7**) (**Scheme 1**).

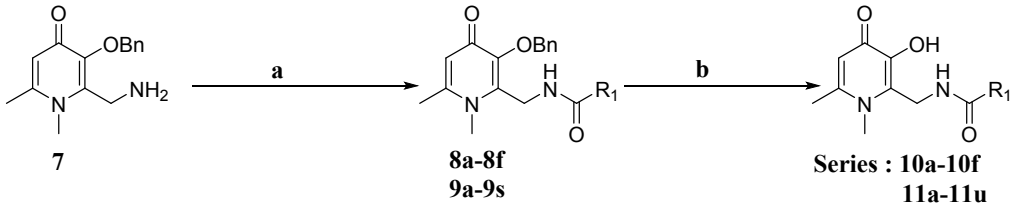
Scheme 1. Synthetic route to compound 7.

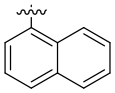
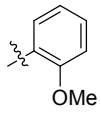
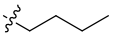
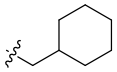
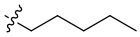
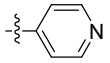
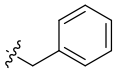
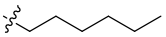
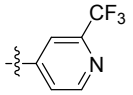
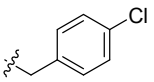
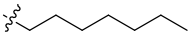
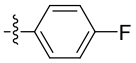
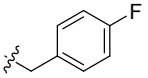
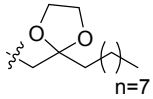
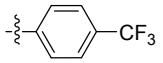
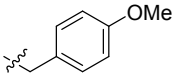
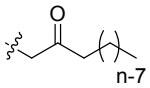
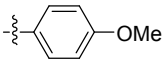
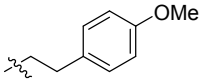
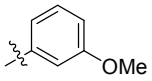
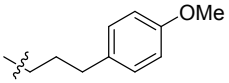
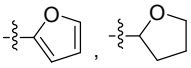


Reagents and conditions：(a) Formaldehyde, NaOH, H_2O, rt, overnight; (b) NaOH, BnCl, MeOH, 80 ℃, overnight; (c) THP, PTS, dry DCM, rt, 3 h; (d) 33-35% $MeNH_2$, EtOH, 75 °C, overnight; (e) 2 mol/L HCl, EtOH, 80 ℃, 4 h; (f) Phthalimide, Ph_3P, diethyl azodicarboxylate (DEAD), dry THF, 0 ℃-rt., overnight; (g) NH_2NH_2, EtOH, 80 ℃, 4 h.

Acylation or sulfonylation of the amine (**7**) with different acid chlorides or sulfuryl chlorides in the presence of the catalyst pyridine afforded the derivatives **8a–8f**, **9a–9u** and **12a–12g**. Subsequent removal of the benzyl protecting group by hydrogenolysis with Pd/C gave compounds **10a–10f**, **11a–11u**, **13a–13g** (**Scheme 2**, **Scheme 3**).

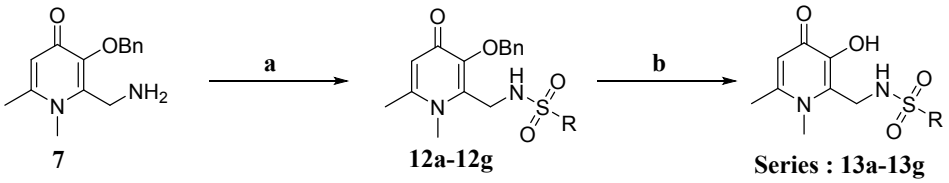
Scheme 2. Synthetic route to 10a–10f, 11a–11u derivatives.

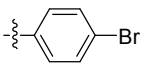
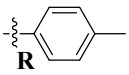
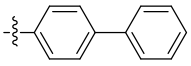
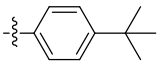
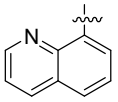
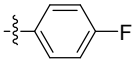
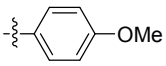
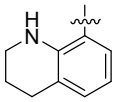


Compound	R	Compound	R	Compound	R
8a, 10a		9f, 11f		9n, 11n	
8b, 10b		9g, 11g		9o, 11o	
8c, 10c		9h, 11h		9p, 11p	
8d, 10d		9i, 11i		9q, 11q	
8e, 10e		9j, 11j		9r, 11r	
8f		9k, 11k		9s, 11s	
10f		9l, 11l		9t, 11t	
9a, 11a		9m, 11m		9u, 11u	
9b, 11b					
9c, 11c					
9d, 11d					
9e, 11e					

Reagents and conditions: (a) R_1-CO_2Cl, dry DMF, pyridine, 0 ℃-rt, 4 h; or (**8f**, **9m**) RCOOH, EDCI, DMAP, rt., overnight; (b) H_2, 10% Pd/C, MeOH, overnight or (**10f**) ATF: H_2O (1:1), rt., 4 h and H_2, 10% Pd/C, MeOH, overnight.

Scheme 3. Synthetic route to 13a–13g.

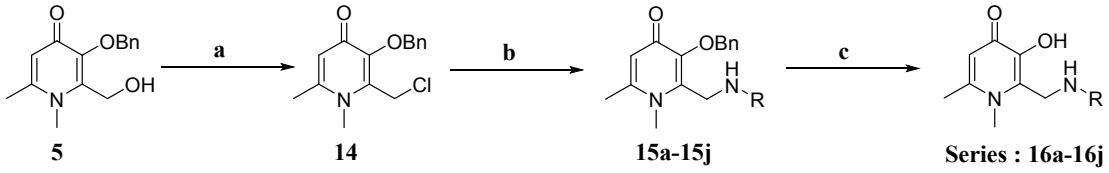


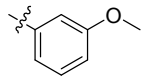
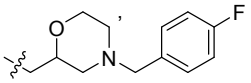
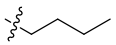
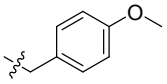
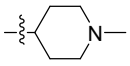
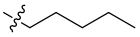
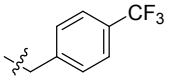
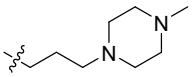
Compound	R	Compound	R	Compound	R
12a		12c, 13c		12f, 13f	
13a		12d, 13d		12g	
12b, 13b		12e, 13e		13g	

Reagents and conditions: (a) R-SO_2Cl, dry DMF, pyridine, 0 ℃-rt, overnight; (b) H_2, 10% Pd/C, MeOH, overnight.

Synthesis of the target compounds 16a–16j. Compounds **16a–16j** were synthesized in three steps from compound **5** as shown in **Scheme 4**. The 2-(1′-hydroxyl) group of **5** was chlorinated using thionyl chloride to obtain **14**. The chlorine in **14** was replaced by various primary amines to give **15a**–**15j**. Finally, the benzyl protecting group was hydrogenolyzed using Pd/C to obtain **16a**–**16j** (**Scheme 4**).

Scheme 4. Synthetic route to 16a–16g derivatives.



Compound	R	Compound	R	Compound	R
15a, 16a		**15e, 16e**		**15i, 16i**	
15b, 16b		**15f, 16f**		**15j, 16j**	
15c, 16c		**15g, 16g**			
15d, 16d		**15h, 16h**			

Reagents and conditions: (a) $SOCl_2$; (b) R-NH_2, dry DMF, TEA, 75 °C, 5-6 h (c) H_2, 10% Pd/C, MeOH, overnight.

Biological Evaluation.

Determination of minimum inhibitory concentrations (MIC). We tested the effect of target compounds on bacterial growth (**Table 1**) and our results showed that at a concentration of 512 μM, these synthetic derivatives exhibited no influence on the growth of *P. aeruginosa* PAO1.

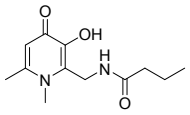
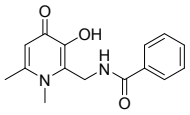
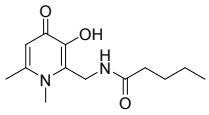
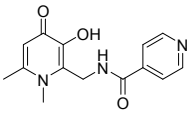
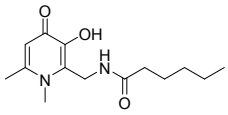
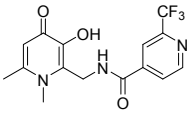
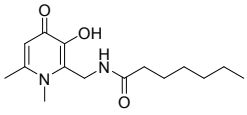
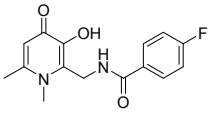
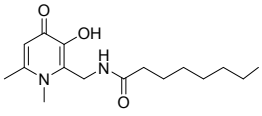
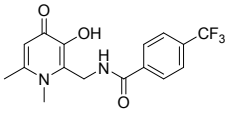
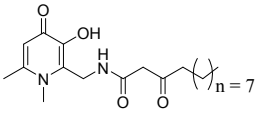
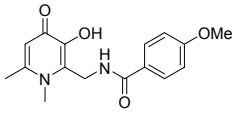
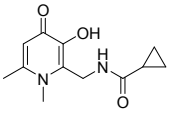
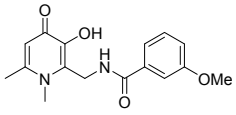
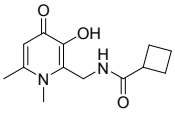
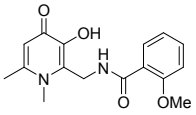
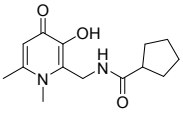
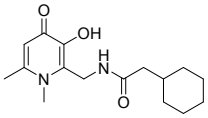
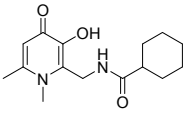
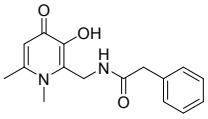
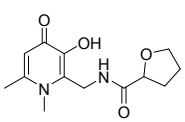
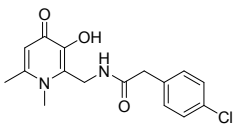
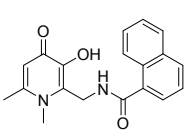
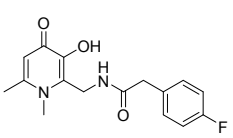
Anti-biofilm screening and SAR study. The inhibitory effect of compounds on biofilm was determined by a crystal violet staining assay. Azithromycin and our previously reported active pyranone derivative (**6a**)[50] were utilized as positive controls. Azithromycin and **6a** displayed IC_{50} values in the staining assay of 6.76 ± 2.28 μM and 20.31 ± 0.50 μM, respectively. As detailed in **Table 1**, some target

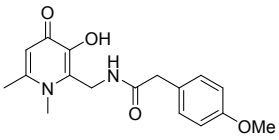
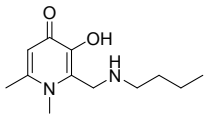
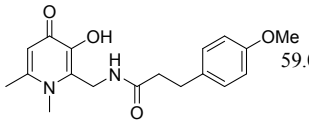
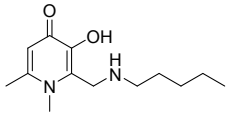
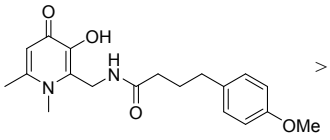
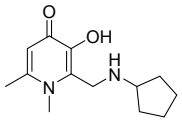
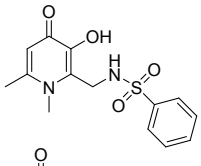
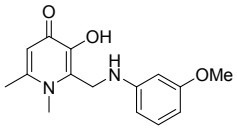
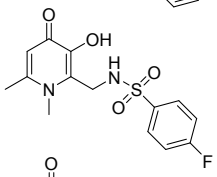
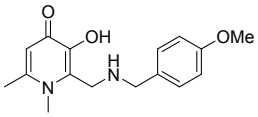
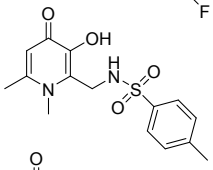
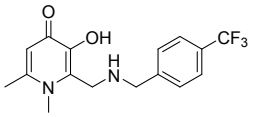
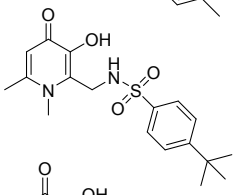
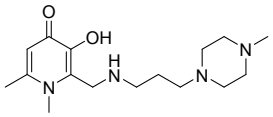
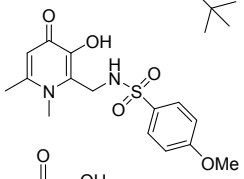
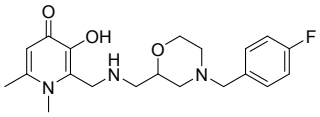
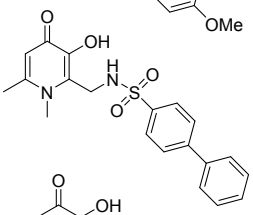
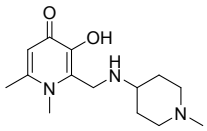
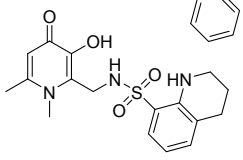
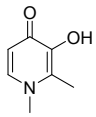
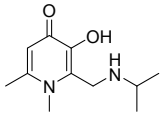
compounds with an amide linker exhibited biofilm inhibitory activity (IC_{50} < 100 μM) and **10d**, a target compound with an alkyl chain of six carbons, displayed the best anti-biofilm effect with an IC_{50} value of 6.57 ± 0.74 μM. The results showed that both extending (**10e**, **10f**) and shortening (**10a**, **10b**, **10c**) the length of the alkyl chain sharply reduced the biofilm inhibitory activity. To study the effect of the R group on biofilm formation, compounds with various aliphatic and aromatic rings introduced into the R group (**11a**–**11u)** were synthesized. Compounds **11a**–**11u** with aliphatic and aromatic rings displayed lower bioactivities (IC_{50} > 26.49 ± 1.62 μM) than **10d** (IC_{50} = 6.57 ± 0.74 μM). Interestingly, we found that compound **11e** with a tetrahydrofuran in the side chain had the highest activity to inhibit biofilm with an IC_{50} value of 26.49 ± 1.62 μM, while compounds substituted with cycloalkanes (**11a**–**11d**) revealed much decreased effect on biofilm inhibition. We investigated if the substituents on the phenyl ring and the number of carbon atoms between the cyclic group and the amide bond have any influence on anti-biofilm activity and found that target compounds (**11j**–**11n**) containing various groups on the phenyl ring exhibited weak biofilm inhibitory activity with IC_{50} values > 38.42 ± 10.18 μM. In contrast, *p*-methoxyphenyl derivative (**11l**) showed a better anti-biofilm effect than the *m*-methoxyphenyl derivative **11m** or the *o*-methoxyphenyl derivative **11n**. Compounds **11s**, **11t**, and **11u** with 1, 2, and 3 carbons between the cyclic group and the amido bond of the side chain, respectively and electron donating groups on the benzene ring were synthesized in a manner similar to that used for **11l** and it was found that the increases in the chain length drastically reduced the biofilm inhibitory

activity of these compounds.

To explore the influence of the linker on anti-biofilm activity, the amide linker was replaced by a sulfonamide, providing compounds **13a**–**13g**. Compounds with a sulfonamide bond as a linker retained some anti-biofilm activity, but most of them exhibited a biofilm inhibitory activity (IC_{50} > 51.63 ± 8.61 μM) which is lower than that of **10d**. We explored the amino bond as a linker in compounds **16a**–**16j**. The anti-biofilm activities of compounds **16a**–**16j** which contain the amino bond as a linker were still weaker than that of **10d**. On the basis of these results, some preliminary SARs of the 3-hydroxy-1,6-dimethylpyridin-4(1*H*)-one derivatives can be summarized as follows: (1) compounds in the amide-linked series with six carbon alkyl chains (**10d**) exhibited efficient biofilm inhibitory activity; (2) compounds in the amide-linked series with various aliphatic and aromatic rings introduced into the chain exhibited reduced activity, and the length of the chain between the cyclic group and the linker had a dramatic negative influence on the biofilm inhibitory activity; (3) replacement of the amide bond with a sulfonamide or amino bond failed to increase the biofilm inhibitory activity.

Table 1. Biological evaluation of derivatives against the *P. aeruginosa* PAO1.

Compound	Structure	$IC_{50}^{a,b}$(μM)	Compound	Structure	$IC_{50}^{a,b}$(μM)
10a		45.14 ± 5.22	**11g**		41.67 ± 1.37
10b		56.88 ± 0.44	**11h**		> 100
10c		57.94 ± 2.13	**11i**		> 100
10d		6.57 ± 0.74	**11j**		> 100
10e		> 100	**11k**		61.05 ± 10.29
10f		> 100	**11l**		38.42 ± 10.18
11a		> 100	**11m**		> 100
11b		64.13 ± 0.62	**11n**		69.69 ± 5.79
11c		65.07 ± 4.57	**11o**		46.78 ± 9.37
11d		63.43 ± 7.62	**11p**		28.12 ± 6.74
11e		26.49 ± 1.62	**11q**		> 100
11f		75.56 ± 2.87	**11r**		54.95 ± 8.92

Compound	Structure	IC_{50}[a,b] (μM)	Compound	Structure	IC_{50}[a,b] (μM)
11s		65.45 ± 7.29	**16b**		52.45 ± 7.84
11t		59.07 ± 8.87	**16c**		> 100
11u		> 100	**16d**		14.92 ± 1.49
13a		> 100	**16e**		> 100
13b		> 100	**16f**		> 100
13c		59.31 ± 2.19	**16g**		> 100
13d		51.63 ± 8.61	**16h**		16.89 ± 0.43
13e		> 100	**16i**		> 100
13f		> 100	**16j**		34.96 ± 8.78
13g		35.31 ± 8.49	**DFP**		> 100
16a		24.53 ± 6.53			

[a]IC_{50} = concentration of inhibitor needed to inhibit biofilm formation by 50%. [b] For most compounds, the screening concentration of IC_{50} was 20-100 μM.

Compound 10d prevents P. aeruginosa biofilm formation without interfering with bacterial growth. Preliminary screening of biofilm inhibitory activity in the *P. aeruginosa* PAO1 strain indicated that compound **10d** has the best activity. To evaluate the potential anti-biofilm activity of **10d** comprehensively, its effects on the biofilm formation in *P. aeruginosa* PAO1 strains at concentrations of 1.25, 2.5, 5, 10 and 20 μM were measured. The results showed that **10d** reduced *P. aeruginosa* PAO1 biofilm biomass development in a dose-dependent manner and that it also has biofilm inhibitory activity with an inhibition rate >25% at concentrations as low as 1.25 μM (**Figure 3A**). The results of crystal violet staining assay in a glass tube confirmed that **10d** inhibits the formation of biofilm in a concentration-dependent manner (**Figure 3B**). Confocal laser scanning microcopy was used to observe the effect of **10d** on biofilms and, as shown in **Figure 3C**, after treatment with **10d** at concentrations of 1.25, 2.5, 5, 10 and 20 μM, the biofilm (green fluorescence) formation was reduced in a dose-dependent manner. We confirmed that **10d** (**Figure S311 A**) has no inhibitory effect on the growth of bacteria at 1.25, 2.5, 5, 10, 20 μM concentrations. These results show that **10d** could act as a biofilm inhibitor by acting on specific targets related to biofilm regulation rather than by inhibiting bacterial growth. The potential targets and inhibitory mechanisms of the compounds were explored systematically.

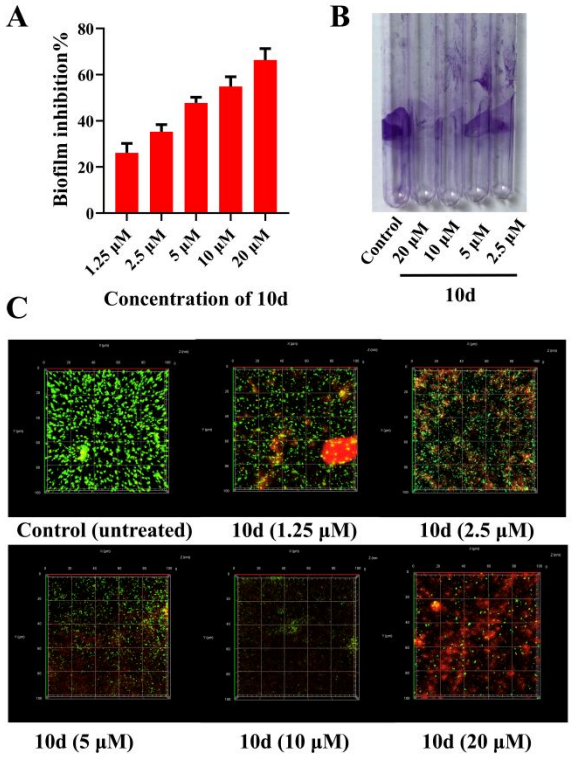


Figure 3. Effects of **10d** on *P. aeruginosa* biofilm formation. (A) Biofilm formation at different concentrations of **10d** (1.25, 2.5, 5, 10, 20 μM) for 24 h in a microtiter plate. Bacterial counts were quantified in triplicate. (B) Biofilm formation at different concentrations of **10d** (1.25, 2.5, 5, 10, 20 μM) for 24 h in a glass tube. Bacterial counts were quantified in triplicate. (C) 3D image of *P.aeruginosa* PAO1 biofilm after incubation with **10d** (1.25, 2.5, 5, 10, 20 μM) for 24 h. Control was untreated. The experiments were done in triplicate.

Compound 10d functions as an iron chelator, inducing P. aeruginosa–related iron deficiency. Considering that iron ions have a regulatory effect on bacterial biofilm formation, we first verified the iron-chelating ability of **10d** and the influence of iron content on bacteria. The pFe(III) value, defined as the negative logarithm of free iron concentration in a solution, was selected as the parameter with which to assess the ligand affinity for the metal under biological conditions[21] and pFe(III) of

10d was 20.6. The pFe(III) values of **10d** were determined using a fluorescence method with deferiprone (DFP) as the reference compound[22]. The results showed that **10d** exhibits satisfactory chelating ability with a pFe(III) value of 22.00 ± 0.14, which is stronger than the DFP pFe(III) value of 20.81 ± 0.44. The ability of **10d** to cause bacterial iron deficiency and inhibit bacterial biofilm formation was investigated. We analyzed the effect of various concentrations (1.25, 2.5, 5, 10, 20 μM) of **10d** (**Table 2**) on the iron content in *P. aeruginosa* PAO1 through inductively coupled plasma mass spectrometry. We found that the iron concentration in the bacteria was 0.88 ± 0.30 μM and 1.23 ± 0.24 μM after the addition of compound **10d** (20 μM) and positive control DFP (20 μM), which is significantly lower than that in the control group (1.91 ± 0.80 μM). Additionally, these data revealed that **10d** reduced the iron concentration in bacteria in a concentration-dependent manner, exhibiting a strong iron-chelating ability which could lead to iron deficiency in bacteria. We also found that the effect of compound **10d** on iron chelation was as strong as the positive control DFP, and the effects of compound **10d** on the induction of iron deficiency and the inhibition of biofilm formation in bacteria are considerably superior to those of DFP. The biofilm inhibition rate of DFP is >100 μM. These differences suggest that compound **10d** could exert an efficient anti-biofilm activity dependent on its iron ion chelation ability, but also could do so by chelating irons and supporting specific interactions with some targets in the iron acquisition system. One possibility is that the chelated iron ion is possibly used as a Trojan horse molecule to locate targets, and the lower activity of DFP could be attributed to its inability to bind to specific targets.

Table 2. Iron content in *P. aeruginosa* PAO1.

Compound	Concentration (μM)	Total Fe[a] (μM)
10d	0	1.91 ± 0.80
	1.25	1.55 ± 0.69
	2.5	1.56 ± 0.71
	5	1.17 ± 0.50
	10	0.97 ± 0.31
	20	0.88 ± 0.30
DFP	20	1.23 ± 0.24

[a]All dates represent mean ± S.D. from different experiments performed in triplicate.

Compound 10d competes strongly with Pvd. To investigate the validity of the aforementioned hypothesis, the effects of **10d** on the iron acquisition system were analyzed. Production of siderophores and utilization is one of the major strategies deployed by bacteria to access iron under iron-limiting conditions[23]. *P. aeruginosa* excretes two principal siderophores, namely pyoverdine[24] (Pvd) and pyochelin[25] (Pch). Pvd and Pch can naturally produce fluorescence in *P. aeruginosa* under iron-limiting conditions. Consequently, the effects of **10d** on the intercellular levels of Pvd and Pch were first evaluated by fluorescence detection in ABTGC medium (B-medium (0.1% $MgCl_2$, 0.1% $CaCl_2$, 0.1% $FeCl_3$) supplemented with 10% A10, 0.2% glucose and 0.2% casamino acids) containing 10 μM (iron-normal medium) and 50 μM (iron-sufficient medium) of $FeCl_3$. We found that **10d** (**Figure 4A**) strongly increases the level of Pvd in a concentration-dependent manner to counteract iron deficiency in an ABTGC medium containing 10 μM $FeCl_3$. However, iron-chelating agents such as DFP show negligible effects on the production of Pvd at all tested concentrations (1.25, 2.5, 5, 10, 20 μM). Furthermore, **10d** (**Figure 4B**) and

DFP have only a slight influence on the production of Pch. These results indicated that **10d** may have a high-affinity for the Pvd iron acquisition system that regulates the iron acquisition pathway, thus causing iron deficiency and inhibition of biofilm formation. The secretions of Pvd and Pch in *P. aeruginos*a were nearly undetectable under the condition of sufficient supplementation with $FeCl_3$ (50 μM in the ABTGC medium), where **10d** exhibited less effect on the levels of Pvd and Pch (**Figure S312**).

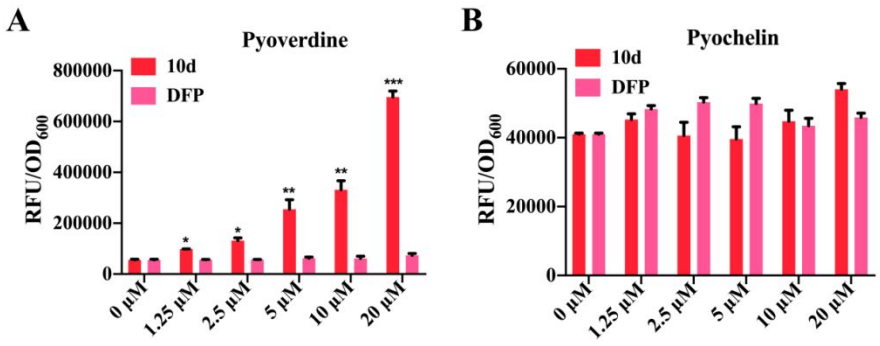


Figure 4. (A) Effects of **10d** on the production of iron-free pyoverdine at different concentrations (1.25, 2.5, 5, 10, 20 μM) in iron supplemented ABTGC (10 μM of $FeCl_3$). (B) Effects of **10d** on the production of iron-free pyochelin at different concentrations (1.25, 2.5, 5, 10, 20 μM) in iron supplemented ABTGC (10 μM of $FeCl_3$). The experiments were done in triplicate. Error bars are means ± SDs. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.00$.

Inactivation of anti-biofilm activity of 10d in Pvds mutant and overexpressed strains of fpvA. FpvA is a high-affinity outer membrane receptor of Pvd in *P. aeruginosa*, and is controlled by two distinct ECF sigma factors, namely σ^{FpvI} and σ^{PvdS}. The σ^{PvdS} factor is required for activating the synthesis of Pvd and the expression of FpvA and is encoded by the *Pvds* gene[26] and strains with *Pvds* mutants

showed negligible ability to acquire iron through the high-affinity pyoverdine iron acquisition system. To investigate the mechanisms of **10d** in the regulation of the high-affinity Pvd iron acquisition system and to identify its potential target, *Pvds* mutant and *fpvA*-overexpressed strains were selected to evaluate the regulatory role of **10d** in the high-affinity Pvd iron acquisition system. As depicted in **Figure 5A**, the inhibitory effects of **10d** on biofilm formation in the *Pvds* mutant strain (the high-affinity Pvd iron acquisition system deficient strain) was significantly reduced and the inhibitory effect of **10d** at 20 μM was decreased to approximately 20%. These results confirmed that **10d** can act on the high-affinity Pvd iron acquisition system and indicated that **10d** might serve as a biofilm inhibitor. We tested the effect of **10d** on biofilm inhibition in the *fpvA*-overexpressed strains, and our results showed that **10d** has a weak activity to inhibit biofilm in *fpvA*-overexpressed *P. aeruginosa* (**Figure 5B**). Taken together, our data suggest that **10d** is better able than the normal iron-chelating agent DFP to induce iron deficiency and biofilm inhibition in *P. aeruginosa* and that **10d** could compete with Pvd by specifically interacting with the siderophore receptor FpvA.

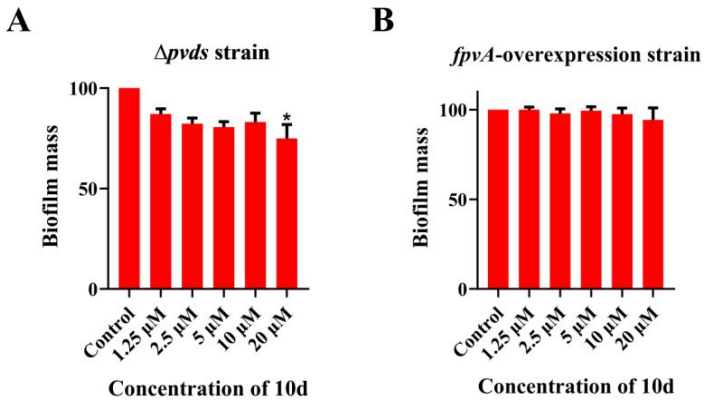


Figure 5. (A) Effects of **10d** on biofilm formation in *Pvds* mutant strain. (B) Effects of **10d** on biofilm formation in *fpvA*-overexpression strain. The experiments were done in triplicate. Error bars are means ± SDs. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.00$.

In silico study on 10d with FpvA target. We tried regular free docking between FpvA and **10d** in the presence or absence of Fe^{3+} in the binding pocket. Our results suggested that the **10d** is not comparable to Pvd from either the structural or energy aspects because of its relatively smaller size and weaker interaction. As mentioned above, **10d** competes strongly with Pvd, which raises the question of how the competition between such different molecules proceeds. After observing the Pvd-Fe structure and studying the quantitative reaction of **10d** and Fe^{3+}, we assumed that a sexadentate chelation of **10d** and iron ion could be the key to the competitive binding.

Tripolymers of **10d** were built and energy optimized in the opls3 force field in Maestro, Schrödinger. 529 reasonable conformations in two groups (cis- and trans-tripolymers) were obtained by conformational searching[27]. Following a two round strategy docking, many preferable conformations in the active pocket were obtained, most of them showing a better score (< -9) than Pvd (-7.37) in the same site and also

with favorable energy affinities (**Figure 6**). A 40 ns MD simulation was completed on the FpvA-**10d**-Fe complex, to confirm the affinity and potential conformational change between the FpvA and the test compound. As shown in **Figure 6B**, the binding of the **10d**-Fe tripolymer was located firmly in the binding site within the beta barrel domain of FpvA[28]. Two critical interactions were observed during the simulation: the hydrogen bonds with ARG69 and TYR661, along with the alkyl tails inserted into hydrophobic cavities surrounded by PHE660, TYR661, MET608 and ALA64. An MM-PBSA calculation based on the last 15 ns trajectories gave a satisfactory ΔG of -43.56 kcal/mol which is consistent with our hypothesis that **10d** competes with Pvd. Notably, although most of the FpvA structure remained stable, the conformation of the signal domain was most flexible during the MD simulation. This non negligible conformation change indicated the potential biological effects caused by the **10d**-Fe chelate.

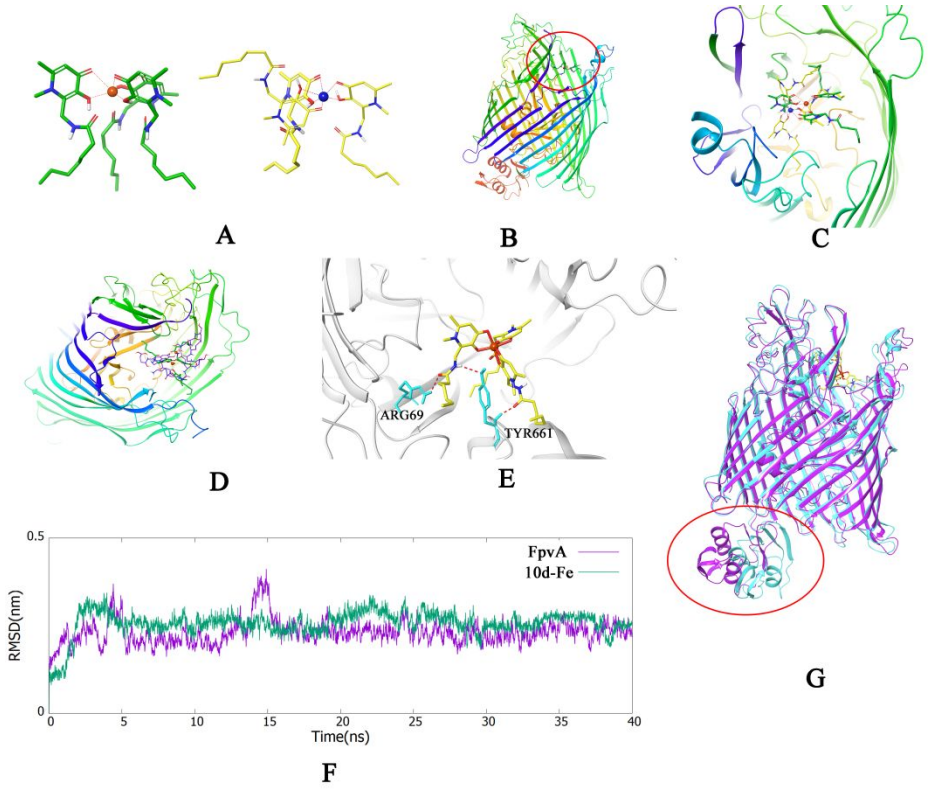


Figure 6. Docking conformation of **10d** tripolymer in FpvA. (A) Structures of cis- and trans-tripolymers. (B) tripolymer in the binding pocket of Pvd, (PDB ID 2W6T). (C) cis-(-10.499) and trans-(-9.182) tripolymers docking results. (D) cis-tripolymer (-10.499) and Pvd (-7.37). cis- and trans-tripolymers are depicted in green and yellow cylinders, respectively, Pvd is in purple. (E) key interactions between **10d**-Fe and FpvA in MD simulation. (F) RMSD of **10d**-Fe and FpvA during simulation. (G) signal domain conformation change in md simulation.

10d inhibits QS-controlled P. aeruginosa virulence. On the basis of the potential of **10d** as a dual-acting biofilm inhibitor and the fact that **10d** has approximately 20% biofilm inhibitory activity in the high-affinity Pvd iron acquisition system deficient strain (*Pvds* mutant strain), we tested the effect of **10d** on the QS systems, another putative regulatory pathway of compound **10d**. Because QS systems are responsible for the regulation of secretion of the virulence factor in *P. aeruginosa*

PAO1[29], the effect of **10d** on three virulence factors were examined. The virulence factors include elastase, rhamnolipid, and pyocyanin and they are regulated by the *las*, *rhl*, and *pqs* systems, respectively. The results are presented in **Figure 7**. As expected, **10d** significantly attenuates the activity of elastase (**Figure 7A**) and decreases the levels of rhamnolipid (**Figure 7B**) and pyocyanin (**Figure 7C**) in *P. aeruginosa* PAO1 in a concentration-dependent manner. Together, these results demonstrate that the inhibitory effect of **10d** on the production of three virulences might be related to the QS system.

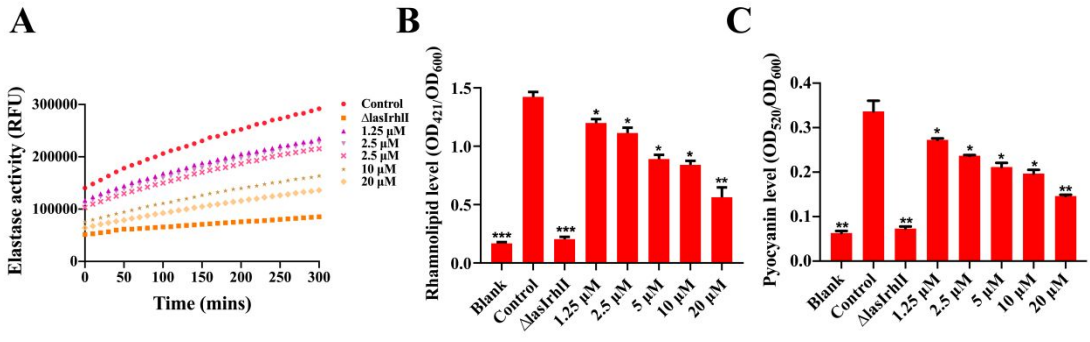


Figure 7. Effects of **10d** on the production of virulence factors at different concentrations (1.25, 2.5, 5, 10, 20 μM). (A) Elastase activity. (B) Rhamnolipid production. (C) Pyocyanin production. Error bars are means ± SDs. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

10d Inhibits the fluorescence of QS reporter strains. Three reporter strains, PAO1-*lasB*-*gfp*, PAO1-*rhlA*-*gfp*, and PAO1-*pqsA*-*gfp*[30] were used to further explore the influence of **10d** on the three QS systems. The green fluorescence protein (*gfp*) gene was integrated downstream from the promoters of *lasB*, *rhlA*, and *pqsA*, respectively, and the production of green fluorescent protein (GFP) indicated the activity of these promoters. As depicted in **Figures 8A-8C**, compound **10d**

significantly inhibits the fluorescence of PAO1-*lasB-gfp*, PAO1-*rhlA-gfp*, and PAO1-*pqsA-gfp* strains in a dose-dependent manner (1.25, 2.5, 5, 10, 20 μM). To verify that **10d** targets the corresponding QS genes rather than the GFP protein, the PAO1-*gfp* strain was used as a control strain to investigate the influence of **10d** on the fluorescence of the GFP protein. It was shown that **10d** has no effect on the fluorescence of GFP (**Figure 8D**). These experimental results indicate a direct inhibitory effect of **10d** on the QS pathway. Collectively, our data demonstrated that the biofilm inhibition of **10d** involved both iron uptake and QS systems.

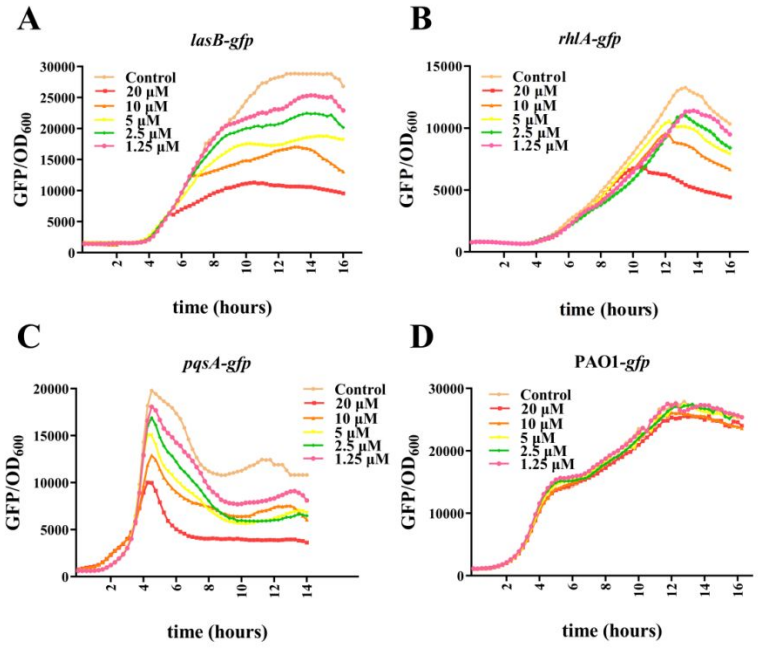


Figure 8. Dosage-dependent inhibition curves of **10d** incubated with the following QS monitors (A) PAO1-*lasB-gfp*, (B) PAO1-*rhlA-gfp* and (C) PAO1-*pqsA-gfp*. (D) PAO1- *gfp*. The experiments were done in triplicate.

10d Suppresses swimming and swarming motilities of P. aeruginosa. Flagellar and pili motility are necessary for *P. aeruginosa* biofilm development[31]. During the development of the *P. aeruginosa* biofilm, swarming and swimming motility are

mediated by flagella. These motility modes are used for colony expansion along a semisolid surface, which is correlated with the capacity of *P. aeruginosa* to colonize *in vivo*[32]. Twitching motility is mediated by pili, a motility mode commonly observed in dense aggregates with cell-to-cell contact[33,34], and plays a major role in the later stages of biofilm development. We assessed the effect of **10d** on the swimming, swarming, and twitching motilities of *P. aeruginosa* PAO1 by inoculating *P. aeruginosa* PAO1 after overnight culture onto motility plates. It was found that **10d** (**Figure 9A, B**) suppressed flagellum-driven swimming and swarming motilities in a concentration-dependent manner at concentrations of 1.25, 2.5, 5, 10 and 20 μM. This result was supported by the previously established anti-biofilm activity of **10d**. However, **10d** (**Figure 9C**) had no effect on the twitching motility of *P. aeruginosa* PAO1. This phenomenon was inconsistent with the report that indicated twitching motility was stimulated by lactoferrin[15] acting as an iron chelator, which can function as a biofilm inhibitor. This surprising result related to **10d** contrasts with the findings related to lactoferrin in a published study[15]. An earlier study showed that the twitching motility was positively correlated with the presence of *lasR*, *lasI*, and *rhlI* genes[35], and the ability of **10d** to inhibit the QS pathway was shown in the same study. Accordingly, the unchanged influence on the twitching motility of **10d** could be attributed to the suppression of twitching motility caused by the inhibition of the QS pathway which cancels the stimulation caused by **10d** as an iron chelator. Therefore, **10d** exhibits no effect on the twitching motility of *P. aeruginosa* PAO1. This result further confirmed that **10d** is a dual-acting biofilm inhibitor rather than a single target

iron-chelating biofilm inhibitor. Furthermore, according to the characteristics of the mechanism of the biological effects of **10d**, we speculate that **10d** may mainly act on the PVD receptor FpvA to cause iron deficiency at low concentrations, which will stimulating twitching motility, and thus cause results in d and e (**Figure 9C**) to likely exceed that of blank control.

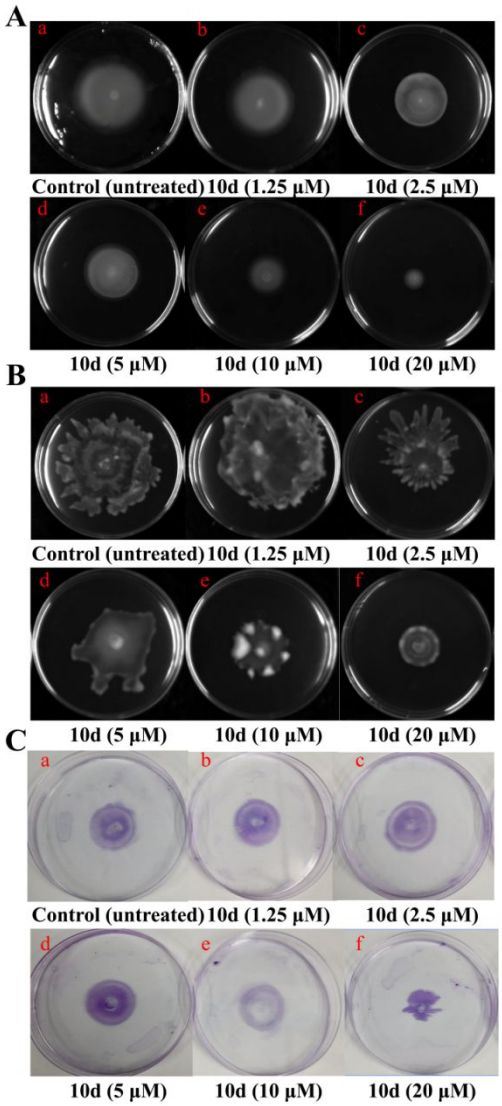


Figure 9. Motility inhibition assays of *P. aeruginosa* PAO1. Three motility assays were conducted on plates containing **10d** on agar at different concentrations (1.25, 2.5, 5, 10, 20 μM). (A) Swimming motility. (B) Swarming motility. (C) Twitching motility. The results shown are

representative of results from three independent experiments.

Through a series of evaluations of biological characterization, we demonstrated that **10d** can not only selectively act on the Pvd receptors FpvA to cause iron deficiency but also can interfere with the QS pathway to reduce QS-regulated virulence factors (elastase, rhamnolipid, and pyocyanin) as well as the bacterial motility in *P. aeruginosa.*

Effect of 10d on cytotoxicity. We evaluated the toxicity of **10d** in the African green monkey kidney cell line Vero and the noncancerous human hepatocyte cell line LO2 using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The chelating agent DFP can affect the iron content in normal cells and possesses cytotoxicity within a certain range[36-38]. We found that **10d** (**Figure. 10**) is less cytotoxic than DFP to Vero and LO2 cells, and has less effect on the viability of Vero and LO2 cells at concentrations up to 50 μM. Taken together, these results indicated that compound **10d** has low toxicity in Vero and LO2 cells.

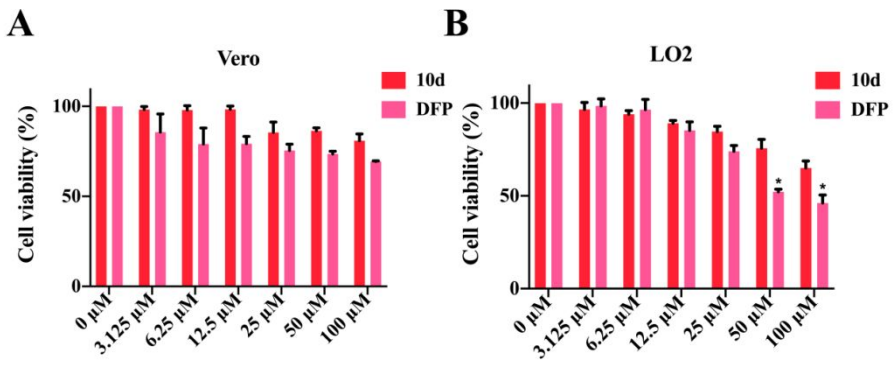


Figure 10. Vero (A) and LO2 (B) cells were treated with **10d** at various concentrations (0, 3.125, 6.25, 12.5, 25, 50, 100 μM) for 24 h. Cell viability was determined using an MTT assay. Error bars are means ± SDs. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.00$.

10d Reduces P. aeruginosa acute virulence in cells. To assess the potential of the synthesized compounds for reduction of *P. aeruginosa* virulence, we evaluated the ability of **10d** *in vitro* to block *P. aeruginosa* acute virulence against the African green monkey kidney cell line Vero and noncancerous human hepatocyte cell line LO2 using cell viability as a readout. Cell survival was quantified 4 h post-infection with *P. aeruginosa* in the presence or absence of **10d**. *P. aeruginosa* was pre-treated with different concentrations of **10d** (0, 1.25, 2.5, 5, 10, and 20 μM) for 24 h. The Vero and LO2 cells were infected with *P. aeruginosa* for 4 h. Subsequent MTT assay showed that the pre-treatment with **10d** significantly and dose-dependently promoted the survival of infected Vero and LO2 cells (**Figure 11**). Notably, in the presence of compound **10d** (20 μM), the cell survival rate of Vero was increased from 41.5% to 96.4% (**Figure 11A**) and that of LO2 was enhanced from 40.7% to 89.9% (**Figure 11B**). These data indicate that **10d** has considerable therapeutic potential as a dual biofilm inhibitor in acute *P. aeruginosa* infections.

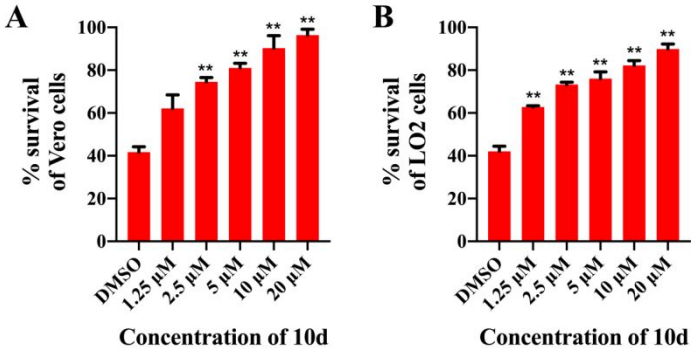


Figure 11. **10d** rescues *P. aeruginosa* cell cytotoxicity. (A) anti-virulence efficacy in *Vero* cell infection assays of **10d** at various concentrations (1.25, 2.5, 5, 10, 20 μM). (B) anti-virulence efficacy in LO2 cell infection assays of **10d** at various concentrations (1.25, 2.5, 5, 10, 20 μM). Error bars are means ± SDs. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.00$.

Compound 10d prevents biofilm formation in clinical strains of P. aeruginosa. Finally, we evaluated the biofilm inhibitory effect of **10d** against four multidrug resistant (MDR) strains obtained from the First Affiliated Hospital of Jinan University Clinical Laboratory. Notably, **10d** (**Table 3**) exhibits efficient biofilm inhibition activity against MDR strains, in cases in which modern antibiotic therapy failed. This result indicates that **10d** could be used clinically as a potential biofilm inhibitor.

Table 3. The biofilm inhibition rate of 10d against clinical strains of *P. aeruginosa.*

Strain	Inhibition rate[a] (%)				
	20 μM	10 μM	5 μM	2.5 μM	1.25 μM
1121	23.17±10.72	4.41±1.01	10.56±1.82	7.68±6.40	11.80±3.84
1129	40.02±7.32	30.44±4.32	30.61±4.33	21.29±2.04	12.66±1.94
1167	51.42±3.34	47.44±0.78	43.69±1.63	34.65±3.35	34.52±4.38
FB	14.37±3.54	18.38±2.99	3.57±1.24	-8.67±4.69	-5.12±0.71

[a]All data represent mean ± S.D. from different experiments performed in triplicate.

CONCLUSION

Bacterial antibiotic resistance presents a considerable challenge to clinical efforts to combat infection. One mechanism of infection involves the formation of bacterial biofilms[39], and consequently, biofilm inhibitors may be part of a new strategy to overcome bacterial antibiotic resistance[40]. In this study, potential dual-acting biofilm inhibitors using hydroxypyridone[41,42] as an iron chelator combined with quorum-sensing signal molecules *N*-acyl-homoserine lactone (Acyl-HSL) and *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (OdDHL) were designed and

synthesized. Most of the compounds showed potential biological activity, and among them, compound **10d** exhibited promising anti-biofilm activity for both standard strain PAO1 and clinical multidrug resistant strains. This shows that 3-hydroxy-1,6-dimethylpyridin-4(1*H*)-one is an effective anti-biofilm pharmacophore structure. Further mechanistic studies have confirmed that **10d** can act both as an iron chelator to compete strongly with Pvd, and as an inhibitor of the fluorescence of QS-controlled PAO1-*lasB*-*gfp*, PAO1-*rhlA*-*gfp* and PAO1-*pqsA*-*gfp* strains in a concentration-dependent manner. We proved that as a dual-acting biofilm inhibitor, **10d** can selectively act on both the Pvd receptor FpvA to cause iron deficiency and on the QS pathway to reduce QS-regulated virulence factors (elastase, rhamnolipid, and pyocyanin) in *P. aeruginosa.* These encouraging results demonstrate that the development of dual-acting agents with both iron-uptake and QS inhibitory effects is a novel and feasible strategy for constructing effective biofilm inhibitors for the treatment of *P. aeruginosa* infections in the future.

EXPERIMENTAL SECTION

Organic Synthesis.

General experimental. All solvents and reagents used were purchased from commercial sources and used as received unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX400 spectrometer at 400 MHZ or a NT-300 WB spectrometer at 300 MHz. Chemical shifts (δ) are reported in ppm relative to the internal standard tetramethylsilane. High-resolution mass spectra (HRMS) were obtained on a Finnigan MAT-90 spectrometer or an Agilent 6210 series LC/MSD TOF mass spectrometer. Melting point analysis was determined with a digital melting point apparatus (Briwell/GH60). Melting points are uncorrected. Thin layer chromatography (TLC) was performed using Merck 60 F254 silica gel plates. The purity of the compounds was checked by high-performance liquid chromatography (HPLC). HPLC analyses were conducted with a Thermo Scientific Dionex Ultimate 3000 series consisting of a RS pump, an automatic solvent degasser, and a manual injector. Separations were carried out using a reversed-phase C_{18} analytical column. All compounds were obtained with a purity > 95%.

3-Hydroxy-2-(hydroxymethyl)-6-methyl-4H-pyran-4-one (1). Allomaltol (37.00 g, 290.00 mmol) was dissolved in water (400 mL) and NaOH (13.00 g, 320.00 mmol) was added, 37% aqueous formaldehyde solution (27 mL) was added dropwise, and the mixture was stirred at rt overnight. After the reaction was completed, the pH was adjusted to 1 with concentrated HCl and the mixture was then cooled to 0 °C. A large amount of solids were precipitated and were filtered and dried to give **1** as a white

solid (42.70 g, 93.20%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.26 (s, 3H), 4.39 (s, 2H), 5.36 (brs, 1H), 6.22 (s, 1H), 8.87 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.4, 165.1, 149.9, 141.7, 111.6, 55.5, 19.8. HRMS (ESI): calcd for $C_7H_9O_4$ $[M + H]^+$ = 157.0495, found $[M + H]^+$ = 157.0495. mp 158.3-159.2 °C.

3-(Benzyloxy)-2-(hydroxymethyl)-6-methyl-4H-pyran-4-one (2). Compound **1** (42.70 g, 270.00 mmol) was dissolved in MeOH (300 mL), NaOH (12.00 g, 300.00 mmol) was added, and benzyl bromide (46.20 g, 270.00 mmol) was slowly added dropwise. The reaction was completed after reflux for 12 h. The reaction solution was concentrated under reduced pressure, and the residue was dissolved in H_2O (300 mL) and extracted three times with DCM, then washed twice with 5% aqueous sodium hydroxide solution and saturated brine to collect the organic phase. The combined organic layer was dried with anhydrous Na_2SO_4, concentrated *in vacuo* and the crude product was recrystallized from DCM/petroleum ether to afford the solid **2** (56. 60 g, 85.20%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.26 (s, 3H), 4.28 (d, J = 6.0 Hz, 2H), 5.02 (s, 2H), 5.36 (t, J = 6.0 Hz, 1H), 6.27 (s, 1H), 7.3-7.45 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.4, 165.4, 159.7, 142.1, 137.3, 129.0, 128.8, 128.6, 114.6, 73.8, 55.7, 19.5. HRMS (ESI): calcd for $C_{14}H_{15}O_4$ $[M + H]^+$ = 247.0965, found $[M + H]^+$ = 247.0965. mp 113.2-114.0 °C.

3-(Benzyloxy)-2-(hydroxymethyl)-1,6-dimethylpyridin-4(1H)-one (5). Compound **2** (50.00 g, 200.00 mmol) was dissolved in anhydrous DCM (250 mL), 3,4-dihydropyran (33.30 g, 400.00 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added, then allowed to react at room temperature (rt) for 2 h. Then the

reaction solution was washed twice with 5% acqueous Na_2CO_3 solution, washed twice with H_2O, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain **3** as a yellow oil of. The yellow oil (**3**) was dissolved in EtOH (100 mL) and methylamine (100 mL). The reaction mixture was sealed in a thick-walled glass tube and stirred at 70 °C for 24 h. Next, the mixture was concentrated *in vacuo* to obtain a brown liquid (**4**). The brown liquid **4** was dissolved in EtOH (20 mL), and 2N HCl (25 mL) was added for reflux for 4 h. The solvent was concentrated *in vacuo* prior to addition of H_2O (150 mL). Subsequent adjustment of the aqueous fraction to pH 9 with 10N NaOH solution was followed by extraction 3 times with DCM . The organic phase was dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give a crude product, which was recrystallized from MeOH/Et_2O, afford the solid **5** (39.60 g, 76.50%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.29 (s, 3H), 3.60 (s, 3H), 4.56 (d, J = 6.0 Hz, 2H), 5.03 (s, 2H), 5.50 (t, J = 6.0 Hz, 1H), 6.17 (s, 1H), 7.20-7.45 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.8, 148.3, 145.1, 143.3, 138.2, 128.7, 128.2, 117.7, 73.1, 54.3, 35.9, 20.4. HRMS (ESI): calcd for $C_{15}H_{18}NO_3$ $[M + H]^+$ = 260.1281, found $[M + H]^+$ = 260.1286. mp 145.2-146.4 °C.

2-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)iso-indoline-1,3-dione (6). Compound **5** (39.00 g, 150.00 mmol), phthalimide (26.50 g, 180.00 mmol), and triphenylphosphine (47.20 g, 180.00 mmol) were placed in a dry 500 mL two-neck flask and protected under nitrogen. Next, 200 mL of anhydrous THF was added and the reaction mixture was placed in ice H_2O at 0 °C for 30 min, and then

diethyl azodicarboxylate (31.30 g, 180.00 mmol) was slowly added dropwise. After the addition was complete, the temperature was slowly raised to rt. Stirring overnight at rt, a large amount of precipitate was formed in the reaction solution, which was filtered. The filter cake was washed with cold THF to obtain 49.40 g of a white solid (**6**). Yield: 84.50%. This white solid was directly used in the next step.

2-(Aminomethyl)-3-(benzyloxy)-1,6-dimethylpyridin-4(1H)-one (7). Compound **6** (48.00 g, 120.00 mmol) was dissolved in EtOH (300 mL), 90 mL of 5.5% hydrazine hydrate was added and the mixture was heated under reflux for 3 h. Then the reaction mixture was concentrated under reduced pressure. 200 mL of H_2O was added to the residue, followed by 10 N NaOH solution, adjusting to pH = 12, extracted three times with DCM, dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure. Column chromatography eluted with DCM/MeOH = 10/1. **7** is a white solid (25.00 g, 78.10%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H), 3.59 (s, 3H), 3.73 (s, 2H), 5.08 (s, 2H), 6.14 (s, 1H), 7.20-7.45 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.6, 147.9, 145.3, 144.5, 138.3, 129.0, 128.7, 128.3, 117.6, 72.6, 36.9, 35.6, 20.6. HRMS (ESI): calcd for $C_{15}H_{19}N_2O_2$ $[M + H]^+$ = 259.1441, found $[M + H]^+$ = 259.1441. mp 147.2-148.6 °C.

1. General procedure for the reaction of 8a–8f, 9a–9u, 12a–12g (Procedure A).

Procedure A: A solution of R-COCl or R-SO_2Cl (2.40 mmol, 1.2 equiv) in dry DMF (2 mL) was added dropwise to a solution of compound **7** (2.00 mmol, 1 equiv) in DMF (10 mL) comprising 0.80 mL of pyridine (10.00 mmol, 5 equiv) at 0 °C. under nitrogen. The reaction mixture was then stirred under nitrogen at rt for 4 h.

Then the solvent was removed under high vacuum. The residue was dissolved in DCM (50 mL), washed with 1 N HCl (20 mL) and H_2O (2 × 20 mL), dried over anhydrous Na_2SO_4 and filtered, and then the solvent was removed *in vacuo*. Following this, the residue was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid or a colorless sticky liquid.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-butyramide (8a). The reaction was carried out according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and butyryl chloride (0.26 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford colorless sticky liquid **8a** (0.54 g, 82.50%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.84 (t, J = 7.4 Hz, 3H), 1.19-1.30 (m, 2H), 1.44-1.60 (m, 2H), 2.08 (t, J = 7.3 Hz, 2H), 2.29 (s, 3H), 3.44 (s, 3H), 4.38 (d, J = 4.9 Hz, 2H), 5.09 (s, 2H), 6.21 (s, 1H), 7.29-7.45 (s, 5H), 8.05 (t, J = 4.5 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.6, 172.5, 148.4, 146.1, 140.9, 138.8, 128.8, 128.7, 128.3, 117.9, 115.0, 72.8, 37.4, 36.1, 34.8, 20.6, 19.1, 14.1. HRMS (ESI): calcd for $C_{19}H_{25}N_2O_3$, $[M + H]^+$ = 329.1860, found $[M + H]^+$ = 329.1867.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)pent-anamide (8b). The reaction was prepared according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and valeryl chloride (0.29 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford colorless sticky liquid **8b** (0.58 g, 84.80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, J = 6.9 Hz, 3H), 1.12-1.30 (m, 2H), 1.41-1.53 (m, 2H), 2.11 (t, J = 7.4 Hz, 2H), 2.29

(s, 3H), 3.43 (s, 3H), 4.37 (d, J = 4.9 Hz, 2H), 5.09 (s, 2H), 6.19 (s, 1H), 7.33-7.49 (s, 5H), 8.07 (t, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.7, 172.5, 148.3, 146.2, 140.8, 138.2, 128.8, 128.7, 128.3, 117.9, 72.7, 36.1, 35.2, 34.8, 27.8, 22.3, 20.7, 14.2. HRMS (ESI): calcd for $C_{20}H_{27}N_2O_3$ $[M + H]^+$ = 343.2016, found $[M + H]^+$ = 343.2016.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)hexan-amide (8c). The reaction was prepared according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and hexanoyl chloride (0.29 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford colorless sticky liquid **8c** (0.61 g, 84.90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.84 (t, J = 6.9 Hz, 3H), 1.12-1.30 (m, 4H), 1.41-1.53 (m, 2H), 2.10 (t, J = 7.4 Hz, 2H), 2.29 (s, 3H), 3.43 (s, 3H), 4.37 (d, J = 4.9 Hz, 2H), 5.09 (s, 2H), 6.20 (s, 1H), 7.33-7.49 (s, 5H), 8.06 (t, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.7, 172.5, 148.3, 146.2, 140.8, 138.2, 128.8, 128.7, 128.3, 117.9, 72.7, 36.1, 35.5, 34.8, 31.4, 25.3, 22.3, 20.6, 14.3. HRMS (ESI): calcd for $C_{21}H_{29}N_2O_3$ $[M + H]^+$ = 357.2173, found $[M + H]^+$ = 357.2173.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)heptan-amide (8d). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and heptanoyl chloride (0.30 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford colorless sticky liquid **8d** (0.66 g, 89.60%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.84 (t, J = 6.6 Hz, 3H), 1.18-1.29 (m, 6H), 1.44-1.55 (m, 2H), 2.10 (t, J = 7.4 Hz,

2H), 2.29 (s, 3H, CH_3), 3.43 (s, 3H), 4.38 (d, J = 4.9 Hz, 2H), 5.09 (s, 2H), 6.19 (s, 1H), 7.30-7.50 (s, 5H), 8.04 (t, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.7, 172.5, 148.3, 146.2, 140.8, 138.2, 128.8, 128.7, 128.3, 117.9, 72.7, 36.1, 35.5, 34.8, 31.4, 28.8, 25.6, 22.4, 20.6, 14.6. HRMS (ESI): calcd for $C_{22}H_{31}N_2O_3$ $[M + H]^+$ = 371.2329, found $[M + H]^+$ = 371.2329.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)octan-amide (8e). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and octanoyl chloride (0.39 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford colorless sticky liquid **8e** (0.62 g, 80.50%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, J = 6.7 Hz, 3H), 1.15-1.30 (m, 8H), 1.44-1.55 (m, 2H), 2.10 (t, J = 7.4 Hz, 2H), 2.28 (s, 3H), 3.43 (s, 3H), 4.38 (d, J = 4.9 Hz, 2H), 5.09 (s, 2H), 6.19 (s, 1H), 7.29-7.51 (s, 5H), 8.07 (t, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.7, 172.5, 148.3, 146.2, 140.8, 138.2, 128.8, 128.7, 128.7, 128.3, 117.9, 72.7, 36.1, 35.5, 34.8, 31.6, 29.1, 28.9, 25.7, 22.5, 20.6, 14.4. HRMS (ESI): calcd for $C_{23}H_{33}N_2O_3$ $[M + H]^+$ = 385.2468, found $[M + H]^+$ = 385.2468.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-(2-nonyl-1,3-dioxolan-2-yl)acetamide (8f). Compound **7** (0.50 g, 2.00 mmol), 1-ethyl-3(3-dimethylpropylamine)carbodiimide hydrochloride (EDCI) (0.38 g, 2.00 mmol), 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (0.24 g, 2.00 mmol) and 2-(2-nonyl-1,3-dioxolan-2-yl)acetic acid (0.77 g, 3.00 mmol) in anhydrous DCM were stirred under nitrogen at rt. After completion judged by TLC, brine was added.

The organic phase was separated, washed with brine and 5% $NaHCO_3$, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography (MeOH:DCM = 1:25) to afford the white solid **8f** (0.58 g, 58.40%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, J = 5.7 Hz, 3H), 1.15-1.30 (m, 14H), 1.49 (t, J = 7.4 Hz, 1H), 1.70 (s, 1H), 2.07 (t, J = 7.4 Hz, 1H), 2.12 (s, 3H), 2.41 (s, 1H), 3.33-3.40 (m, 2H), 3.45 (s, 3H), 3.79-3.85 (m, 2H), 4.37 (d, J = 4.9 Hz, 2H), 5.09 (s, 2H), 6.20 (s, 1H), 7.30-7.40 (s, 5H), 8.00 (t, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.7, 172.5, 168.6, 148.2, 146.2, 140.8, 140.7, 138.2, 128.8, 128.7, 128.3, 118.0, 109.6, 72.8, 64.7, 43.5, 37.5, 36.1, 31.8, 29.7, 29.5, 29.5, 29.4, 29.4, 29.2, 25.7, 23.5, 22.6, 20.6, 14.4. HRMS (ESI): calcd for $C_{29}H_{43}N_2O_5$ $[M + H]^+$ = 499.3166, found $[M + H]^+$ = 499.3166. mp 110.6-111.7 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)cyclo-propane-carboxamide (9a). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and cyclopropanecarbonyl chloride (0.25 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9a** (0.50 g, 76.00%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.62-0.72 (m, 4H), 1.53-1.65 (m, 1H), 2.29 (s, 3H), 3.44 (s, 3H), 4.39 (d, J = 5.0 Hz, 2H), 5.09 (s, 2H), 6.18 (s, 1H), 7.32-7.51 (m, 5H), 8.35 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.1, 172.5, 148.3, 146.1, 140.8, 138.1, 128.9, 128.7, 128.3, 118.0, 72.7, 36.1, 35.0, 20.6, 13.8, 6.9. HRMS (ESI): calcd for $C_{19}H_{23}N_2O_3$ $[M + H]^+$ = 327.1703, found $[M + H]^+$ = 327.1703. mp 198.1-199.7 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)cyclo-butane-carboxamide (9b). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, mg, 2.00 mmol) and cyclobutanecarbonyl chloride (0.28 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9b** (0.51 g, 74.70%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.71-1.22 (m, 6H), 2.28 (s, 3H), 2.97-3.08 (m, 1H), 3.41 (s, 3H), 4.27 (d, J = 5.0 Hz, 2H), 5.08 (s, 2H), 6.19 (s, 1H), 7.30-7.46 (m, 5H), 7.92 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.3, 172.5, 148.3, 146.1, 140.8, 138.2, 128.9, 128.7, 128.3, 117.9, 72.7, 40.2, 36.1, 34.8, 25.1, 20.6, 18.3. HRMS (ESI): calcd for $C_{20}H_{25}N_2O_3$ $[M + H]^+$ = 341.1860, found $[M + H]^+$ = 341.1860. mp 183.6-184.7 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-cyclopentane-carboxamide (9c). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and cyclopentanecarbonyl chloride (0.32 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9c** (0.58 g, 81.60%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.42-1.79 (m, 8H), 2.29 (s, 3H), 2.55-2.63 (m, 1H), 3.42 (s, 3H), 4.37 (d, J = 4.9 Hz, 2H), 5.08 (s, 2H), 6.19 (s, 1H), 7.32-7.51 (m, 5H), 8.05 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.8, 172.50, 148.3, 145.1, 140.9, 138.2, 128.2, 128.7, 128.3, 117.9, 72.8, 44.4, 36.1, 34.9, 30.5, 26.1, 20.6. HRMS (ESI): calcd for $C_{21}H_{27}N_2O_3$ $[M + H]^+$ = 355.2016, found $[M + H]^+$ = 355.2016. mp 180.1-180.7 °C.

***N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-cyclohexane-carboxamide*(*9d*).** The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and cyclohexanecarbonyl chloride (0.35 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9d** (0.62 g, 83.80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.11-1.39 (m, 6H), 1.61-1.80 (m, 4H), 2.21-2.30 (m, 1H), 2.29 (s, 3H), 3.41 (s, 3H), 4.36 (d, J = 5.0 Hz, 2H), 5.08 (s, 2H), 6.19 (s, 1H), 7.30-7.46 (m, 5H), 7.95 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.6, 172.5, 148.3, 146.2, 140.9, 138.2, 128.8, 128.7, 128.3, 117.9, 72.8, 44.2, 36.1, 29.6, 25.9, 25.7, 20.6. HRMS (ESI): calcd for $C_{22}H_{29}N_2O_3$ $[M + H]^+$ = 369.2173, found $[M + H]^+$ = 369.2173. mp 197.3-198.0 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)furan-2-carboxamide (9e). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and furan-2-carbonyl chloride (0.31 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9e** (0.53 g, 74.70%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.47 (s, 3H), 4.56 (d, J = 4.9 Hz, 2H), 5.14 (s, 2H), 6.21 (s, 1H), 6.62 (dd, J = 3.5 Hz, J = 1.7 Hz, 1H), 7.19 (dd, J = 3.5 Hz, J = 0.8 Hz, 1H), 7.30-7.50 (m, 5H), 7.85 (dd, J = 1.7 Hz, J = 0.8 Hz, 1H), 8.58 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 158.2, 148.3, 147.7, 146.3, 145.8, 140.3, 138.3, 128.8, 128.7, 128.3, 117.9, 114.4, 112.3, 72.5, 36.3, 35.0, 20.6. HRMS (ESI): calcd for $C_{20}H_{21}N_2O_4$ $[M + H]^+$ = 353.1496, found $[M + H]^+$ = 353.1496. mp 178.7-179.6 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-1-napht hamide (9f). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 1-naphthoyl chloride (0.46 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9f** (0.66 g, 79.80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.31 (s, 3H), 3.60 (s, 3H), 4.72 (d, J = 4.7 Hz, 2H), 5.15 (s, 2H), 6.22 (s, 1H), 7.26-7.40 (m, 3H), 7.46-7.62 (m, 6H), 7.92-8.06 (m, 2H), 8.15-8.22 (m, 1H), 8.88 (t, J = 4.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 169.0, 148.4, 146.4, 140.4, 138.3, 134.3, 133.6, 130.5, 130.2, 128.7, 128.7, 128.3, 127.3, 126.7, 126.1, 125.7, 125.3, 118.0, 72.7, 36.4, 35.7, 20.6. HRMS (ESI): calcd for $C_{26}H_{25}N_2O_3$ $[M + H]^+$ = 413.1860, found $[M + H]^+$ = 413.1861. mp 206.9-208.1 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)benz-am ide (9g). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and benzoyl chloride (0.34 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9g** (0.65 g, 89.30%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.30 (s, 3H), 3.50 (s, 3H), 4.62 (d, J = 4.6 Hz, 2H), 5.14 (s, 2H), 6.23 (s, 1H), 7.26-7.35 (m, 3H), 7.42-7.55 (m, 5H), 7.85-7.93 (m, 2H), 8.72 (t, J = 4.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 166.9, 148.3, 146.4, 140.5, 138.3, 134.3, 131.9, 128.8, 128.7, 128.2, 127.9, 117.9, 72.7, 36.3, 35.9, 20.6. HRMS (ESI): calcd for $C_{22}H_{23}N_2O_3$ $[M + H]^+$ = 363.1703, found $[M + H]^+$ = 363.1703. mp 172,1-172.8 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-isonicot

inamide(9h). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and isonicotinoyl chloride (0.30 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white liquid **9h** (0.58 g). Yield: 99.70%. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.71 (s, 2H), 8.58 (s, 1H), 7.85 (s, 2H), 7.24 (s, 5H), 6.26 (s, 1H), 5.03 (s, 2H), 4.64 (d, J = 4.8 Hz, 2H), 3.54 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.9, 20.9, 35.9, 36.6, 36.6, 73.5, 118.3, 121.4, 128.3, 128.4, 128.5, 140.5, 150.4, 165.5, 172.5.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-(trifluoromethyl)isonicotinamide (9i). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 2-(trifluoromethyl) isonicotinoyl chloride (0.46 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white liquid **9i** (0.44 g). Yield: 63.70%. ^{1}H NMR (300 MHz, Chloroform-*d*): δ 9.81 (s, 1H), 8.77 (d, J = 4.8 Hz, 1H), 8.39 (s, 1H), 8.25 (d, J = 4.6 Hz, 1H), 7.14 (s, 5H), 6.14 (s, 1H), 4.88 (s, 2H), 4.65 (d, J = 3.9 Hz, 2H), 3.53 (s, 3H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.9, 29.3, 36.8, 73.7, 117.9, 118.7, 124.2, 127.7, 128.2, 128.5, 136.4, 141.3, 142.0, 148.9, 150.6, 164.2, 172.2.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-fluoroBenzamide (9j). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 4-fluorobenzoyl chloride (0.38 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM =

1:25) to afford the white solid **9j** (0.65 g, 85.90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.29 (s, 3H), 3.49 (s, 3H), 4.61 (d, J = 4.6 Hz, 2H), 5.13 (s, 2H), 6.22 (s, 1H), 7.26-7.50 (m, 7H), 7.90-8.01 (m, 2H), 8.78 (t, J = 4.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.6, 166.1, 165.9, 162.8, 148.3, 146.4, 140.4, 138.2, 130.8, 130.6, 128.8, 128.7, 128.2, 117.9, 115.7, 115.5, 72.7, 49.1, 36.3, 25.9, 20.6. HRMS (ESI): calcd for $C_{22}H_{22}FN_2O_3$ $[M + H]^+$ = 381.1609, found $[M + H]^+$ = 381.1609. mp 204.9-205.6 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-(trifluoromethyl)benzamide (9k). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 4-(trifluoromethyl)benzoyl chloride (0.50 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9k** (0.67 g, 78.10%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.64 (s, 3H), 3.92 (s, 3H), 4.85 (d, J = 4.6 Hz, 2H), 5.22 (s, 2H), 7.36-7.55 (m, 6H), 7.85 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 8.1 Hz, 1H), 9.15 (t, J = 4.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 164.2, 153.1, 147.9, 143.5, 136.8, 129.1, 129.0, 128.9, 125.8, 114.7, 75.0, 36.3, 21.4. HRMS (ESI): calcd for $C_{23}H_{22}F_3N_2O_3$ $[M + H]^+$ = 431.1577, found $[M + H]^+$ = 431.1577. mp 236.2-236.9 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-methoxybenzamide (9l). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 4-methoxybenzoyl chloride (0.41 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM =

1:25) to afford the white solid **9l** (0.61 g, 81.50%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.29 (s, 3H), 3.48 (s, 3H), 3.80 (s, 3H), 4.59 (d, J = 4.6 Hz, 2H), 5.13 (s, 2H), 6.22 (s, 1H), 6.98 (d, J = 8.8 Hz, 2H), 7.19 (dd, J = 3.5 Hz, 2H), 7.25-7.49 (m, 5H), 7.86 (d, J = 8.8 Hz, 1H), 8.55 (t, J = 4.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 166.3, 162.2, 148.3, 146.3, 140.7, 138.3, 129.8, 128.8, 128.7, 128.3, 126.4, 117.9, 113.9, 72.7, 55.8, 36.3, 35.7, 20.6. HRMS (ESI): calcd for $C_{23}H_{25}N_2O_4$ $[M + H]^+$ = 393.1809, found $[M + H]^+$ = 393.1808. mp 205.4-206.9 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-3-meth oxybenzamide (9m). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 3-methoxybenzoyl chloride (0.41 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9m** (0.61 g, 81.60%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 3H), 3.49 (s, 3H), 3.79 (s, 3H), 4.61 (d, J = 4.7 Hz, 2H), 5.13 (s, 2H), 6.21 (s, 1H), 7.06-7.15 (m, 1H), 7.25-7.40 (m, 4H), 7.46-7.53 (m, 4H), 8.76 (t, J = 4.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.6, 166.6, 159.6, 148.3, 146.4, 140.5, 138.3, 135.7, 129.8, 128.8, 128.7, 128.7, 128.2, 120.2, 117.9, 117.7, 113.1, 72.7, 55.8, 36.3, 35.9, 20.6. HRMS (ESI): calcd for $C_{23}H_{25}N_2O_4$ $[M + H]^+$ = 393.1809, found $[M + H]^+$ = 393.1809 . mp 170.9-171.5 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-meth oxybenzamide (9n). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 2-methoxybenzoyl chloride (0.41 g, 2.40 mmol). The crude product was purified by column chromatography

(MeOH:DCM = 1:25) to afford the white solid **9n** (0.63 g, 83.20%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.30 (s, 3H), 3.60 (s, 3H), 3.75 (s, 3H), 4.63 (d, J = 5.4 Hz, 2H), 5.17 (s, 2H), 6.21 (s, 1H), 7.00-7.04 (m, 1H), 7.09-7.12 (m, 1H), 7.28-7.33 (m, 1H), 7.44-7.52 (m, 5H), 7.66-7.70 (m, 1H), 8.40 (t, J = 5.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.5, 165.7, 157.2, 148.2, 146.2, 141.0, 138.4, 132.7, 130.6, 128.8, 128.7, 128.3, 123.5, 120.9, 118.1, 112.4, 72.5, 56.3, 36.4, 35.6, 20.7. HRMS (ESI): calcd for $C_{23}H_{25}N_2O_4$ $[M + H]^+$ = 393.1809, found $[M + H]^+$ = 393.1809. mp 112.4-113.9 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-cyclo hexylacetamide (9o). Compound **7** (0.50 g, 2.00 mmol), EDCI (0.38 g, 2.00 mmol), DMAP (0.24 g, 2.00 mmol) and cyclohexylacetic acid (0.38 g， 2.40 mmol) in anhydrous DCM were stirred under nitrogen at rt. After completion judged by TLC, brine was added. The organic phase was separated, washed with brine and 5% $NaHCO_3$, dried over Na_2SO_4, and concentrated *in vacuo*. Then the residue was purified by flash column chromatography (MeOH:DCM = 1:25) to afford the white solid **9o** (0.59 g, 76.90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.82-0.95 (m, 2H), 1.16-1.27 (m, 3H), 1.53-1.72 (m, 6H), 1.99 (d, J = 6.9 Hz, 2H), 2.29 (s, 3H), 3.43 (s, 3H), 4.37 (d, J = 4.8 Hz, 2H), 5.08 (s, 2H), 6.2 (s, 1H), 7.30-7.49 (m, 5H), 8.07 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 171.9, 148.3, 146.2, 140.7, 138.2, 128.7, 128.7, 128.3, 72.70, 43.4, 36.1, 35.1, 33.0, 26.3, 26.1, 20.6. HRMS (ESI): calcd for $C_{23}H_{31}N_2O_3$ $[M + H]^+$ = 383.2329, found $[M + H]^+$ = 383.2329 . mp 190.3-191.0 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-phenylacetamide (9p). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 2-phenylacetyl chloride (0.37 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9p** (0.61 g, 80.70%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.38 (s, 3H), 3.45 (s, 2H), 4.38 (d, J = 4.9 Hz, 2H), 5.08 (s, 2H), 6.21 (s, 1H), 7.15-7.45 (m, 10H), 8.40 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 170.6, 148.3, 146.2, 140.6, 138.1, 136.6, 129.4, 128.8, 128.7, 128.3, 126.9, 118.0, 72.8, 42.5, 36.1, 35.1, 20.6. HRMS (ESI): calcd for $C_{23}H_{25}N_2O_3$ $[M + H]^+$ = 377.1860, found $[M + H]^+$ = 377.1860. mp 189.6-190.9 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-(4-chlorophenyl)acetamide (9q). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 2-(4-chlorophenyl) acetyl chloride (0.45 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH: DCM = 1:25) to afford the white solid **9q** (0.67 g, 81.60%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.39 (s, 3H), 3.46 (s, 2H), 4.37 (d, J = 4.9 Hz, 2H), 5.08 (s, 2H), 6.21 (s, 1H), 7.26-7.44 (m, 9H), 8.41 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 170.2, 148.3, 146.2, 140.5, 138.1, 135.6, 131.6, 131.3, 128.8, 128.7, 128.6, 128.3, 118.0, 72.8, 41.6, 40.8, 36.1, 35.1, 20.6. HRMS (ESI): calcd for $C_{23}H_{24}ClN_2O_3$ $[M + H]^+$ = 411.1470, found $[M + H]^+$ = 411.1470. mp 209.5-210.8 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-(4-flu

orophenyl)acetamide (9r). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 2-(4-fluorophenyl) acetyl chloride (0.41 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH: DCM = 1:25) to afford the white solid **9r** (0.61 g, 77.90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.39 (s, 3H), 3.44 (s, 2H), 4.37 (d, J = 4.9 Hz, 2H), 5.08 (s, 2H), 6.21 (s, 1H), 7.06-7.12 (m, 2H), 7.25-7.45 (m, 7H), 8.39 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 170.5, 148.3, 146.2, 140.6, 138.1, 132.8, 131.3, 131.2, 128.8, 128.7, 128.3, 118.0, 115.5, 115.2, 110.0, 72.8, 41.5, 36.1, 35.1, 20.6. HRMS (ESI): calcd for $C_{23}H_{24}FN_2O_3$ $[M + H]^+$ = 395.1765, found $[M + H]^+$ = 395.1768. mp 199.6-200.8 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-(4-methoxyphenyl)acetamide (9s). Compound **7** (0.50 g, 2.00 mmol), EDCI (0.38 g, 2.00 mmol), DMAP (0.24 g, 2.00 mmol) and 4-methoxyphenylacetic acid (0.40 g, 2.40 mmol) in anhydrous DCM were stirred under nitrogen at r.t. After completion judged by TLC, brine was added. The organic phase was separated, washed with brine and 5% $NaHCO_3$, dried over Na_2SO_4, and concentrated *in vacuo*. Then, the crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9s** (0.68 g, 83.60%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.36 (s, 2H), 3.38 (s, 3H), 3.72 (s, 3H), 4.36 (d, J = 4.9 Hz, 2H), 5.07 (s, 2H), 6.21 (s, 1H), 6.85 (d,J = 8.0 Hz, 2H), 7.17 (d,J = 8.0 Hz, 2H), 7.32-7.43 (m, 5H), 8.31 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 171.0, 158.4, 148.3, 146.2, 140.7, 138.1, 130.4, 128.8, 128.5, 128.3, 118.0, 114.1, 72.8, 55.5, 41.6, 36.1, 35.1, 20.6.

HRMS (ESI): calcd for $C_{24}H_{27}N_2O_4$ $[M + H]^+$ = 407.1965, found $[M + H]^+$ = 407.1965. mp 224.9-226.1 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-3-(4-methoxyphenyl)propanamide (9t). Compound **7** (0.50 g, 2.00 mmol), EDCI (0.38 g, 2.00 mmol), DMAP (0.24 g, 2.00 mmol) and 4-(4-methoxyphenyl) propionic acid (0.43 g, 2.40 mmol) in anhydrous DCM were stirred under nitrogen at rt. After completion judged by TLC, brine was added. The organic phase was separated, washed with brine and 5% $NaHCO_3$, dried over Na_2SO_4, and concentrated *in vacuo*. Then, the crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9t** (0.64 g, 75.50%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.40 (t, J = 7.6 Hz, 2H), 2.77 (t, J = 7.6 Hz, 2H), 3.27 (s, 3H), 3.70 (s, 3H), 4.37 (d, J = 5.0 Hz, 2H), 5.08 (s, 2H), 6.18 (s, 1H), 6.83 (d，J = 8.6 Hz, 2H), 7.10 (d,J = 8.6 Hz, 2H), 7.31-7.45 (m, 5H), 8.11 (t, J = 5.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 171.9, 158.0, 148.2, 146.2, 140.7, 138.2, 133.4, 129.7, 128.8, 128.7, 128.3, 117.9, 114.1, 72.7, 55.4, 37.4, 36.0, 34.8, 30.6, 20.5. HRMS (ESI): calcd for $C_{25}H_{29}N_2O_4$ $[M + H]^+$ = 421.2122, found $[M + H]^+$ = 421.2122. mp 137.9-139.2 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-(4-methoxyphenyl)butanamide (9u). Compound **7** (0.50 g, 2.00 mmol), EDCI (0.38 g, 2.00 mmol), DMAP (0.24 g, 2.00 mmol) and 4-(4-methoxyphenyl) butyric acid (0.47 g, 2.40 mmol) in anhydrous DCM were stirred under nitrogen at rt. After completion judged by TLC, brine was added. The organic phase was separated, washed with brine

and 5% $NaHCO_3$, dried over Na_2SO_4, and concentrated *in vacuo*. Then, the crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **9u** (0.68 g, 78.30%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.73-1.80 (m, 2H), 2.11 (t, J = 7.5 Hz, 2H), 2.28 (s, 3H), 2.48 (t, J = 7.6 Hz, 2H), 3.43 (s, 3H), 3.71 (s, 3H), 4.39 (d, J = 5.0 Hz, 2H), 5.09 (s, 2H), 6.19 (s, 1H), 6.83 (d,J = 8.0 Hz, 2H), 7.07 (d,J = 8.0 Hz, 2H), 7.31-7.45 (m, 5H), 8.07 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.5, 148.3, 146.2, 140.8, 138.2, 134.0, 129.7, 128.8, 128.7, 128.3, 117.9, 114.1, 72.7, 55.4, 36.1, 35.0, 34.8, 34.3, 27.69, 20.6. HRMS (ESI): calcd for $C_{26}H_{31}N_2O_4$ $[M + H]^+$ = 435.2276, found $[M + H]^+$ = 435.2276. mp 147.5-149.0 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-brom obenzenesulfonamide (12a). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 4-bromobenzenesulfonyl chloride (0.61 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **12a** (0.70 g, 73.90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.54 (s, 3H), 4.05 (d, J = 5.6 Hz, 2H), 4.95 (s, 2H), 6.18 (s, 1H), 7.11-7.31 (m, 5H), 7.69-7.64 (m, 2H), 7.76-7.81 (m, 2H), 8.20 (t, J = 5.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.4, 148.5, 146.2, 138.9, 138.5, 137.9, 132.8, 129.1, 128.6, 128.5, 128.2, 127.1, 36.0, 20.5. HRMS (ESI): calcd for $C_{21}H_{22}BrN_2O_4S$ $[M + H]^+$ = 477.0478, found $[M + H]^+$ = 477.0478. mp 249.6-251.4 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-fluor

obenzenesulfonamide (12b). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 4-fluorobenzenesulfonyl chloride (0.47 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **12b** (0.65 g, 77.70%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.55 (s, 3H), 4.03 (d, J = 5.6 Hz, 2H), 4.96 (s, 2H), 6.17 (s, 1H), 7.14-7.33 (m, 5H), 7.36-7.45 (m, 2H), 7.82-7.88 (m, 2H), 8.14 (t, J = 5.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.4, 148.5, 146.2, 138.6, 136.0, 136.0, 130.2, 130.1, 128.6, 128.4, 128.2, 118.0, 117.0, 116.7, 72.6, 36.0, 29.5, 20.5. HRMS (ESI): calcd for $C_{21}H_{22}FN_2O_4S$ $[M + H]^+$ = 417.1279, found $[M + H]^+$ = 417.1279. mp 233.1-233.9 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-methylbenzenesulfonamide (12c). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 4-methylbenzenesulfonyl chloride (0.46 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **12c** (0.60 g, 73.20%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 2.40 (s, 3H), 3.55 (s, 3H), 4.03 (d, J = 5.6 Hz, 2H), 4.95 (s, 2H), 6.17 (s, 1H), 7.15-7.39 (m, 5H), 7.68 (d, J = 8.0 Hz, 2H), 7.98 (d, J = 8.7 Hz, 2H), 8.01 (t, J = 5.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.4, 148.5, 146.2, 143.4, 138.8, 138.0, 136.8, 130.2, 128.6, 128.5, 127.1, 118.0, 72.6, 40.5, 36.0, 21.5, 20.5. HRMS (ESI): calcd for $C_{22}H_{25}N_2O_4S$ $[M + H]^+$ = 413.1530, found $[M + H]^+$ = 413.1530. mp 244.9-245.8 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-(tert-

butyl)benzenesulfonamide (12d). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 4-(*tert*-butyl)benzenesulfonyl chloride (0.56 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **12d** (0.62 g, 68.60%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.31 (s, 9H), 2.27 (s, 3H), 3.55 (s, 3H), 4.05 (d, J = 5.8 Hz, 2H), 4.99 (s, 2H), 6.16 (s, 1H), 7.21-7.31 (m, 5H), 7.57 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 8.6 Hz, 2H), 8.09 (t, J = 5.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.4, 156.0, 148.5, 146.1, 138.8, 138.0, 137.0, 128.6, 128.5, 128.1, 126.9, 126.5, 118.0, 72.5, 36.0, 35.3, 31.3, 20.5. HRMS (ESI): calcd for $C_{25}H_{31}N_2O_4S$ $[M + H]^+$ = 455.1999, found $[M + H]^+$ = 455.2002. mp 224.4-225.0 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-meth oxybenzenesulfonamide (12e). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and 4-methoxybenzenesulfonyl chloride (0.49 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **12e** (0.61 g, 71.70%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.55 (s, 3H), 3.85 (s, 3H), 4.42 (d, J = 5.8 Hz, 2H), 4.95 (s, 2H), 6.17 (s, 1H), 7.08 (d, J = 8.9 Hz, 2H), 7.15-7.31 (m, 5H), 7.74 (d, J = 8.9 Hz, 2H), 8.50 (t, J = 5.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.4, 162.8, 148.5, 146.2, 138.8, 158.0, 131.3, 129.3, 128.6, 128.5, 128.1, 118.0, 114.8, 72.6, 56.1, 36.0, 20.5. HRMS (ESI): calcd for $C_{22}H_{24}N_2O_5S$ $[M + H]^+$ = 429.1479, found $[M + H]^+$ = 429.1479. mp 210.2-211.4 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-[1,1'-bi

phenyl]-4-sulfonamide (12f). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and [1,1'-biphenyl]-4-sulfonyl chloride (0.61 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **12f** (0.64 g, 67.90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H), 3.56 (s, 3H), 4.09 (d, J = 5.7 Hz, 2H), 4.97 (s, 2H), 6.18 (s, 1H), 7.15-7.20 (m, 5H), 7.45-7.69 (m, 3H), 7.72-7.78 (m, 2H), 7.76-7.78 (m, 4H), 8.17 (t, J = 5.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.4, 148.5, 146.2, 144.6, 138.9, 138.7, 138.5, 138.0, 129.7, 129.1, 128.5, 128.1, 127.9, 127.8, 127.5, 118.0, 72.6, 36.0, 20.5. HRMS (ESI): calcd for $C_{27}H_{26}N_2O_4S$ $[M + H]^+$ = 475.1686, found $[M + H]^+$ = 475.1686. mp 205.0-205.8 °C.

N-((3-(Benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-quinoli ne-8-sulfonamide (12g). The reaction was conducted according to the general procedure A, using compound **7** (0.50 g, 2.00 mmol) and quinoline-8-sulfonyl chloride (0.55 g, 2.40 mmol). The crude product was purified by column chromatography (MeOH:DCM = 1:25) to afford the white solid **12g** (0.67 g, 74.90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.16 (s, 3H), 3.53 (s, 3H), 4.12 (d, J = 6.1 Hz, 2H), 4.85 (s, 2H), 6.04 (s, 1H), 6.92-6.98 (m, 2H), 7.10-7.25 (m, 3H),7.61 (t, J = 6.1 Hz, 1H), 7.65-7.74 (m, 2H), 8.31 (d, J = 7.8 Hz, 2H), 8.55 (dd, J = 8.4 Hz, J = 1.8 Hz, 2H), 9.00 (dd, J = 4.2 Hz, J = 1.8 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.2, 151.8, 148.1, 145.9, 143.0, 138.9, 137.5, 135.6, 134.4, 131.7, 128.5, 128.0, 126.2, 123.1, 117.9, 72.1, 36.1, 20.4. HRMS (ESI): calcd for $C_{24}H_{23}N_3O_4S$ $[M + H]^+$ = 450.1482, found $[M + H]^+$ = 450.1481. mp 210.1-211.8 °C.

2. General procedure for the reaction of 10a–10f, 11a–11s, 13a–13g (Procedure B).

Procedure B: A solution of **10a**–**10f**, **11a**–**11s**, **13a**–**13g** (1.0 equiv) in MeOH/H_2O (4:1) was subjected to hydrogenolysis in the presence of 10% Pd/C catalyst (10% reactant mass) overnight. Following filtration, the filtrate was concentrated *in vacuo* and the crude material was recrystallized from MeOH/Et_2O to give a white solid.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)butyramide (10a). Compound **10a** was prepared according to general procedure B, yielding a white solid with 95.52% purity. Yield: 89.20%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, J =7.2 Hz, 3H), 1.45-1.60 (m, 2H), 2.09 (t, J = 7.4 Hz, 2H), 2.29 (s, 3H), 3.48 (s, 3H), 4.43 (s, 2H), 6.13 (s, 1H), 8.17 (t, J = 5.2 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.7, 169.8, 146.5, 146.2, 129.3, 129.3, 112.8, 37.4, 35.9, 34.5, 20.7, 19.1, 14.1. HRMS (ESI): calcd for $C_{12}H_{19}N_2O_3$ $[M + H]^+$ = 239.1389, found $[M + H]^+$ = 239.1390. mp 218.6.1-219.9 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)pentan-amide (10b). Compound **10b** was prepared according to general procedure B, yielding a white solid with 97.04% purity. Yield: 90.60%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.83 (t, J =7.3 Hz, 3H, CH_2CH_3), 1.17-1.29 (m, 2H), 1.42-1.52 (m, 2H), 2.15 (t, J = 7.4 Hz, 2H), 2.57 (s, 3H), 3.89 (s, 3H), 4.58 (d, J = 5.2 Hz, 2H), 7.34 (s, 1H), 8.85 (t, J = 5.2 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.0, 159.9, 149.0, 143.1, 140.7, 113.2, 35.1, 35.0, 27.7, 22.2, 21.1, 14.1. HRMS (ESI): calcd for $C_{13}H_{21}N_2O_3$ $[M + H]^+$ = 253.1547, found $[M + H]^+$ = 253.1547. mp 231.8-232.9 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)hexan-amide (10c). Compound **10c** was prepared according to general procedure B, yielding a white solid with 95.65% purity. Yield: 91.80%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, J = 6.9 Hz, 3H, CH_2CH_3), 1.15-1.35 (m, 3H), 1.44-1.57 (m, 2H), 2.10 (t, J = 7.5 Hz, 2H), 2.29 (s, 3H), 3.47 (s, 3H), 4.42 (d, J = 5.0 Hz, 2H), 6.13 (s, 1H), 8.15 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.8, 169.8, 146.5, 146.16, 129.3, 112.8, 35.9, 35.5, 34.5, 31.4, 25.4, 22.3, 20.7, 14.3. HRMS (ESI): calcd for $C_{14}H_{23}N_2O_3$ $[M + H]^+$ = 267.1703, found $[M + H]^+$ = 267.1702. mp 210.1-211.8 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)heptan-amide (10d). Compound **10d** was prepared according to general procedure B, yielding a white solid with 96.22% purity. Yield: 93.10%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, J = 6.0 Hz, 3H), 1.21-1.29 (m, 6H), 1.46-1.52 (m, 2H), 2.11 (t, J = 7.4 Hz, 2H), 2.29 (s, 3H), 3.47 (s, 3H), 4.42 (d, J = 4.9 Hz, 2H), 6.12 (s, 1H), 8.17 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.7, 169.8, 146.5, 129.3, 112.8, 35.9, 35.5, 34.4, 31.5, 28.8, 25.7, 22.5, 20.7, 14.4. HRMS (ESI): calcd for $C_{15}H_{25}N_2O_3$ $[M + H]^+$ = 281.1860, found $[M + H]^+$ = 281.1865. mp 211.3-212.1 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)octanamide (10e). Compound **10e** was prepared according to general procedure B, yielding a white solid with 97.64% purity. Yield: 93.00%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, J = 7.5 Hz, 3H), 1.15-1.33 (m, 8H), 1.44-1.58 (m, 2H), 2.10 (t, J = 7.4 Hz, 2H), 2.29 (s, 3H), 3.59 (s, 3H), 4.22 (d, J = 5.0 Hz, 2H), 6.13 (s, 1H), 8.17 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.8, 169.8, 146.5, 146.2, 129.3, 112.8,

110.0, 35.9, 35.5, 34.4, 31.6, 29.1, 28.9, 25.7, 22.5, 20.7, 14.4. HRMS (ESI): calcd for $C_{16}H_{27}N_2O_3$ $[M + H]^+ = 295.2016$, found $[M + H]^+ = 295.2006$. mp 197.3-198.8 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-3-oxo-dodecanamide (10f). The compound **8f** was dissolved in 20 ml of a trifluoroacetic acid/H_2O (1:1) mixed solution and stirred at rt for 4 h to complete the reaction. Then, the excess trifluoroacetic acid was neutralized with saturated $NaHCO_3$, and extracted with DCM. The DCM layer was dried over anhydrous Na_2SO_4, and the liquid was evaporated under reduced pressure to obtain a crude product, which was directly subjected to the next reaction. Then crude product was dissolved in MeOH and the solution was subjected to hydrogenolysis in the presence of 10% Pd/C catalyst (10% reactant mass) for overnight. Following filtration, the filtrate was concentrated in vacuo. The crude material recrystallized from MeOH/Et_2O to give a white solid with 95.20% purity. Yield: 82.30%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, J =6.0 Hz, 3H), 1.10-1.26 (m, 12H), 1.44 (t, J = 6.0 Hz, 2H), 2.30 (s, 3H), 3.36 (s, 2H), 3.51 (s, 3H), 4.45 (s, 2H), 6.14 (s, 1H), 8.39 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 205.4, 169.8, 166.6, 146.3, 129.0, 112.9, 50.6, 42.6, 40.5, 36.0, 34.6, 31.8, 29.3, 29.1, 28.9, 23.4, 33.6, 20.7, 15.6, 14.4; HRMS (ESI): calcd for $C_{20}H_{33}N_2O_4$ $[M + H]^+ = 365.2435$, found $[M + H]^+ = 365.2435$. mp 162.9-163.7 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)cyclo-propanecarboxamide (11a). Compound **11a** was prepared according to general procedure B, yielding a white solid with 95.17% purity. Yield: 96.10%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.62-0.70 (m, 4H), 1.58-1.67 (m, 1H), 2.30 (s, 3H), 3.49 (s, 3H), 4.45

(d, J = 4.9 Hz, 2H), 6.13 (s, 1H), 8.44 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.2, 169.9, 146.5, 146.2, 129.3, 112.7, 36.0, 34.7, 20.7, 13.8, 6.9. HRMS (ESI): calcd for $C_{12}H_{17}N_2O_3$ $[M + H]^+$ = 237.1234, found $[M + H]^+$ = 237.1234. mp 240.2-241.6 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)cyclo-butanecarboxamide (11b). Compound **11b** was prepared according to general procedure B, yielding a white solid with 95.76% purity. Yield: 88.20%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.70-1.91 (m, 2H), 1.95-2.05 (m, 2H), 2.09-2.20 (m, 2H), 2.30 (s, 3H), 3.10-3.15 (m, 1H), 3.46 (s, 3H), 4.42 (d, J = 4.7 Hz, 2H), 6.12 (s, 1H), 8.05 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.4, 169.9, 146.2, 129.3, 112.8, 35.9, 34.5, 25.1, 20.7, 18.3. HRMS (ESI): calcd for $C_{13}H_{19}N_2O_3$ $[M + H]^+$ = 251.1390, found $[M + H]^+$ = 251.1396. mp 225.0-226.9 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)cyclo-pentanecarboxamide (11c). Compound **11c** was prepared according to general procedure B, yielding a white solid with 96.46% purity. Yield: 91.20%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.48-1.74 (m, 8H), 2.29 (s, 3H), 2.51-2.53 (m, 1H), 3.46 (s, 3H), 4.43 (s, 2H), 6.13 (s, 1H), 8.15 (t, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.9, 169.8, 146.5, 146.2, 129.2, 112.7, 44.3, 35.9, 34.5, 30.5, 26.2, 20.7. HRMS (ESI): calcd for $C_{14}H_{21}N_2O_3$ $[M + H]^+$ = 265.1547, found $[M + H]^+$ = 265.1548. mp 272.0-273.2 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)cyclo-hexanecarboxamide (11d). Compound **11d** was prepared according to general procedure B, yielding a white solid with 95.32% purity. Yield: 90.40%. ^{1}H NMR (300 MHz,

DMSO-d_6): δ 1.02-1.40 (m, 5H), 1.55-1.75 (m, 5H), 2.19 (t, J = 7.0 Hz, 1H), 2.56 (s, 3H), 2.51-2.53 (m, 1H), 3.86 (s, 3H), 4.46 (s, J = 4.7 Hz, 2H), 7.29 (s, 1H), 8.65 (t, J = 5.2 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.8, 160.2, 149.0, 143.2, 140.4, 113.2, 44.1, 35.0, 29.5, 25.8, 25.6, 21.0. HRMS (ESI): calcd for $C_{15}H_{23}N_2O_3$ $[M + H]^+$ = 279.1703, found $[M + H]^+$ = 279.1702. mp 277.5-278.6 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)tetra-hydrofuran-2-carboxamide (11e). Compound **11e** was prepared according to general procedure B, yielding a white solid with 95.56% purity. Yield: 83.00%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.74-1.92 (m, 3H), 2.05-2.16 (m, 1H), 2.29 (s, 3H), 3.47 (s, 3H), 3.60-3.76 (m, 1H), 3.84-3.91 (m, 1H), 4.22-4.26 (m, 1H), 4.44 (d, J = 4.7 Hz, 2H), 6.12 (s, 1H), 8.04 (t, J = 5.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.1, 169.8, 146.3, 129.0, 112.8, 78.0, 36.0, 34.5, 30.4, 25.5, 20.7. HRMS (ESI): calcd for $C_{13}H_{19}N_2O_4$ $[M + H]^+$ = 267.1339, found $[M + H]^+$ = 267.1333. mp 226.4-227.7 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-1-naphthamide (11f). Compound **11f** was prepared according to general procedure B, yielding a white solid with 95.57% purity. Yield: 90.10%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.13 (s, 3H), 3.63 (s, 3H), 4.76 (d, J = 4.7 Hz, 2H), 6.15 (s, 1H), 7.50-7.65 (m, 4H), 7.95-8.05 (m, 2H), 8.20-8.25 (m, 1H), 8.94 (t, J = 4.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.1, 169.8, 146.3, 129.0, 112.8, 78.0, 36.0, 34.5, 30.4, 25.5, 20.7. HRMS (ESI): calcd for $C_{19}H_{19}N_2O_3$ $[M + H]^+$ = 323.1390, found $[M + H]^+$ = 323.1386. mp 253.9-255.1 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)benzamide

(11g). Compound **11g** was prepared according to general procedure B, yielding a white solid with 96.02% purity. Yield: 93.70%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.57 (s, 3H), 3.96 (s, 3H), 4.82 (d, J = 4.8 Hz, 2H), 7.35 (s, 1H), 7.42-7.50 (m, 2H), 7.52-7.58 (m, 2H), 7.90-7.96 (m, 2H), 9.31 (t, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.5, 159.9, 149.0, 143.3, 140.5, 133.5, 132.2, 128.8, 128.0, 113.2, 36.0, 21.1. HRMS (ESI): calcd for $C_{15}H_{17}N_2O_3$ $[M + H]^+$ = 273.1234, found $[M + H]^+$ = 273.1233. mp 270.7-271.9 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-isonicotinamide (11h). Compound **11h** was prepared according to general procedure B, yielding a white solid (0.35 g) with 95.33% purity. Yield: 79.20%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (t, J = 4.8 Hz, 1H), 8.82–8.59 (m, 2H), 7.90–7.71 (m, 2H), 6.15 (s, 1H), 4.67 (d, J = 4.7 Hz, 2H), 3.52 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, DMSO): δ 20.8, 35.7, 36.1, 112.7, 121.9, 128.3, 141.3, 146.5, 146.6, 150.6, 165.4, 169.9. HRMS (ESI): calcd for $C_{14}H_{16}N_3O_3$ $[M + H]^+$ = 274.1186, found $[M + H]^+$ = 274.1184. mp 260.6-261.7 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-(trifluoromethyl)isonicotinamide (11i). Compound **11i** was prepared according to general procedure B, yielding a white solid (0.11 g) with 95.38% purity. Yield: 99.60%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.30 (t, J = 4.4 Hz, 1H), 8.93 (d, J = 5.0 Hz, 1H), 8.31 (s, 1H), 8.14 (d, J = 4.9 Hz, 1H), 6.16 (s, 1H), 4.70 (d, J = 4.6 Hz, 2H), 3.53 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, DMSO): δ 20.8, 35.8, 36.1, 112.8, 118.8, 123.3, 125.8, 128.1, 143.3, 146.6, 146.7, 151.5, 164.0, 169.9. HRMS (ESI): calcd for

$C_{15}H_{15}N_3O_3F_3$ $[M + H]^+ = 342.1060$, found $[M + H]^+ = 342.1041$. mp 283.5-285.2 °C.

4-Fluoro-N-((3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-benzamide (11j). Compound **11j** was prepared according to general procedure B, yielding a white solid with 95.95% purity. Yield: 95.50%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.58 (s, 3H), 3.95 (s, 3H), 4.81 (d, J = 4.7 Hz, 2H), 7.25-7.33 (m, 2H), 7.36 (s, 1H), 7.96-8.06 (m, 2H), 9.34 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.3, 162.9, 159.9, 149.0, 143.3, 140.5, 130.7, 130.2, 115.6, 113.2, 36.0, 21.1. HRMS (ESI): calcd for $C_{15}H_{16}FN_2O_3$ $[M + H]^+ = 291.1139$, found $[M + H]^+ = 291.1134$. mp 269.3-270.5 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-(trifluoromethyl)benzamide (11k). Compound **11k** was prepared according to general procedure B, yielding a white solid with 95.14% purity. Yield: 84.90%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.59 (s, 3H), 3.95 (s, 3H), 4.85 (d, J = 4.7 Hz, 2H), 7.37 (s, 1H), 7.85 (d, J = 8.2 Hz, 2H), 8.13 (d, J = 8.1 Hz, 2H), 9.50 (t, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.0, 159.8, 149.0, 143.4, 140.3, 137.5, 129.0, 125.7, 113.2, 49.0, 36.0, 21.1. HRMS (ESI): calcd for $C_{16}H_{16}F_3N_2O_3$ $[M + H]^+ = 341.1108$, found $[M + H]^+ = 341.1108$. mp 286.9-288.0 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-methoxybenzamide (11l). Compound **11l** was prepared according to general procedure B, yielding a white solid with 99.93% purity. Yield: 97.20%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.29 (s, 3H), 3.52 (s, 3H), 3.80 (s, 3H), 4.64 (d, J = 4.4 Hz, 2H), 6.15 (s, 1H), 6.98 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 8.5 Hz, 2H), 8.64 (t, J = 4.5 Hz, 1H). ^{13}C

NMR (75 MHz, DMSO-d_6): δ 169.8, 166.3, 162.1, 146.5, 129.8, 129.0, 126.5, 113.9, 112.7, 55.8, 36.0, 35.5, 20.7. HRMS (ESI): calcd for $C_{16}H_{19}N_2O_4$ $[M + H]^+$ = 303.1339, found $[M + H]^+$ = 303.1338. mp 234.6-235.7 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-3-methoxy benzamide (11m). Compound **11m** was prepared according to general procedure B, yielding a white solid with 98.02% purity. Yield: 93.60%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.50 (s, 3H), 3.80 (s, 3H), 3.84 (s, 3H), 4.78 (s, 2H), 7.00 (s, 1H), 7.09 (d,J = 7.3 Hz, 1H), 7.36 (d,J = 7.3 Hz, 2H), 7.45-7.55 (m, 2H), 9.18 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.0, 159.6, 148.3, 144.3, 137.2, 135.2, 129.3, 120.3, 118.0, 113.1, 79.8, 79.5, 79.2, 55.8, 38.7, 35.9, 21.0. HRMS (ESI): calcd for $C_{16}H_{19}N_2O_4$ $[M + H]^+$ = 303.1339, found $[M + H]^+$ = 303.1322. mp 188.9-190.2 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-methoxy benzamide (11n). Compound **11n** was prepared according to general procedure B, yielding a white solid with 96.27% purity. Yield: 96.20%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.31 (s, 3H), 3.64 (s, 3H), 3.86 (s, 3H), 4.68 (d, J = 5.3 Hz, 2H), 6.15 (s, 1H), 7.02 (t, J = 7.4 Hz, 1H), 7.22 (d, J = 8.2 Hz, 1H), 7.46 (t, J = 7.4 Hz, 1H), 7.73 (dd, J = 7.6 Hz, J = 1.6 Hz, 1H), 8.54 (t, J =5.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.8, 165.6, 157.3, 146.4, 146.3, 132.8, 130.7, 129.9, 120.9, 113.1, 112.4, 79.8, 79.4, 79.1, 56.4, 49.1, 36.2, 35.3, 20.8. HRMS (ESI): calcd for $C_{16}H_{19}N_2O_4$ $[M + H]^+$ = 303.1339, found $[M + H]^+$ = 303.1339. mp 197.6-198.8 °C.

2-Cyclohexyl-N-((3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)acetamide (11o). Compound **11o** was prepared according to general procedure B,

yielding a white solid with 95.12% purity. Yield: 88.60%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.81-0.95 (m, 2H), 1.05-1.27 (m, 3H), 1.50-1.72 (m, 5H), 2.00 (d, J = 6.8 Hz, 2H), 2.29 (s, 3H), 3.47 (s, 3H), 4.41 (d, J = 4.9 Hz, 2H), 6.13 (s, 1H), 8.16 (t, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.0, 169.8, 146.5, 146.2, 129.2, 112.8, 43.3, 35.9, 35.1, 34.5, 33.0, 26.3, 26.1, 20.7. HRMS (ESI): calcd for $C_{16}H_{25}N_2O_3$ $[M + H]^+$ = 293.1860, found $[M + H]^+$ = 293.1866. mp 242.2-243.9 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-phenylacetamide (11p). Compound **11p** was prepared according to general procedure B, yielding a white solid with 95.29% purity. Yield: 93.90%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.42 (s, 3H), 3.46 (s, 2H), 4.44 (d, J = 4.8 Hz, 2H), 5.77 (s, 1H), 6.14 (s, 1H), 7.18-7.34 (m, 5H), 8.48 (t, J = 6.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.7, 169.8, 146.5, 146.3, 136.7, 129.4, 129.1, 128.8, 126.8, 112.9, 55.4, 42.5, 35.9, 34.7, 20.7. HRMS (ESI): calcd for $C_{16}H_{19}N_2O_3$ $[M + H]^+$ = 287.1390, found $[M + H]^+$ = 287.1391. mp 231.0-232.4 °C.

2-(4-Chlorophenyl)-N-((3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)acetamide (11q). Compound **11q** was prepared according to general procedure B, yielding a white solid with 97.49% purity. Yield: 88.40%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.56 (s, 3H), 3.52 (s, 2H), 3.87 (s, 3H), 4.61 (d, J = 5.1 Hz, 2H), 7.27 (s, 1H), 7.33-7.40 (m, 4H), 9.13 (t, J = 5.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 171.2, 159.8, 148.9, 143.2, 140.5, 135.3, 131.7, 131.4, 129.5, 128.6, 113.2, 49.0, 35.3, 21.1. HRMS (ESI): calcd for $C_{16}H_{18}ClN_2O_3$ $[M + H]^+$ = 321.1000, found $[M + H]^+$ = 321.1002. mp 261.5-262.6 °C.

2-(4-Fluorophenyl)-N-((3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl) methyl)acetamide (11r). Compound **11r** was prepared according to general procedure B, yielding a white solid with 96.57% purity. Yield: 90.10%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H), 3.42 (s, 3H), 3.45 (s, 2H), 4.43 (d, J = 4.9 Hz, 2H), 6.14 (s, 1H), 7.06-7.17 (m, 2H), 7.24-7.32 (m, 2H), 8.48 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.6, 169.8, 163.1, 159.9, 146.5, 146.3, 132.9, 131.3, 129.1, 115.5, 115.2, 112.9, 49.1, 35.9, 34.7, 20.7. HRMS (ESI): calcd for $C_{16}H_{18}FN_2O_3$ [M + H]$^+$ = 305.1296, found [M + H]$^+$ = 305.1299. mp 231.7-232.6 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-2-(4-metho xyphenyl)acetamide (11s). Compound **11s** was prepared according to general procedure B, yielding a white solid with 96.89% purity. Yield: 91.30%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.55 (s, 3H), 3.42 (s, 2H), 3.71 (s, 3H), 3.86 (s, 3H), 4.59 (d, J = 5.0 Hz, 2H), 6.83 (d,J = 8.3 Hz, 2H), 7.17 (d,J = 8.1 Hz, 2H), 7.31 (s, 1H), 9.05 (t, J = 5.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.1, 160.1, 158.4, 148.8, 143.3, 140.4, 130.5, 128.1, 114.1, 113.2, 79.9, 79.5, 79.2, 55.5, 41.3, 35.3, 21.1. HRMS (ESI): calcd for $C_{17}H_{21}N_2O_4$ [M + H]$^+$ = 317.1496, found [M + H]$^+$ = 317.1496. mp 206.2-207.5 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-3-(4-metho xyphenyl)propanamide (11t). Compound **11t** was prepared according to general procedure B, yielding a white solid with 97.59% purity. Yield: 96.70%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.43 (t, J = 7.5 Hz, 2H), 2.54 (s, 3H), 2.75 (t, J = 7.5 Hz, 2H), 3.70 (s, 3H), 3.76 (s, 3H), 4.56 (d, J = 5.1 Hz, 2H), 7.32 (s, 1H), 6.78 (d,J = 8.6 Hz,

2H), 7.07 (d,*J* = 8.5 Hz, 2H), 6.18 (s, 1H), 8.84 (t, *J* = 5.3 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.0, 160.0, 157.9, 148.9, 143.1, 140.5, 133.1, 129.6, 114.0, 113.2, 55.4, 37.2, 35.0, 30.5, 21.0. HRMS (ESI): calcd for $C_{18}H_{23}N_2O_4$ $[M + H]^+$ = 331.1652, found $[M + H]^+$ = 331.1658. mp 209.6-211.0 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-(4-methoxyphenyl)butanamide (11u). Compound **11u** was prepared according to general procedure B, yielding a white solid with 95.68% purity. Yield: 92.40%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.76-1.80 (m, 2H), 2.15 (t, *J* = 6.5 Hz, 2H), 2.46 (s, 3H), 2.48 (t, *J* = 7.6 Hz, 2H), 3.71 (s, 3H), 3.75 (s, 3H), 4.54 (s, 2H), 6.81 (d,*J* = 8.1 Hz, 2H), 6.19 (s, 1H), 7.07 (d,*J* = 8.0 Hz, 2H), 8.60 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.3, 157.9, 148.0, 144.4, 133.9, 129.6, 114.1, 113.1, 79.8, 79.5, 79.2, 55.4, 38.2, 34.9, 34.3, 27.6, 21.0. HRMS (ESI): calcd for $C_{19}H_{25}N_2O_4$ $[M + H]^+$ = 345.1809, found $[M + H]^+$ = 345.1808. mp 148.2-149.9 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-benzenesulfonamide (13a). Compound **13a** was prepared according to general procedure B, yielding a white solid with 96.38% purity. Yield: 86.00%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.42 (s, 3H), 3.72 (s, 3H), 4.25 (s, 2H), 6.59 (s, 1H), 7.60-7.69 (m, 3H), 7.82-7.85 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.6, 148.1, 144.2, 139.9, 133.3, 129.7, 127.1, 112.8, 37.9, 37.6, 20.8. HRMS (ESI): calcd for $C_{14}H_{17}N_2O_4S$ $[M + H]^+$ = 309.0904, found $[M + H]^+$ = 309.0904 . mp 210.7-211.6 °C.

4-Fluoro-N-((3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)benzenesulfonamide (13b). Compound **13b** was prepared according to general

procedure B, yielding a white solid with 96.58% purity. Yield: 85.20%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H), 3.54 (s, 3H), 4.14 (s, 2H), 6.08 (s, 1H), 7.40-7.58 (m, 2H), 7.85-7.94 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.7, 163.0, 146.8, 146.2, 136.5, 130.2, 127.0, 117.0, 116.7, 112.8, 110.0, 38.2, 35.8, 20.6. HRMS (ESI): calcd for $C_{14}H_{16}FN_2O_4S$ $[M + H]^+$ = 327.0908, found $[M + H]^+$ = 327.0809. mp 222.5-223.8 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-methylbenzenesulfonamide (13c). Compound **13c** was prepared according to general procedure B, yielding a white solid with 96.54% purity. Yield: 87.40%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H), 2.40 (s, 3H), 3.54 (s, 3H), 4.10 (s, 2H), 6.09 (s, 1H), 7.42 (d, J = 8.0 Hz, 2H), 7.74 (d, J = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.7, 146.8, 146.2, 143.4, 137.2, 130.1, 127.1, 112.8, 38.2, 35.7, 21.5, 20.6. HRMS (ESI): calcd for $C_{15}H_{19}N_2O_4S$ $[M + H]^+$ = 323.1060, found $[M + H]^+$ = 323.1060. mp 246.3-247.2 °C.

4-(tert-Butyl)-N-((3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)benzenesulfonamide (13d). Compound **13d** was prepared according to general procedure B, yielding a white solid with 95.62% purity. Yield: 85.70%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.32 (s, 9H), 2.26 (s, 3H), 3.54 (s, 3H), 4.14 (s, 2H), 6.07 (s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.7, 162.6, 156.0, 146.7, 146.1, 137.4, 127.1, 126.9, 126.5, 112.7, 110.0, 38.2, 35.8, 35.3, 31.3, 20.7. HRMS (ESI): calcd for $C_{18}H_{25}N_2O_4S$ $[M + H]^+$ = 365.1530, found $[M + H]^+$ = 365.1530. mp 229.2-231.1 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-methoxy benzenesulfonamide (13e). Compound **13e** was prepared according to general procedure B, yielding a white solid with 95.75% purity. Yield: 83.50%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H), 3.54 (s, 3H), 3.86 (s, 3H), 4.09 (s, 2H), 6.09 (s, 1H), 7.14 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.8, 162.8, 146.8, 146.2, 131.7, 129.3, 127.2, 114.8, 112.7, 56.1, 38.2, 35.7, 20.6. HRMS (ESI): calcd for $C_{15}H_{19}N_2O_4S$ $[M + H]^+$ = 339.1009, found $[M + H]^+$ = 339.1009 . mp 216.7-218.0 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-[1,1'-biphe nyl]-4-sulfonamide (13f). Compound **13f** was prepared according to general procedure B, yielding a white solid with 96.13% purity. Yield: 80.50%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.25 (s, 3H), 3.56 (s, 3H), 4.19 (s, 2H), 6.07 (s, 1H), 7.42-7.56 (m, 3H), 7.76 (d, J = 7.3 Hz, 2H), 7.86-7.94 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.7,146.8, 146.2, 144.6, 139.0, 138.9, 129.6, 129.0, 127,9, 127.7, 127.6, 112.7, 38.2, 35.8, 20.6. HRMS (ESI): calcd for $C_{20}H_{21}N_2O_4S$ $[M + H]^+$ = 385.1217, found $[M + H]^+$ = 385.1220. mp 241.1-242.6 °C.

N-((3-Hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-1,2,3,4-tetr ahydroquinoline-8-sulfonamide (13g). Compound **13g** was prepared according to general procedure B, yielding a white solid with 96.20% purity. Yield: 80.50%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.73-1.87 (m, 2H), 2.25 (s, 3H), 2.73 (t, 2H, J = 6.2 Hz), 3.30-3.60 (m, 2H), 3.49 (s, 3H), 4.08 (s, 2H), 6.03 (t, J = 2.7 Hz, 1H), 6.07 (s, 1H), 6.50 (t, J = 7.6 Hz, 1H), 7.08 (dd, J = 7.2 Hz, J = 1.5 Hz, J = 7.3 Hz, 1H), 7.39

(dd, J = 8.0 Hz, J = 1.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.7,146.6, 146.2, 142.9, 134.0, 128.0, 127.2, 122.9, 118.6, 114.1, 112.7, 37.8, 35.6, 27.7, 20.7. HRMS (ESI): calcd for $C_{17}H_{22}N_3O_4S$ $[M + H]^+$ = 364.1326, found $[M + H]^+$ = 364.1326. mp 230.2-231.1 °C.

3-(Benzyloxy)-2-(chloromethyl)-1,6-dimethylpyridin-4(1H)-one(14).

Compound **5** (5.00 g, 0.02 mol) was dissolved in $SOCl_2$ (15 mL) and stirred at rt for 2.5 h. The reaction was quenched by the slow addition of saturated sodium carbonate solution. The mixture was extracted with DCM (3 × 100 mL) and organic fractions were combined and dried. The solvent was concentrated to dryness under vacuum, and the residue was purified by column chromatography (MeOH:DCM = 1:20) to afford a white solid **14** (5.10 g, 91.80%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.54 – 7.30 (m, 5H), 6.25 (s, 1H), 5.16 (s, 2H), 4.84 (s, 2H), 3.59 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.6, 36.0, 37.5, 73.0, 118.4, 128.5, 128.8, 129.0, 137.8, 139.5, 145.8, 148.9, 172.3. mp 173.2-174.5 °C.

3. General procedure for the reaction of 15a–15j (Procedure C).

Procedure C: A solution of compound **14** (1.50 mmol, 1.0 equiv) and amine (3.00 mmol, 2.0 equiv) in DMF was added to 0.4 mL of triethylamine (3.00 mmol, 2.2 equiv). The mixture was heated to 75 °C for 3 h. The mixture was extracted with DCM three times and organic layers were combined and dried. Then the solvent was concentrated to dryness under vacuum and the residue was purified by column chromatography (MeOH:DCM = 1:30) to afford the oily liquid.

3-(Benzyloxy)-2-((isopropylamino)methyl)-1,6-dimethylpyridin-4(1H)-one (15a). The reaction was conducted according to the general procedure C, using

compound **14** (0.40 g, 1.50 mmol) and isopropylamine (0.18 g, 3.00 mmol) and yielding the white oily liquid **15a** (0.22 g). Yield: 58.20%. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.34 (dd, J = 5.6, 12.6 Hz, 4H), 6.35 (s, 1H), 5.29 (s, 2H), 3.63 (s, 2H), 3.59 (s, 2H), 2.70 (dt, J = 6.2, 12.4 Hz, 1H), 2.29 (s, 3H), 0.98 (d, J = 6.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.8, 22.3, 35.5, 42.0, 49.3, 73.0, 77.2, 118.5, 128.2, 128.5, 129.4, 137.5, 145.2, 147.0, 173.4.

3-(Benzyloxy)-2-((butylamino)methyl)-1,6-dimethylpyridin-4(1H)-one (15b). The reaction was conducted according to the general procedure C, using compound **14** (0.40 g, 1.50 mmol) and butylamine (0.22 g, 3.00 mmol) and yielding the white oily liquid **15b** (389.00 mg). Yield: 82.50%. 1H NMR (400 MHz, Chloroform-d): δ 7.41–7.29 (m, 5H), 6.34 (s, 1H), 5.25 (s, 2H), 3.59 (s, 3H), 3.53 (s, 2H), 2.40 (t, J = 6.4 Hz, 2H), 2.27 (s, 3H), 1.33–1.22 (m, 5H), 0.87 (t, J = 6.8 Hz, 3H). 13C NMR (101 MHz, CDCl3): δ 13.9, 20.4, 20.8, 31.7, 44.5, 49.7, 73.0, 118.4, 128.1, 128.4, 129.3, 137.5, 142.2, 145.0, 147.1, 173.5.

3-(Benzyloxy)-1,6-dimethyl-2-((pentylamino)methyl)pyridin-4(1H)-one (15c). The reaction was conducted according to the general procedure C, using compound **14** (0.40 g, 1.50 mmol) and amylamine (0.26 g, 3.00 mmol) and yielding the white oily liquid **15c** (0.42 g). Yield: 84.70%. 1H NMR (400 MHz, chloroform-d): δ 7.35 (dt, J = 6.5, 13.0 Hz, 5H), 6.36 (s, 1H), 5.28 (s, 2H), 3.61 (s, 3H), 3.55 (s, 2H), 2.41 (t, J = 6.9 Hz, 2H), 2.29 (s, 3H), 1.38–1.18 (m, 6H), 0.89 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3): δ 14.0, 20.8, 22.5, 29.4, 29.4, 35.4, 44.5, 50.0, 72.9, 118.5, 128.2, 128.4, 129.4, 137.6, 142.1, 147.0, 173.5.

3-(Benzyloxy)-2-((cyclopentylamino)methyl)-1,6-dimethylpyridin-4(1H)-one (15d). The reaction was conducted according to the general procedure C, using compound **14** (0.40 g, 1.50 mmol) and cyclopentylamine (0.26 g, 3.00 mmol) yielding

the yellow oily liquid **15d** (0.34 g). Yield: 70.00%. ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.39–7.30 (m, 5H), 6.34 (s, 1H), 5.27 (s, 2H), 3.60 (s, 3H), 3.52 (s, 2H), 2.87 (p, J = 6.4 Hz, 1H), 2.27 (s, 3H), 1.76–1.43 (m, 7H), 1.21–1.08 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.8, 24.0, 32.6, 35.4, 43.3, 60.1, 72.9, 118.4, 128.2, 128.5, 129.5, 137.5, 142.4, 144.9, 147.0, 173.5.

3-(Benzyloxy)-2-(((3-methoxyphenyl)amino)methyl)-1,6-dimethylpyridin-4(1H)-one (15e). The reaction was conducted according to the general procedure C, using compound **14** (0.40 g, 1.50 mmol) and m-methoxyaniline (0.37 g, 3.00 mmol) yielding the yellow oily liquid **15e** (0.35 g). Yield: 64.00%. ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.37–7.29 (m, 5H), 7.10 (t, J = 8.1 Hz, 1H), 6.41–6.32 (m, 2H), 6.20–6.06 (m, 2H), 5.32 (s, 2H), 4.09 (s, 2H), 3.79 (s, 3H), 3.50 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.8, 35.6, 39.3, 55.1, 72.8, 99.5, 103.6, 106.4, 118.5, 128.3, 128.5, 129.7, 130.0, 137.1, 140.8, 145.1, 147.4, 148.4, 160.8, 173.3.

3-(Benzyloxy)-2-(((4-methoxybenzyl)amino)methyl)-1,6-dimethylpyridin-4(1H)-one (15f). The reaction was conducted according to the general procedure C, using compound **14** (0.40 g, 1.50 mmol) and 4-methoxybenzylamine (0.41 g, 3.00 mmol) yielding the yellow oily liquid **15f** (0.50 g). Yield: 87.70%. ^{1}H NMR (300 MHz, chloroform-*d*): δ 7.36–7.30 (m, 3H), 7.24 (d, J = 6.9 Hz, 1H), 7.15 (d, J = 8.6 Hz, 2H), 6.87–6.82 (m, 2H), 6.36 (s, 1H), 5.28 (s, 2H), 3.81 (d, J = 2.7 Hz, 4H), 3.50 (d, J = 2.4 Hz, 7H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.8, 35.3, 43.7, 53.4, 55.3, 72.9, 100.0, 113.7, 118.5, 128.2, 128.5, 129.5, 131.4, 137.4, 145.0, 147.0, 158.8, 173.5.

3-(Benzyloxy)-1,6-dimethyl-2-(((4-(trifluoromethyl)benzyl)amino)-methyl)pyridin-4(1H)-one (15g). The reaction was conducted according to the general procedure C, using compound **14** (0.40 g, 1.50 mmol) and 4-(trifluoromethyl)benzylamine (0.53 g, 3.00 mmol) yielding the yellow oily liquid **15g** (0.46 g). Yield: 73.20%. ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.52 (d, J = 6.9 Hz, 2H), 7.42 (d, J = 5.1 Hz, 2H), 7.29 (dt, J = 7.3, 15.1 Hz, 5H), 7.16 (t, J = 6.4 Hz, 1H), 6.37 (s, 1H), 5.28 (s, 2H), 3.61 (s, 2H), 3.54 (s, 5H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.8, 35.4, 44.0, 53.3, 72.9, 118.6, 123.9, 124.0, 124.0, 124.1, 124.8, 124.8, 124.8, 124.9, 128.2, 128.4, 128.7, 129.5, 131.6, 137.4, 140.3, 141.6, 145.1, 147.1, 173.4.

3-(Benzyloxy)-1,6-dimethyl-2-(((3-(4-methylpiperazin-1-yl)propyl)amino)-methyl)pyridin-4(1H)-one (15h). The reaction was conducted according to the general procedure C, using compound **14** (0.40 g, 1.50 mmol) and 1-(3-aminopropyl)-4-methylpiperazine (0.47 g, 3.00 mmol) yielding the yellow oily liquid **15h** (368.00 mg). Yield: 61.30%. ^{1}H NMR (300 MHz, chloroform-*d*): δ 7.41–7.30 (m, 5H), 6.37 (s, 1H), 5.28 (s, 2H), 3.60 (s, 3H), 3.54 (s, 2H), 2.44 (t, J = 6.9 Hz, 8H), 2.31 (d, J = 9.7 Hz, 9H), 1.51 (p, J = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.8, 26.8, 35.4, 44.5, 45.9, 48.3, 53.1, 55.0, 56.5, 72.9, 118.4, 128.1, 128.4, 129.4, 137.5, 142.3, 144.9, 147.1, 173.5.

3-(Benzyloxy)-2-((((4-(4-fluorobenzyl)morpholin-2-yl)methyl)amino)methyl)-1,6-dimethylpyridin-4(1H)-one (15i). The reaction was conducted according to the general procedure C, using compound **14** (0.40 g, 1.50 mmol) and 4-[(4-fluorophenyl)methyl]-2-morpholinemethanamine (0.63 g, 3.00 mmol) yielding the white oily liquid **15i** (0.91 g). Yield: 95.20%. ^{1}H NMR (400 MHz, chloroform-*d*):

δ 7.39 (d, J = 6.5 Hz, 2H), 7.37–7.31 (m, 3H), 7.29 (d, J = 2.9 Hz, 3H), 7.02 (t, J = 8.6 Hz, 2H), 6.36 (s, 1H), 5.26 (s, 2H), 3.83 (d, J = 11.3 Hz, 1H), 3.70–3.40 (m, 9H), 2.64 (dd, J = 11.4, 17.0 Hz, 2H), 2.52 (dd, J = 7.7, 12.4 Hz, 1H), 2.42 (dd, J = 3.8, 12.4 Hz, 1H), 1.84 (t, J = 10.6 Hz, 4H), 1.41–1.17 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.8, 35.4, 44.5, 52.0, 53.0, 56.3, 62.4, 66.5, 73.1, 74.8, 118.5, 128.1, 128.4, 129.3, 130.6, 130.7, 137.5, 141.7, 147.0, 160.9, 163.3, 173.5.

3-(Benzyloxy)-1,6-dimethyl-2-(((1-methylpiperidin-4-yl)amino)methyl)pyridin-4(1H)-one (15j). The reaction was conducted according to the general procedure C, using compound **14** (0.40 g, 1.50 mmol) and 1-methylpiperidin-4-amine (0.34 g, 3.00 mmol) yielding the white oily liquid **15j** (0.36 g). Yield: 50.60%. ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.31 (td, J = 4.5, 7.5 Hz, 5H), 6.32 (s, 1H), 5.24 (s, 2H), 4.68 (s, 1H), 3.57 (s, 3H), 3.55 (s, 2H), 2.72 (d, J = 11.8 Hz, 3H), 2.25 (s, 3H), 2.22 (s, 4H), 1.91 (t, J = 11.5 Hz, 2H), 1.24 (t, J = 11.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.8, 32.1, 35.4, 41.6, 46.1, 54.4, 73.0, 118.4, 128.2, 128.5, 129.4, 129.4, 137.5, 142.4, 145.0, 147.1, 173.5.

4. General procedure for the reaction of 16a–16j (Procedure D).

Procedure D: A solution of **15a–15j** (1.0 equiv) in MeOH was subjected to hydrogenolysis in the presence of 10% Pd/C catalyst (10% reactant mass) overnight. Following filtration, the filtrate was concentrated *in vacuo* and the crude material recrystallized from MeOH/diethyl ether to give a white solid.

3-Hydroxy-2-((isopropylamino)methyl)-1,6-dimethylpyridin-4(1H)-one (16a). Compound **16a** was prepared according to general procedure D, yielding the white solid (0.11 g) with 97.33% purity. Yield: 73.80%. ^{1}H NMR (400 MHz, DMSO-d_6): δ

6.11 (s, 1H), 3.89 (s, 2H), 3.65 (s, 3H), 2.86 (p, J = 6.2 Hz, 1H), 2.29 (s, 3H), 1.08 (d, J = 6.3 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.7, 22.5, 35.8, 41.5, 49.2, 112.6, 130.4, 145.8, 146.5, 169.7. HRMS (ESI): calcd for $C_{11}H_{19}N_2O_2$ $[M + H]^+$ = 211.1441, found $[M + H]^+$ = 211.1452. mp 158.4-159.6 °C.

2-((Butylamino)methyl)-3-hydroxy-1,6-dimethylpyridin-4(1H)-one (16b). Compound **16b** was prepared according to general procedure D, yielding the white solid (0.17 g) with 95.72% purity. Yield: 51.10%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.06 (s, 1H), 3.78 (s, 2H), 3.61 (s, 3H), 2.50 (t, J = 7.0 Hz, 2H), 2.25 (s, 3H), 1.30 (ddt, J = 7.4, 14.3, 36.3 Hz, 4H), 0.82 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.3, 20.7, 31.9, 35.5, 43.9, 48.9, 112.4, 130.9, 145.6, 146.4, 169.7. HRMS (ESI): calcd for $C_{12}H_{21}N_2O_2$ $[M + H]^+$ = 225.1598, found $[M + H]^+$ = 225.1612. mp 151.3-152.7 °C.

3-Hydroxy-1,6-dimethyl-2-((pentylamino)methyl)pyridin-4(1H)-one (16c). Compound **16c** was prepared according to general procedure D, yielding the white solid (0.24 g) with 97.64% purity. Yield: 84.30%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.09 (s, 1H), 3.81 (s, 2H), 3.65 (s, 3H), 2.51 (d, J = 6.5 Hz, 2H), 2.29 (s, 3H), 1.48–1.33 (m, 2H), 1.25 (s, 4H), 0.85 (d, J = 5.9 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.4, 20.7, 22.5, 29.5, 29.5, 35.5, 44.0, 49.3, 112.3, 130.9, 145.6, 146.4, 169.7. HRMS (ESI): calcd for $C_{13}H_{23}N_2O_2$ $[M + H]^+$ = 239.1754, found $[M + H]^+$ = 239.1764. mp 103.0-104.2 °C.

2-((Cyclopentylamino)methyl)-3-hydroxy-1,6-dimethylpyridin-4(1H)-one (16d). Compound **16d** was prepared according to general procedure D, yielding a red

solid (0.17 g) with 96.67% purity. Yield: 64.80%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.10 (s, 1H), 3.82 (s, 2H), 3.65 (s, 3H), 3.06 (p, J = 6.1 Hz, 1H), 2.29 (s, 3H), 1.86–1.15 (m, 8H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.7, 24.0, 32.7, 35.6, 42.8, 59.8, 112.5, 131.0, 145.6, 146.4, 169.7. HRMS (ESI): calcd for $C_{13}H_{21}N_2O_2$ $[M + H]^+$ = 237.1598, found $[M + H]^+$ = 237.1606. mp 160.0-161.0 °C.

3-Hydroxy-2-(((3-methoxyphenyl)amino)methyl)-1,6-dimethylpyridin-4(1H)-one (16e). Compound **16e** was prepared according to general procedure D, yielding the red solid (0.15 g) with 97.86% purity. Yield: 55.20%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.96 (t, J = 8.2 Hz, 1H), 6.30 (d, J = 6.6 Hz, 2H), 6.14 (d, J = 9.1 Hz, 2H), 6.03 (t, J = 4.4 Hz, 1H), 4.34 (d, J = 4.9 Hz, 2H), 3.66 (s, 3H), 3.58 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.7, 36.0, 38.8, 55.2, 98.1, 102.5, 105.8, 112.5, 129.5, 130.0, 145.7, 146.8, 150.0, 160.9, 169.8. HRMS (ESI): calcd for $C_{15}H_{19}N_2O_3$ $[M + H]^+$ = 275.1390, found $[M + H]^+$ = 275.1396. mp 203.4-203.6 °C.

3-Hydroxy-2-(((4-methoxybenzyl)amino)methyl)-1,6-dimethylpyridin-4(1H)-one (16f). Compound **16f** was prepared according to general procedure D, yielding the red solid (0.28 g) with 98.53% purity. Yield: 73.60%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.35–7.17 (m, 2H), 6.94–6.74 (m, 2H), 6.09 (s, 1H), 3.78 (s, 2H), 3.73 (s, 3H), 3.65 (s, 2H), 3.62 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.7, 35.5, 43.4, 52.5, 55.5, 112.4, 114.0, 129.6, 130.9, 132.9, 145.6, 146.4, 158.6, 169.7. HRMS (ESI): calcd for $C_{16}H_{21}N_2O_3$ $[M + H]^+$ = 289.1547, found $[M + H]^+$ = 289.1551. mp 180.0-181.3 °C.

3-Hydroxy-1,6-dimethyl-2-(((4-(trifluoromethyl)benzyl)amino)methyl)pyridin-4(1H)-one (16g). Compound **16g** was prepared according to general procedure D, yielding the red solid (0.24 g) with 95.78% purity. Yield: 67.50%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.99–7.17 (m, 4H), 6.08 (s, 1H), 3.82 (d, J = 9.7 Hz, 4H), 3.64 (s,

3H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.7, 35.5, 43.4, 52.3, 112.3, 123.7, 123.7, 123.8, 123.8, 123.8, 124.6, 124.7, 124.7, 124.7, 129.4, 130.5, 132.5, 142.8, 145.7, 146.5, 169.7. HRMS (ESI): calcd for $C_{16}H_{18}N_2O_2F_3$ $[M + H]^+$ = 327.1315, found $[M + H]^+$ = 327.1329. mp 169.3-170.6 °C.

3-Hydroxy-1,6-dimethyl-2-(((3-(4-methylpiperazin-1-yl)propyl)amino)methyl)-pyridin-4(1H)-one (16h). Compound **16h** was prepared according to general procedure D, yielding the yellow solid (0.21 g) with 98.60% purity. Yield: 72.80%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.10 (s, 1H), 3.80 (s, 2H), 3.64 (s, 3H), 2.54 (dd, J = 8.2, 15.0 Hz, 3H), 2.30 (d, J = 6.3 Hz, 11H), 2.14 (s, 3H), 1.56 (p, J = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.7, 26.8, 35.5, 43.9, 46.1, 47.6, 53.1, 55.1, 56.5, 112.4, 130.8, 145.6, 146.4, 169.7. HRMS (ESI): calcd for $C_{16}H_{29}N_4O_2$ $[M + H]^+$ = 309.2285, found $[M + H]^+$ = 309.2294. mp 99.6-100.8 °C.

2-((((4-(4-Fluorobenzyl)morpholin-2-yl)methyl)amino)methyl)-3-hydroxy-1,6-dimethylpyridin-4(1H)-one (16i). Compound **16i** was prepared according to general procedure D, yielding a red solid (0.37 g) with 97.27% purity. Yield: 72.80%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.35–7.29 (m, 2H), 7.14 (t, J = 8.8 Hz, 2H), 6.09 (s, 1H), 3.77 (dd, J = 8.3, 17.2 Hz, 3H), 3.58 (s, 3H), 3.54–3.46 (m, 2H), 3.43 (d, J = 3.4 Hz, 2H), 2.69 (d, J = 11.1 Hz, 1H), 2.58 (dd, J = 6.8, 12.1 Hz, 2H), 2.27 (s, 3H), 2.03 (td, J = 3.5, 11.6 Hz, 1H), 1.83–1.70 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.7, 35.5, 43.9, 51.7, 53.1, 56.7, 61.8, 66.3, 75.1, 112.4, 115.4, 130.7, 131.1, 131.2, 134.4, 145.6, 146.4, 160.5, 162.9, 169.7. HRMS (ESI): calcd for $C_{20}H_{27}N_3O_3F$ $[M + H]^+$ = 376.2031, found $[M + H]^+$ = 376.2039. mp 154.9-156.9 °C.

3-Hydroxy-1,6-dimethyl-2-(((1-methylpiperidin-4-yl)amino)methyl)pyridin-4(1H)-one (16j). Compound **16j** was prepared according to general procedure D, yielding a yellow solid (0.15 g) with 97.34% purity. Yield: 61.40%. ^{1}H NMR (400

MHz, DMSO-d_6): δ 6.09 (s, 1H), 3.83 (s, 2H), 3.65 (s, 3H), 2.68 (d, J = 11.5 Hz, 2H), 2.42–2.32 (m, 1H), 2.29 (s, 3H), 2.13 (s, 3H), 1.86 (dt, J = 11.1, 19.6 Hz, 4H), 1.28 (q, J = 13.6, 14.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.7, 32.4, 35.5, 41.3, 46.4, 54.3, 54.6, 112.3, 131.3, 145.4, 146.4, 169.7. HRMS (ESI): called for $C_{14}H_{24}N_3O_2$ $[M + H]^+ = 266.1863$, found $[M + H]^+ = 266.1871$. mp 112.4-113.6 °C.

Biological *assay*s.

Metal chelating assay. For the determination of pFe(III) values, the metal chelation assay was performed in DMSO and 3-morpholinopropanesulfonic acid (MOPS, Solarbio, China) buffer using a fluorescence spectrophotometer (Perkin-Elmer) with emission mode of excitation wavelength (435 nm) and emission wavelength from 440 nm to 550 nm. MOPS buffer was treated with Chelex 100 before use. CP645[20] was synthesized as described. Fluorescence of CP645 (6 μM, 4 mL, total volume) was quenched by the addition of Fe-NTA (2 μM, 4 mL, total volume) mixed for 30 min prior to the addition of **10d** (60 μM, 4 mL). The fluorescence of **10d** was detected after 48 h. The maximum fluorescence intensities of CP645-Fe complex (6 μM:2 μM) and CP645 (6 μM) were set at 0% and 100% respectively, based on this, the relative fluorescence intensity of the compounds mixed with CP645-Fe[22] was calculated using the equation: fluorescence(%) = 11.743 pFe(III)-169. Quoted values are the mean of three independent determinations.

Determination of minimum inhibitory concentrations (MIC). A broth-microdilution method from standards published by the Clinical and Laboratory Standards Institute[5,43] was employed to determine compounds' minimum inhibitory

concentrations (MIC). Briefly, two-fold serial dilutions of compounds were conducted in Muller-Hinton broth (Sangon Biotech, China), at a volume of 200 μL per well in 96-well U-bottomed polystyrene microtiter plates (Corning/Costar, NY, USA). Each well was inoculated with 200 μL of the standardized *P. aeruginosa* inoculum, yielding a final bacterial concentration achieving an OD_{600} of 0.05. The final tested concentrations of antimicrobial agents ranged from 0 μM to 521 μM for compounds.

Growth curve assay. To construct growth curves, overnight cultures of *P. aeruginosa* PAO1 inoculum were diluted in 100 mL of fresh LB broth to achieve a cell suspension optical density at 600 nm (OD_{600}) of 0.05. The growth curve was recorded every 10 min with Tecan Infinate 200 Pro plate reader (Tecan Group Ltd., Mannedorf, Switzerland) for 16 h.

Biofilm formation assay. Measurement of compounds in biofilm formation by Crystal violet assay[44]. *P. aeruginosa* PAO1 was grown overnight in LB medium at 37 ^{o}C for 24 h. Subsequently, the evaluation of biofilm formation using sterile 96-well flat-bottomed polystyrene microtiter plates (Corning/Costar, NY, USA) involved culture of 150 μl of bacterial (OD_{600} = 0.05) in ABTGC medium at 37 ^{o}C for 24 h. Subsequently, supernatant cells were removed and the biofilms were washed three times with sterile phosphate buffered saline (PBS), then MeOH as a fixative. After 30 min, the MeOH was removed, and the microtiter plates were dried at rt. Crystal violet (0.1% in H_2O) was then added to dye the biofilms which were then incubated for 30 min. Crystal violet was removed, microtiter plates were rinsed three times with sterile PBS and add 150 μL of acetic acid (33%) to dissolve the biofilm. The absorbance

values were measured at 570 nm by a microplate reader. All compounds were dissolved in DMSO to make 100 μM stock solutions before transferring to 96-well plates for experiment.

3D imaging of biofilm. To visualize bacterial biofilm formation, the same method as described previously was used for biofilm formation but in a glass-bottom 96-well plate (12–556–38, Fisher Scientific International Inc., Franklin, MA)[45]. After 24 h of incubation at 37 °C, the supernatant was removed, and the well surface was rinsed briefly with 1 × phosphatebuffered saline (PBS) buffer three times. PBS buffer (100 μL) containing 1 μM of SYTO 9 (invitrogen, USA) and 10 μM of propidium iodide (Shanghai Yuanye Bio-Technology Co., Ltd) were added into the well. The plate was incubated for 15 min in the dark. Fluorescent images were acquired with confocal microscope (Zeiss LSM800) for monitoring SYTO 9 and propidium iodide (PI) fluorescence. Images were obtained using an oil immersion 63 × objective lens.

Elastase assay. Overnight culture of *P. aeruginosa* PAO1 in 5 mL of LB medium was performed to a final optical density at 600 nm (OD_{600}) of 0.01. Different concentrations of **10d** (20, 10, 5, 2.5, 1.25 and 0 μM) were incubated for 24 h at 37 °C, with shaking (200 rpm). The supernatant was centrifuged at 10000 rpm for 10 min. Elastase activity was measured by using EnzChekElastase assay kit (Invitrogen, USA), which consisted of BODIPY fluorophore (FL)-labeled DQ elastin conjugate and yields fluorescent fragments upon cleavage by elastase enzyme[46]. The fluorescence signal was recorded every 10 min with Tecan Infinate 200 Pro plate reader (Tecan Group Ltd., Mannedorf, Switzerland; excitation 490 nm, emission 520

nm) for 5 h.

Rhamnolipid assay. Rhamnolipid was quantified according to the method of Koch et al[52], with modifications. A subculture was conducted by directly diluting the overnight culture 1:100 into fresh Minimal Medium (49.3 mM Na_2HPO_4, 50 mM KH_2PO_4, 4.8 mM $MgSO_4$, 7.6 mM $(NH_4)_2SO_4$, 0.6 mM $CaCl_2$, 25 μM $FeSO_4$, 0.162 μM $(NH_4)_6Mo_7O_{24}$, 38 μM $ZnSO_4$, 14 μM $MnCl_2$, 1.6 μM $CuSO_4$, 0.86 μM $CoCl_2$, 1.9 μM boric acid, 5.5 μM $NiCl_2$, 6.72 μM EDTA, 0.6% glycerol in 18 MΩ deionized H2O). The cultures were grown for 24 h at 37 °C, with shaking (200 rpm). The final cell density was measured at 600 nm (OD_{600}) using microplate reader (Bio-Tek). Supernatants were collected by centrifuging at 10000 rpm for 10 min and extracted with Et_2O (twice). Organic fractions were concentrated to yield a white solid. It was resuspended in deionized H2O and added with 0.19% (w/v) orcinol in 50% H_2SO_4. The resulting mixture was incubated at 80 °C for 30 min to give a yellow-orange solution. After cooling to rt, the absorbance was measured at 421 nm and results were normalized with final OD_{600} values.

Pyocyanin assay. Overnight culture of *P. aeruginosa* PA14 was standardized to OD_{600} of 0.05 and diluted 100 times into 25 mL of LB Medium (Sangon Biotech, China) in 250 mL flask. The cultures were grown for 48 h at 37 °C, with shaking (200 rpm). The cultures were then centrifuged for 10 min at 10000 rpm and 8 mL of the supernatants were transferred into new tubes. Briefly, 8 mL of culture supernatant conducted as described above was extracted with chloroform at a ratio of 4:1, followed by extraction with 1 mL of 0.2 M HCl. The absorbance of the upper red

phase was measured using the OD_{520}. The data was normalized by dividing OD_{520} reading with the final OD_{600} values.

GFP reporter strain assays. PAO1-*gfp*, PAO1-*lasB-gfp*, PAO1-*rhlA-gfp* and PAO1-*pqsA-gfp* bioreporter strains were gifts from Singapore Centre for Environmental Life Sciences Engineering of Nanyang Technology University. An overnight culture of reporter strain (grown in LB medium at 37 ^{o}C, 200 rpm) was diluted in ABTGC medium to a final optical density at 600 nm (OD_{600}) of 0.02 (2.5×10^8 CFU/mL). An equal amount of the bacterial suspension was added to the wells to reach final inhibitor concentration. DMSO control (0.1% final concentration) and blank control were used. The microtiter plate was incubated at 37 ^{o}C in Tecan Infinate 200 Pro plate reader (Tecan Group Ltd., Männedorf, Switzerland) to measure the cell density (OD_{600}) and GFP fluorescence (excitation at 485 nm, emission at 535 nm) with 15 min intervals for at least 12 h. The inhibition assays for all test compounds and controls were done in triplicate.

Cytotoxicity Assay. The cytotoxicity of **10d** on Vero cells[47] and LO2 cells[48] was determined by an MTT assay. Cells were seeded into 96-well plate at a density of 5000-15000 cells/well for 16 h. The cells were then washed with phosphate buffered saline (PBS) and treated with varying concentrations of compound (0, 0.01, 0.1, 1, 10, 100 μM) in 200 μL of DMEM (Gibico, China). The cells were incubated with the compound at 37 °C and 5% CO_2 for 16 h. Thiazolyl blue tetrazolium bromide (Sigma Chemical Co.) was added 20 μL (5 mg/mL) to each well of cells. Cells were incubated at 37 °C under 5% CO_2 for 3 h. Finally, the suspension was removed and

150 μL DMSO was added to dissolve the formazan with 10 min shaking. OD_{570} was detected by microplate reader (Bio-Tek). Experiments were performed in triplicate, and the results are shown as the mean ± SD.

Cell viability assay. Cells survival in *P. aeruginosa* infection was assessed as previously described[49]. Briefly *P. aeruginosa* cultures were grown to achieve OD_{600} of 1.2, with or without various concentrations of **10d** at sub-MIC (20, 10, 5, 2.5, 1.25 and 0 μM). The cultures were grown for 24 h at 37 °C, with shaking (200 rpm). The cells were then washed and added to bacterial at a MOI of 100. 4 h postinfection, bacterial cells were killed with 500 μg/mL gentamycin for 2 h at 37 °C in 5% CO_2 and then washed away three with PBS. Cells were incubated with 500 μg/mL MTT for 16 h in 5% CO_2 at 37 °C, then medium was removed, and 0.2 mL of dimethyl sulfoxide (DMSO) was added to each well and reacted for 30 min at rt. The amount of MTT formazan was qualified by measuring at 570 nm using ELISA plate reader. Vero (African green monkey kidney cell) and LO2 cells (noncancerous human hepatocyte cell line) were maintained in DMEM medium, respectively.

Swarming motility assay. To monitor swarming, petri dishes were filled with 20 mL of LB medium supplemented with 0.5% (w/v) bacto agar (Becton, Dickinson and Co.), 0.5% (w/v) casamino acids (Becton, Dickinson and Co.) and 0.5% (w/v) glucose (Solarbio, China) in the presence of **10d** (20, 10, 5, 2.5, 1.25 and 0 μM). Petri dishes were dried in a single stack for 1 h at rt and then drop 1 μL PAO1(OD_{600} = 1) onto center of petri dishes and open the petri dishes to dry. The petri dishes were incubated for 16 h at 37 °C.

Swimming motility assay. To monitor swimming, petri dishes contained 20 mL of LB medium supplemented with 0.3% (w/v) bacto agar, 0.5% (w/v) casamino acids and 0.5% (w/v) glucose in the presence of **10d** (20, 10, 5, 2.5, 1.25 and 0 μM). Petri dishes were dried in a single stack for 1h at rt and then drop 1 μL PAO1(OD_{600} = 1) into the bottom of the petri dishes and open the petri dishes to dry. The petri dishes were incubated for 16 h at 37 °C.

Twitching motility assay. To monitor twitching, petri dishes were charged with 20 mL of LB medium supplemented with 1% (w/v) bacto agar, 0.5% (w/v) casamino acids and 0.5% (w/v) glucose in the presence of **10d** (20, 10, 5, 2.5, 1.25 and 0 μM). Petri dishes were dried in a single stack for 1h at room temperature and then drop 1 μL PAO1 (OD_{600} = 1) into the bottom of the petri dishes and open the petri dishes to dry. The petri dishes were incubated for 16 h at 37 °C.

Pyoverdine assay. An overnight culture of *P. aeruginosa* PAO1 (grown in LB medium at 37 °C, 200 rpm) was diluted in ABTGC medium to a final optical density at 600 nm (OD_{600}) of 0.02 (2.5×10^8 CFU/mL). Different concentrations of **10d** (20, 10, 5, 2.5, 1.25 and 0 μM) were distributed to 10 μM or 50 μM of $FeCl_3$ ABTGC medium. The microtiter plate was incubated at 37 °C in microplate reader (Bio-Tek) to measure the cell density (OD_{600}), Pyoverdine[50] fluorescence (excitation at 400 nm, emission at 447 nm), pyochelin fluorescence (excitation at 350 nm, emission at 430 nm). The experiment assay for all test compounds and controls were done in triplicate.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS). An overnight

culture of *P. aeruginosa* PAO1 was standardized to OD_{600} of 0.05 and diluted 100 times into 50 mL of ABTGC medium in a 250 mL flask. The cultures were grown for 24 h at 37 °C, with shaking (200 rpm). The cultures were centrifuged for 10 min at 4000 rpm, the deposits were transferred into new tubes, and the settling washed twice with 5 mL HEPES buffer solution (Solarbio, China) and centrifuged for 10 min at 4000 rpm. After adding 100 μL lysozyme (Solarbio, China) in 5 mL HEPES (50 mM) cells in the sample solutions were broken by ultrasonic cell breaker for 30 min, and then sample solution was digested by 1 M nitric acid[51]. Finally, the cultures were centrifuged for 10 min at 10000 rpm, and samples were analyzed by inductively coupled plasma mass spectrometry (iCAP 7000 SERIES, Thermo Scientific).

Computational Methods. All molecular docking calculations have been performed using Glide, Schrödinger[53]. The preparation of FpvA structure (PDB ID 2W6T)[28] followed the protein preparation and refinement protocol. After the repair and refinement of the protein, the ligand preparation and conformation search were done via ligprep and Macromodel[27]. An SP docking were first conducted to narrow the number of further evaluation, and the top 100 results from SP docking were submitted to another XP docking to obtain the final score and conformation, the best one of which in FpvA was chosen to the MD simulation. Topology of the protein-ligand complex were conducted and examined via ambertools[28]. The force-field parameters for **10d**-Fe complex were obtained using the Antechamber and Metal Center Parameter Builder MCPB.py[54], AMBERff14SB force fields were used for the protein in the simulation system[55], and TIP3P model was used for the H_2O[56].

Trajectories were produced by using the Gromacs software[57]. NVT and NPT equilibration of the system were performed after the minimization. Then a 40 ns production run under NPT ensemble was conducted. Pressure and temperature were regulated at 1 Atm and 300 K, electrostatic interactions were evaluated using the particle mesh Ewald (PME) scheme.

QM based geometry optimization and force constant calculation were performed in Gaussian09, using B3LYP/6-31G* level[58].

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Additional tables and figures representing NMR spectra (^{1}H and ^{13}C NMR) and HRMS of compounds, the comprehensive statement of confirming purity and the method, HPLC data of final compounds, bacterial growth curves assay, poverdine and pyochelin assay, confocal laser scanning microscopy (CLSM) images of *P. aeruginosa* biofilm formation (PDF). Molecular formula strings file (CSV).

AUTHOR INFORMATION

Corresponding Authors

* Wei-Min Chen: e-mail, twmchen@jnu.edu.cn; phone, +86 20 8522 4497.

* Jing Lin: e-mail, linjing_jnu@163.com; phone, +86 20 8522 1367.

* Ping-Hua Sun: e-mail, pinghuasunny@163.com.

ORCID

Wei-Min Chen: 0000-0002-0292-7841

Notes

All authors declare no competing financial interest.

Yi-Bin Li, Present address: *College of Pharmacy, Southern Medical University, Guangzhou 510515, P. R. China*

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China

(No. 81872776), The Pearl River S&T Nova Program of Guangzhou (No. 201806010116), and the Natural Science Foundation of Guangdong Province (No. 2019A1515011801).

We sincerely thank Dr. Liang Yang from the Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University for gifting the PAO1-*lasB-gfp*, PAO1-*rhlA-gfp*, PAO1-*pqsA-gfp*, high expression *fpvA* and ∆*Pvds* strain. Finally, we also sincerely thank the First Affiliated Hospital of Jinan University Clinical Laboratory for gifting 4 multi drug resistant (MDR) strains.

ABBREVIATIONS USED:

QS, quorum sensing; Pvd, pyoverdin; Pch, pyochelin; *P. aeruginosa, Pseudomonas aeruginosa*; 3-oxo-C12-HSL, N-(3-oxododecanoyl)-L-homoserine lactone; C4-HSL, N-butanoyl-L-homoserine lactone; PQS, 2-heptyl-3-hydroxy-4(1*H*) quinolone; QSIs, QS inhibitors; SAR, structure–activity relationship; MIC, minimum inhibitory concentrations; DFP, deferiprone; GFP, green fluorescent protein; MDR, multidrug resistant; HRMS, high-resolution mass spectra; HPLC, high-performance liquid chromatography; DMSO, dimethyl sulfoxide; DCM, DCM; THF, tetrahydrofuran; EDCI, 1-ethyl-3(3-dimethylpropylamine)carbodiimide hydrochloride; DMAP, 4-(N,N-dimethylamino)pyridine; TLC, thin-layer chromatography; MOPS, 3-morpholinopropanesulfonic acid; PBS, phosphate buffered saline; PI, propidium iodide; OdDHL, N-(3-oxo-dodecanoyl)-L-homoserine lactone; Acyl-HSL, N-acyl-homoserine lactone.

REFERENCES

(1) Liu, Y. C.; Chan, K. G.; Chang, C. Y. Modulation of Host Biology by *Pseudomonas aeruginosa* Quorum Sensing Signal Molecules: Messengers or Traitors. *Front. Microbiol.* **2015**, *6*, 1226-1235.

(2) Choi, H.; Ham, S. Y.; Cha, E.; Shin, Y.; Kim, H. S.; Bang, J. K.; Son, S. H.; Park, H. D.; Byun, Y. Structure-Activity Relationships of 6- and 8-Gingerol Analogs as Anti-Biofilm Agents. *J. Med. Chem.* **2017**, *60*, 9821-9837.

(3) Cao, H.; Krishnan, G.; Goumnerov, B.; Tsongalis, J.; Tompkins, R.; Rahme, L. G. A Quorum Sensing-Associated Virulence Gene of *Pseudomonas aeruginosa* Encodes a LysR-Like Transcription Regulator with a Unique Self-Regulatory Mechanism. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 14613-14618.

(4) Diggle, S. P.; Winzer, K.; Chhabra, S. R.; Worrall, K. E.; Camara, M.; Williams, P. The *Pseudomonas aeruginosa* Quinolone Signal Molecule Overcomes the Cell Density-Dependency of the Quorum Sensing Hierarchy, Regulates Rhl-Dependent Genes at the Onset of Stationary Phase and Can Be Produced in the Absence of LasR. *Mol. Microbiol.* **2003**, *50*, 29-43.

(5) Diggle, S. P.; Cornelis, P.; Williams, P.; Camara, M. 4-Quinolone Signalling in *Pseudomonas aeruginosa*: Old Molecules, New Perspectives. *Int. J. Med. Microbiol.* **2006**, *296*, 83-91.

(6) Haussler, S.; Becker, T. The *Pseudomonas* Quinolone Signal (PQS) Balances Life and Death in *Pseudomonas aeruginosa* Populations. *PLoS Pathog.* **2008**, *4*, e1000166.

(7) Fong, J.; Yuan, M.; Jakobsen, T. H.; Mortensen, K. T.; Delos, S. M.; Chua, S.

L.; Yang, L.; Tan, C. H.; Nielsen, T. E.; Givskov, M. Disulfide Bond-Containing Ajoene Analogues as Novel Quorum Sensing Inhibitors of *Pseudomonas aeruginosa*. *J. Med. Chem.* **2017**, *60*, 215-227.

(8) Manson, D. E.; O'Reilly, M. C.; Nyffeler, K. E.; Blackwell, H. E. Design, Synthesis, and Biochemical Characterization of Non-Native Antagonists of the *Pseudomonas aeruginosa* Quorum Sensing Receptor LasR with Nanomolar IC_{50} Values. *ACS Infect. Dis.* **2020**, *6*, 649−661.

(9) Moore, J. D.; Rossi, F. M.; Welsh, M. A.; Nyffeler, K. E.; Blackwell, H. E. A Comparative Analysis of Synthetic Quorum Sensing Modulators in *Pseudomonas aeruginosa*: New Insights into Mechanism, Active Efflux Susceptibility, Phenotypic Response, and Next-Generation Ligand Design. *J. Am. Chem. Soc.* **2015**, *137*, 14626−14639.

(10) Lee, J.; Wu, J.; Deng Y. Y.; Wang, J.; Wang, C.; Wang, J. H.; Chang, C. Q.; Dong, Y. H.; Williams, P.; Zhang, L. H. A Cell-Cell Communication Signal Integrates Quorum Sensing and Stress Response. *Nat. Chem. Biol.* **2013**, *9,* 339−343.

(11)Biswas, N. N.; Kutty, S. K.; Barraud, N.; Iskander, G. M.; Griffith, R.; Rice, S. A.; Willcox, M.; Black, D. S.; Kumar, N. Indole-Based Novel Small Molecules for the Modulation of Bacterial Signalling Pathways. *Org. Biomol. Chem.* **2015**, *13*, 925-937.

(12) Krewulak, K. D.; Vogel, H. J. Structural Biology of Bacterial Iron Uptake. *Biochim. Biophys. Acta, Biomembr.* **2008**, *1778*, 1781-1804.

(13) Braun, V.; Hantke, K. Recent Insights into Iron Import by Bacteria. *Curr.*

Opin. Chem. Biol. **2011**, *15*, 328-334.

(14) Banin, E.; Vasil, M. L.; Greenberg, E. P. Iron and *Pseudomonas aeruginosa* Biofilm Formation. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 11076-11081.

(15) Singh, P. K.; Parsek, M. R.; Greenberg, E. P.; Welsh, M. J. A Component of Innate Immunity Prevents Bacterial Biofilm Development. *Nature* **2002**, *417*, 552-555.

(16) Gupta, A.; Singh, R. L.; Raghubir, R. Antioxidant Status During Cutaneous Wound Healing in Immunocompromised Rats. *Mol. Cell. Biochem.* **2002**, *241*, 1-7.

(17) Mohammadpour, M.; Behjati, M.; Sadeghi, A.; Fassihi, A. Wound Healing by Topical Application of Antioxidant Iron Chelators: Kojic Acid and Deferiprone. *Int. Wound J.* **2013**, *10*, 260-264.

(18) Ammons, M. C.; Ward, L. S.; Dowd, S.; James, G. A. Combined Treatment of *Pseudomonas aeruginosa* Biofilm with Lactoferrin and Xylitol Inhibits the Ability of Bacteria to Respond to Damage Resulting from Lactoferrin Iron Chelation. *Int. J. Antimicrob. Agents* **2011**, *37*, 316-323.

(19) Ilangovan, A.; Fletcher, M.; Rampioni, G.; Pustelny, C.; Rumbaugh, K.; Heeb, S.; Camara, M.; Truman, A.; Chhabra, S. R.; Emsley, J.; Williams, P. Structural Basis for Native Agonist and Synthetic Inhibitor Recognition by the *Pseudomonas aeruginosa* Quorum Sensing Regulator PqsR (MvfR). *PLoS Pathog.* **2013**, *9,* e1003508.

(20) Ma, Y. M.; Luo, W.; Quinn, P. J.; Liu, Z.; Hider, R. C. Design, Synthesis, Physicochemical Properties, and Evaluation of Novel Iron Chelators with Fluorescent

Sensors. *J. Med. Chem.* **2004**, *47*, 6349-6362.

(21) Liu, Z. D.; Hider, R. C. Design of Clinically Useful Iron(III)-Selective Chelators. *Med. Res. Rev.* **2002**, *22*, 26-64.

(22) Ma, Y. M.; Xie, Y. Y.; Hider, R. C. A Novel Fluorescence Method for Determination of pFe^{3+}. *Analyst* **2013**, *138*, 96-99.

(23) Braun, V.; Killmann, H. Bacterial Solutions to the Iron-Supply Problem. *Trends Biochem. Sci.* **1999**, *24*, 104-109.

(24) Cezard, C.; Farvacques, N.; Sonnet, P. Chemistry and Biology of Pyoverdines, *Pseudomonas* Primary Siderophores. *Curr. Med. Chem.* **2015**, *22*, 165-186.

(25) Llamas, M. A.; Imperi, F.; Visca, P.; Lamont, I. L. Cell-Surface Signaling in *Pseudomonas*: Stress Responses, Iron Transport, and Pathogenicity. *FEMS Microbiol. Rev.* **2014**, *38*, 569-597.

(26) Beare, P. A.; For, R. J.; Martin, L. W.; Lamont, I. L. Siderophore-Mediated Cell Signalling in *Pseudomonas aeruginosa*: Divergent Pathways Regulate Virulence Factor Production and Siderophore Receptor Synthesis. *Mol. Microbiol.* **2003**, *47*, 195-207.

(27) Watts, K. S.; Dalal, P.; Murphy, R. B.; Sherman, W.; Friesner, R. A.; Shelley, J. C. ConfGen: A Conformational Search Method for Efficient Generation of Bioactive Conformers. *J. Chem. Inf. Model.* **2010**, *50*, 534-546.

(28) Greenwald, J.; Nader, M.; Celia, H.; Gruffaz, C.; Geoffroy, V.; Meyer, J. M.; Schalk, I. J.; Pattus, F. FpvA Bound to Non-cognate Pyoverdines: Molecular Basis of Siderophore Recognition by An Iron Transporter. *Mol. Microbiol.* **2009**, *72*,

1246-1259.

(29) Papenfort, K.; Bassler, B. L. Quorum Sensing Signal-Response Systems in Gram-Negative Bacteria. *Nat. Rev. Microbiol.* **2016**, *14*, 576-588.

(30) Jakobsen, T. H.; van Gennip, M.; Phipps, R. K.; Shanmugham, M. S.; Christensen, L. D.; Alhede, M.; Skindersoe, M. E.; Rasmussen, T. B.; Friedrich, K.; Uthe, F.; Jensen, P. O.; Moser, C.; Nielsen, K. F.; Eberl, L.; Larsen, T. O.; Tanner, D.; Hoiby, N.; Bjarnsholt, T.; Givskov, M. Ajoene, a Sulfur-Rich Molecule from Garlic, Inhibits Genes Controlled by Quorum Sensing. *Antimicrob. Agents Chemother.* **2012**, *56*, 2314-2325.

(31) O'Toole, G. A.; Kolter, R. Flagellar and Twitching Motility Are Necessary for *Pseudomonas aeruginosa* Biofilm Development. *Mol. Microbiol.* **1998**, *30*, 295-304.

(32) Shrout, J. D.; Chopp, D. L.; Just, C. L.; Hentzer, M.; Givskov, M.; Parsek, M. R. The Impact of Quorum Sensing and Swarming Motility on *Pseudomonas aeruginosa* Biofilm Formation Is Nutritionally Conditional. *Mol. Microbiol.* **2006**, *62*, 1264-1277.

(33) Merz, A. J.; So, M.; Sheetz, M. P. Pilus Retraction Powers Bacterial Twitching Motility. *Nature* **2000**, *407*, 98-102.

(34) Semmler, A. B.; Whitchurch, C. B.; Mattick, J. S. A Re-Examination of Twitching Motility in *Pseudomonas aeruginosa*. *Microbiol.* **1999**, *145*, 2863-2873.

(35) Aboushleib, H. M.; Omar, H. M.; Abozahra, R.; Elsheredy, A.; Baraka, K. Correlation of Quorum Sensing and Virulence Factors in *Pseudomonas aeruginosa* Isolates in Egypt. *J. Infect. Dev. Ctries.* **2015**, *9*, 1091-1099.

(36) Shalev, O.; Hileti, D.; Nortey, P.; Hebbel, R. P.; Hoffbrand, V. A. Transport of 14C-Deferiprone in Normal, Thalassaemic and Sickle Red Blood Cells. *Br. J. Haematol.* **1999**, *105*, 1081-1083.

(37) Gaboriau, F.; Leray, A. M.; Ropert, M.; Gouffier, L.; Cannie, I.; Troadec, M. B.; Loreal, O.; Brissot, P.; Lescoat, G. Effects of Deferasirox and Deferiprone on Cellular Iron Load in the Human Hepatoma Cell Line HepaRG. *BioMetals* **2010**, *23*, 231-245.

(38) Bose, C.; Megyesi, J. K.; Shah, S. V.; Hiatt, K. M.; Hall, K. A.; Karaduta, O.; Swaminathan, S. Evidence Suggesting a Role of Iron in a Mouse Model of Nephrogenic Systemic Fibrosis. *PLoS One* **2015**, *10*, e0136563.

(39) Russell, A. B.; LeRoux, M.; Hathazi, K.; Agnello, D. M.; Ishikawa, T.; Wiggins, P. A.; Wai, S. N.; Mougous, J. D. Diverse Type VI Secretion Phospholipases Are Functionally Plastic Antibacterial Effectors. *Nature* **2013**, *496*, 508-512.

(40) Parrino, B.; Schillaci, D.; Carnevale, I.; Giovannetti, E.; Diana, P.; Cirrincione, G.; Cascioferro, S. Synthetic Small Molecules as Anti-Biofilm Agents in the Struggle against Antibiotic Resistance. *Eur. J. Med. Chem.* **2019**, *161*, 154-178.

(41) Jadhav, G. P.; Chhabra, S. R.; Telford, G.; Hooi, D. S.; Righetti, K.; Williams, P.; Kellam, B.; Pritchard, D. I.; Fischer, P. M. Immunosuppressive But Non-LasR-Inducing Analogues of the *Pseudomonas aeruginosa* Quorum-Sensing Molecule N-(3-Oxododecanoyl)-l-Homoserine Lactone. *J. Med. Chem.* **2011**, *54*, 3348-3359.

(42) Brown, M. F.; Mitton-Fry, M. J.; Arcari, J. T.; Barham, R.; Casavant, J.; Gerstenberger, B. S.; Han, S.; Hardink, J. R.; Harris, T. M.; Hoang, T.; Huband, M. D.; Lall, M. S.; Lemmon, M. M.; Li, C.; Lin, J.; McCurdy, S. P.; McElroy, E.; McPherson, C.; Marr, E. S.; Mueller, J. P.; Mullins, L.; Nikitenko, A. A.; Noe, M. C.; Penzien, J.; Plummer, M. S.; Schuff, B. P.; Shanmugasundaram, V.; Starr, J. T.; Sun, J.; Tomaras, A.; Young, J. A.; Zaniewski, R. P. Pyridone-Conjugated Monobactam Antibiotics with Gram-Negative Activity. *J. Med. Chem.* **2013**, *56*, 5541-5552.

(43) Koeth, L. M.; DiFranco-Fisher, J. M.; McCurdy, S. A Reference Broth Microdilution Method for Dalbavancin In Vitro Susceptibility Testing of Bacteria that Grow Aerobically. *J. Vis. Exp.* **2015**, *103*, e53028.

(44) Vandeputte, O. M.; Kiendrebeogo, M.; Rajaonson, S.; Diallo, B.; Mol, A.; El, J. M.; Baucher, M. Identification of Catechin as One of the Flavonoids from Combretum Albiflorum Bark Extract that Reduces the Production of Quorum-Sensing-Controlled Virulence Factors in *Pseudomonas aeruginosa* PAO1. *Appl. Environ. Microbiol.* **2010**, *76*, 243-253.

(45) Furiga, A.; Lajoie, B.; El, H. S.; Baziard, G.; Roques, C. Impairment of *Pseudomonas aeruginosa* Biofilm Resistance to Antibiotics by Combining the Drugs with a New Quorum-Sensing Inhibitor. *Antimicrob. Agents Chemother.* **2015**, *60*, 1676-1686.

(46) Tan, S. Y.; Chua, S. L.; Chen, Y. C.; Rice, S. A.; Kjelleberg, S.; Nielsen, T. E.; Yang, L.; Givskov, M. Identification of Five Structurally Unrelated Quorum-Sensing Inhibitors of *Pseudomonas aeruginosa* from a Natural-Derivative Database.

Antimicrob. Agents Chemother. **2013**, *57*, 5629-5641.

(47) Kudumela, R. G.; McGaw, L. J.; Masoko, P. Antibacterial Interactions, Anti-Inflammatory and Cytotoxic Effects of Four Medicinal Plant Species. *BMC Complem. Altern. Med.* **2018**, *18*, 199-206.

(48) Ma, L. L.; Wu, J.; Wang, S. L.; Yang, H.; Liang, D. H.; Lu, Z. Synergistic Antibacterial Effect of Bi_2S_3 Nanospheres Combined with Ineffective Antibiotic Gentamicin against Methicillin-Resistant *Staphylococcus aureus*. *J. Inorg. Biochem.* **2017**, *168*, 38-45.

(49) Maura, D.; Drees, S. L.; Bandyopadhaya, A.; Kitao, T.; Negri, M.; Starkey, M.; Lesic, B.; Milot, S.; Deziel, E.; Zahler, R.; Pucci, M.; Felici, A.; Fetzner, S.; Lepine, F.; Rahme, L. G. Polypharmacology Approaches Against the *Pseudomonas aeruginosa* MvfR Regulon and Their Application in Blocking Virulence and Antibiotic Tolerance. *ACS Chem. Biol.* **2017**, *12*, 1435-1443.

(50) Hannauer, M.; Barda, Y.; Mislin, G. L.; Shanzer, A.; Schalk, I. J. The Ferrichrome Uptake Pathway in *Pseudomonas aeruginosa* Involves an Iron Release Mechanism with Acylation of the Siderophore and Recycling of the Modified Desferrichrome. *J. Bacteriol.* **2010**, *192*, 1212-1220.

(51) Hunter, R. C.; Asfour, F.; Dingemans, J.; Osuna, B. L.; Samad, T.; Malfroot, A.; Cornelis, P.; Newman, D. K. Ferrous Iron Is a Significant Component of Bioavailable Iron in Cystic Fibrosis Airways. *mBio.* **2013**, *4*, 1-8.

(52) Li, Y. B.; Liu, J.; Huang, Z. X.; Yu, J, H.; Lin, J.; Chen, W. M. Design, Synthesis and Biological Evaluation of 2-Substituted

3-Hydroxy-6-Methyl-4*H*-Pyran-4-One Derivatives as *Pseudomonas aeruginosa* Biofilm Inhibitors. *Eur. J. Med. Chem.* **2018**, *158*, 753-766.

(53) Schrödinger Release 2019-1: Induced Fit Docking Protocol; Glide, Schrödinger, LLC, New York, NY, 2016; Prime, Schrödinger, LLC, New York, NY, 2019.

(54) Li, P. F.; Jr. K. M. M. MCPB.py: A Python Based Metal Center Parameter Builder. *J. Chem. Inf. Model.* **2016**, *56*, 599-604.

(55) Maier, J. A.; Martinez, C.; Kasavajhala, K.; Wickstrom, L.; Hauser, K. E.; Simmerling, C. Ff14SB: Improving the Accuracy of Protein Side Chain and Backbone Parameters from Ff99SB. *J. Chem. Theory. Comput.* **2015**, *11*, 3696-3713.

(56) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of Simple Potential Functions for Simulating Liquid Water. *J. Chem. Phys.* **1983**, *79*, 926-935.

(57) Abraham, M. J.; Murtola, T.; Schulz, R.; Páll, S.; Smith, J. C.; Hess, B.; Lindahl, E. GROMACS: High Performance Molecular Simulations through Multi-level Parallelism from Laptops to Supercomputers. *Softwarex* **2015**, *1*, 19-25.

(58) Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang,

M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.

Table of Contents Graphic